SPECIFICATION

HETEROCYCLIC RING AND PHOSPHONOXYMETHYL GROUP SUBSTITUTED PYRIDINE DERIVATIVES, AND ANTIFUNGAL AGENTS CONTAINING SAME

Technical Field

The present invention relates to novel heterocyclic ring and phosphonoxymethyl group substituted pyridine derivatives, and antifungal agents containing the same.

Background Art

In recent years, managements of opportunistic infections have become more and more significant more than ever because of an increase in the number of elderly people and immunocompromised patients as a result of advanced chemotherapies or the like. As demonstrated by the fact that opportunistic infections are occurring one after another by different avirulent pathogen, it has been shown that the problem of infectious disease will not ends as long as there are underlying diseases that diminish the immune functions of patients. Consequently, new strategies for infectious diseases control, including the problem of drug-resistant pathogen, will be one of the important issues in the soon-to-come aged society.

In the field of antifungal agents, heretofore, for instance, amphotericine B which is based on a polyene skeleton, fluconazole, itraconazole and voriconazole which are based on an azole skeleton, or the like, have been developed for the treatment of deep seated mycoses. Most of pre-existing drugs already available

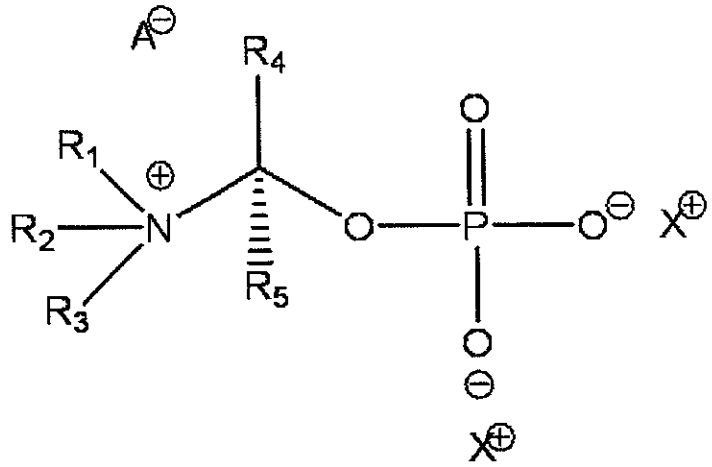
commercially have similar mechanism of action, and currently, the appearance of azole-resistant fungi or the like has been problems.

In recent years, as a 1,3-β-glucan synthetase inhibitor with a novel mechanism, naturally occurring compound-derived cyclic hexapeptides caspofungin and micafungin or the like, have been developed; however, from the fact that these agents only exist in injectable form, they are not yet sufficient practically as antifungal agents.

Since there have been the situations that the pre-existing antifungal agents are insufficient for treatment of the deep seated mycoses, there is a demand and need for development of agents which are based on a novel mechanism and are of high safety. As the related art relevant to antifungal agents based on such a novel mechanism, Patent Documents 1 and 2 describe pyridine derivatives which demonstrates effects against the onset, progress, and persistence of infections by inhibiting the expression of cell wall proteins, inhibiting the cell wall assembly and also adhesion onto cells, and preventing pathogens from showing pathogenicity, with the process which transports GPI (Glycosylphosphatidylinositol)-anchored proteins to the cell wall being inhibited.

In light of this situation, there has been proposed in Patent document 3 a hetero ring-substituted pyridine derivative as an antifungal agent that has excellent antifungal action not seen in conventional antifungal agents, and is also superior in terms of its properties, safety, and metabolic stability.

On the other hand, Patent documents 4 and 5 disclose N-phosphoryloxymethyl prodrugs that are compounds represented by the following formula, as water-soluble prodrugs:



(wherein R_1, R_2, and R_3 represent substituents including a parent tertiary or secondary amine, each of R_4 and R_5 represents an organic or inorganic residue, and X represents a cationic organic or inorganic salt.)

However, the water-soluble prodrugs known up to now do not have an excellent antifungal action based on the inhibition of the GPI-anchored protein transport process, nor are they excellent in terms of solubility in water, stability in an aqueous solution, as well as safety and in-vivo kinetics, and the creation of a better antifungal agent has been desired.

Patent document 1 : International Publication WO 02/04626 pamphlet

Patent document 2 : International Publication WO 05/033079 pamphlet

Patent document 3 : International Publication WO 07/052615 pamphlet

Patent document 4 : U.S. Patent No. 6,235,728 B1

Patent document 5 : Japanese Patent Publication No. 2001-527083

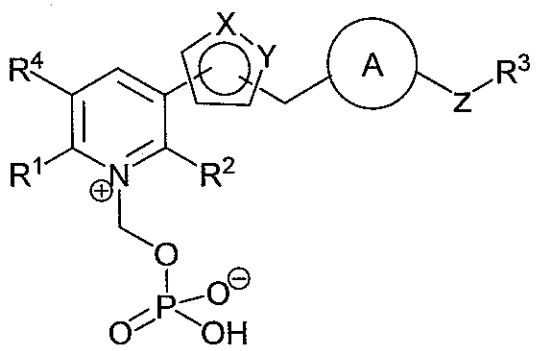
Disclosure of Invention

Problems to be Solved by the Invention

In light of such situations, it is an object of the present invention to provide an antifungal agent that has excellent antifungal action, and is also excellent in terms of

solubility in water, its stability in an aqueous solution, and its safety and in-vivo kinetics.

As a result of diligent research conducted in light of the above situations, the inventors perfected the present invention upon discovering that compounds which are represented by the following formula (I):

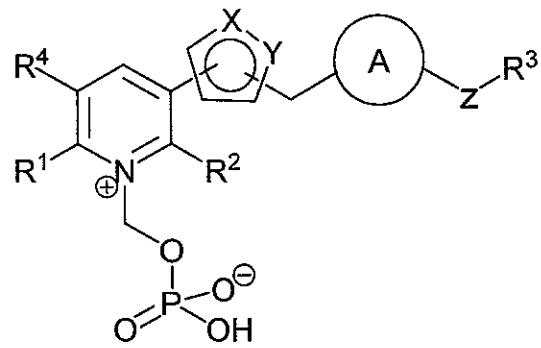


(I)

and has a phosphonoxymethyl group on a nitrogen atom on a pyridine derivative has excellent antifungal action and is also excellent in terms of solubility in water, its stability in an aqueous solution, and its safety and in-vivo kinetics.

Specifically, in a first aspect of the present invention, there is provided:

[1] A compound represented by the following formula (I):



(I)

(wherein

R^1 represents a hydrogen atom, a halogen atom, an amino group, R^{11}-NH- (wherein R^{11} represents a C_{1-6} alkyl group, a hydroxy C_{1-6} alkyl group, a C_{1-6} alkoxy

C_{1-6} alkyl group, or a C_{1-6} alkoxycarbonyl C_{1-6} alkyl group), R^{12}-(CO)-NH- (wherein R^{12} represents a C_{1-6} alkyl group or a C_{1-6} alkoxy C_{1-6} alkyl group), a C_{1-6} alkyl group, a hydroxy C_{1-6} alkyl group, a cyano C_{1-6} alkyl group, a C_{1-6} alkoxy group, or a C_{1-6} alkoxy C_{1-6} alkyl group;

R^2 represents a hydrogen atom, a C_{1-6} alkyl group, an amino group, or a di-C_{1-6} alkylamino group;

one of X and Y represents a nitrogen atom, and the other represents a nitrogen atom or an oxygen atom;

ring A represents a 5- or 6-member heteroaryl ring or a benzene ring, which may have one or two halogen atoms or C_{1-6} alkyl groups;

Z represents a single bond, a methylene group, an ethylene group, an oxygen atom, a sulfur atom, $-CH_2O-$, $-OCH_2-$, -NH-, $-CH_2NH-$, $-NHCH_2-$, $-CH_2S-$, or $-SCH_2-$;

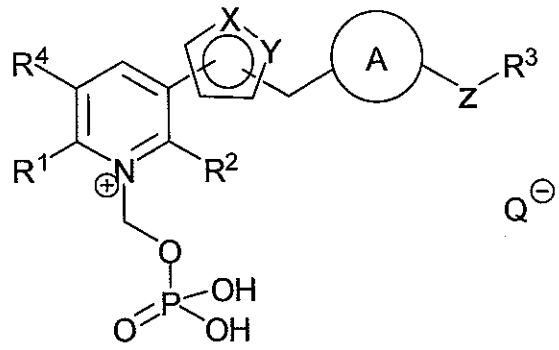
R^3 represents a hydrogen atom or a halogen atom, or a C_{1-6} alkyl group, a C_{3-8} cycloalkyl group, a C_{6-10} aryl group, a 5- or 6-member heteroaryl group, or a 5- or 6-member non-aromatic heterocyclic group, wherein these groups may have one or two substituents selected from a substituent group α; and

R^4 represents a hydrogen atom or a halogen atom;

[substituent Group α]

a halogen atom, a cyano group, a C_{1-6} alkyl group, a C_{1-6} alkoxy group, a C_{1-6} alkoxycarbonyl group, a C_{3-8} cycloalkyl group, a C_{2-6} alkenyl group, and a C_{2-6} alkynyl group), or a salt thereof.

[2] The compound or the salt thereof according to item [1], wherein the salt of the compound represented by the following formula (I) is a salt represented by the following formula (IA):

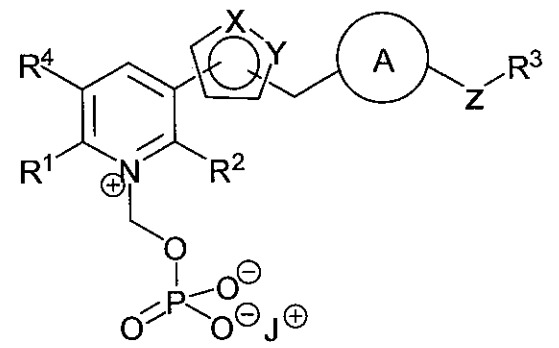


(IA)

wherein

Q^- represents a pharmaceutically acceptable anion.

[3] The compound or the salt thereof according to item [1], wherein the salt of the compound represented by the following formula (I) is a salt represented by the following formula (IB):

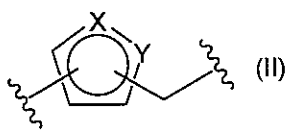


(IB)

wherein

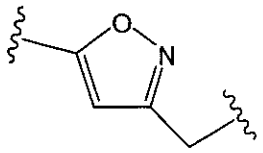
J^+ represents a pharmaceutically acceptable cation.

[4] The compound or the salt thereof according to any one of items [1] to [3], wherein a partial structure represented by the following formula (II):

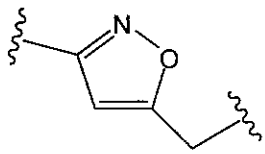


(II)

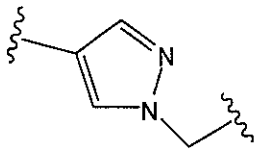
of the compound represented by the above formula (I), (IA), or (IB) represents a partial structure selected from the following group:



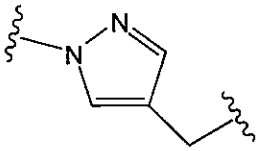
(III)



(IV)



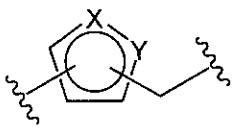
(V)



(VI)

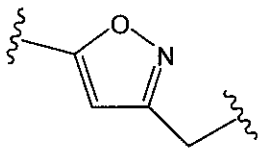
[5] The compound or the salt thereof according to any one of items [1] to [4], wherein one of X and Y represents a nitrogen atom and the other represents an oxygen atom.

[6] The compound or the salt thereof according to item [4], wherein the partial structure represented by the following formula (II):



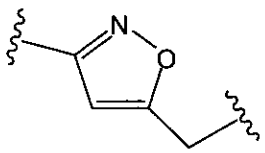
(II)

of the compound represented by the above formula (I), (IA), or (IB) represents a partial structure represented by the following formula (III):



(III)

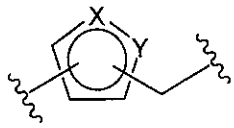
or a partial structure represented by the following formula (IV):



(IV)

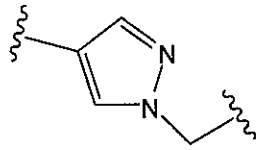
[7] The compound or the salt thereof according to any one of items [1] to [6], wherein both of X and Y represent nitrogen atoms.

[8] The compound or the salt thereof according to item [7], wherein the partial structure represented by the following formula (II):



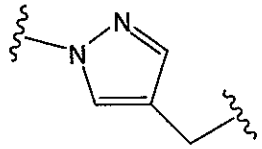
(II)

of the compound represented by the above formula (I), (IA), or (IB) represents a partial structure represented by the following formula (V):



(V)

or a partial structure represented by the following formula (VI):



(VI)

[9] The compound or the salt thereof according to any one of items [1] to [8], wherein R^2 represents an amino group.

[10] The compound or the salt thereof according to item [9], wherein R^1 represents a hydrogen atom, an amino group, or a C_{1-6} alkoxy C_{1-6} alkyl group.

[11] The compound or the salt thereof according to any one of items [1] to [8], wherein R^1 represents an amino group and R^2 represents a hydrogen atom.

[12] The compound or the salt thereof according to any one of items [1] to [11], wherein the ring A represents a pyridine ring, a benzene ring, a furan ring, a thiophene ring, or a pyrrole ring.

[13] The compound or the salt thereof according to item [12], wherein the ring A represents a pyridine ring or a benzene ring.

[14] The compound or the salt thereof according to any one of items [1] to [13], wherein Z represents an oxygen atom, $-CH_2O-$, or $-OCH_2-$.

[15] A pharmaceutical composition comprising: the compound or the salt thereof according to any one of items [1] to [14].

[16] A medicament comprising: the compound or the salt thereof according to any one of items [1] to [14].

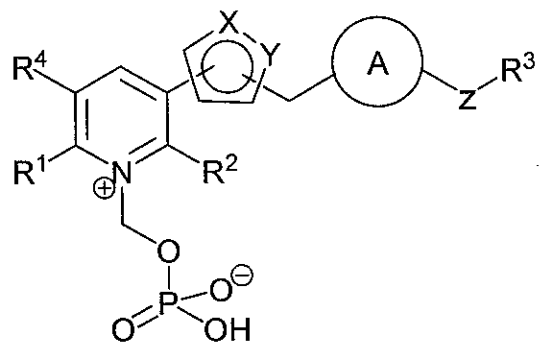
[17] An antifungal agent comprising as an active ingredient the compound or the salt thereof according to any one of items [1] to [14].

[18] A method for preventing and/or treating a fungal disease by administering a pharmaceutically effective amount of the compound or the salt thereof according to any one of items [1] to [14].

[19] A use of the compound or the salt thereof according to any one of items [1] to [14], for manufacture of an antifungal agent.

In addition, in a second aspect of the present invention, there is provided:

[20] A process for producing a compound represented by the following formula (I):



(I)

(wherein

R^1 represents a hydrogen atom, a halogen atom, an amino group, R^{11}-NH- (wherein R^{11} represents a C_{1-6} alkyl group, a hydroxy C_{1-6} alkyl group, a C_{1-6} alkoxy C_{1-6} alkyl group, or a C_{1-6} alkoxycarbonyl C_{1-6} alkyl group), R^{12}-(CO)-NH- (wherein R^{12} represents a C_{1-6} alkyl group or a C_{1-6} alkoxy C_{1-6} alkyl group), a C_{1-6} alkyl group, a hydroxy C_{1-6} alkyl group, a cyano C_{1-6} alkyl group, a C_{1-6} alkoxy group, or a C_{1-6} alkoxy C_{1-6} alkyl group;

R^2 represents a hydrogen atom, a C_{1-6} alkyl group, an amino group, or a di-

C_{1-6} alkylamino group;

one of X and Y represents a nitrogen atom, and the other represents a nitrogen atom or an oxygen atom;

ring A represents a 5- or 6-member heteroaryl ring or a benzene ring, which may have one or two halogen atoms or C_{1-6} alkyl groups;

Z represents a single bond, a methylene group, an ethylene group, an oxygen atom, a sulfur atom, $-CH_2O-$, $-OCH_2-$, $-NH-$, $-CH_2NH-$, $-NHCH_2-$, $-CH_2S-$, or $-SCH_2-$;

R^3 represents a hydrogen atom or a halogen atom, or a C_{1-6} alkyl group, a C_{3-8} cycloalkyl group, a C_{6-10} aryl group, a 5- or 6-member heteroaryl group, or a 5- or 6-member non-aromatic heterocyclic group, wherein these groups may have one or two substituents selected from a substituent group α; and

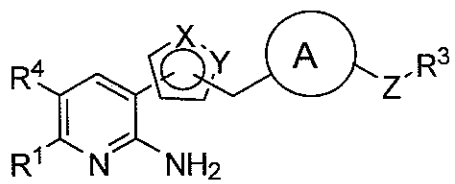
R^4 represents a hydrogen atom or a halogen atom;

[substituent Group α]

a halogen atom, a cyano group, a C_{1-6} alkyl group, a C_{1-6} alkoxy group, a C_{1-6} alkoxycarbonyl group, a C_{3-8} cycloalkyl group, a C_{2-6} alkenyl group, and a C_{2-6} alkynyl group),

comprising the steps of:

reacting a compound represented by the following formula (1) and phosphoric acid di-*tert*-butyl ester chloromethyl ester in the presence of a base; and



(1)

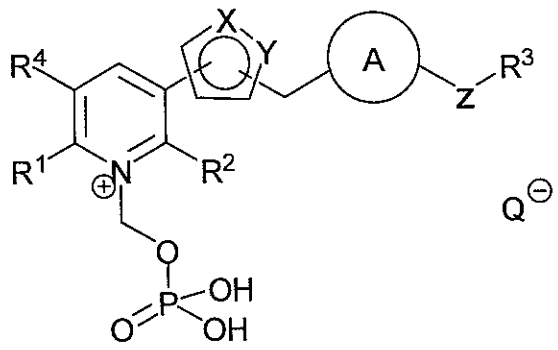
(wherein ring A, R^1, R^3, R^4, X, Y and Z have the same meaning as defined above)

subjecting to a treatment by an acid.

[21] The process according to item [20], wherein the base is sodium iodide.

[22] The process according to item [20] or [21], wherein the acid is trifluoroacetic acid or hydrochloric acid.

[23] A process for producing a salt of a compound represented by the following formula (IA):



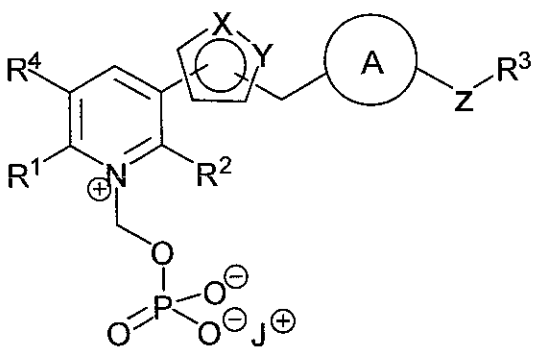
(IA)

(wherein ring A, R^1, R^3, R^4, X, Y and Z have the same meaning as defined above, and Q^- represents a pharmaceutically acceptable anion)

comprising reacting the compound obtained by the process according to any one of items [20] to [22] and an acid.

[24] The process according to item [23], wherein the acid is selected from the group consisting of hydrochloric acid, hydrobromic acid, nitric acid, and sulfuric acid.

[25] A process for producing a salt of a compound represented by the following formula (IB):



(IB)

(wherein ring A, R^1, R^3, R^4, X, Y and Z have the same meaning as defined above, and J^+ represents a pharmaceutically acceptable cation)

comprising reacting the compound obtained by the process according to any one of items [20] to [22] and a base.

[26] The process according to item [25], wherein the base is selected from the group consisting of lithium hydroxide, sodium hydroxide, potassium hydroxide calcium hydroxide and lysine.

Advantageous Effects of the Invention

The compound represented by formula (I), (IA), or (IB) (hereinafter referred to simply as "the compound according to the present invention") 1) acts against the onset, development and persistence of infections by inhibiting fungal GPI biosynthesis, thereby inhibiting expression of cell wall proteins and blocking cell wall assembly while preventing the fungus from attaching to cells so that the pathogen cannot become pathogenic, and 2) is superior in terms of physical properties, and particularly its solubility in water, its stability in an aqueous solution, and its safety and in-vivo kinetics, making the above compounds extremely useful as a preventive or therapeutic agent for fungal infections.

Brief Description of Drawings

Fig. 1 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium anhydrous form (type 1) obtained in Example 2 of the present invention;

Fig. 2 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium anhydrous form (type 2) obtained in Example 3 of the present invention;

Fig. 3 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-

3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium anhydrous form (type 3) obtained in Example 4 of the present invention;

Fig. 4 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium hydrate obtained in Example 5 of the present invention;

Fig. 5 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium acetic acid solvate obtained in Example 6 of the present invention;

Fig. 6 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium amorphous obtained in Example 7 of the present invention;

Fig. 7 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium monohydrochloride obtained in Example 8 of the present invention;

Fig. 8 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium hemisulfate obtained in Example 10 of the present invention;

Fig. 9 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium mononitrate obtained in Example 11 of the present invention;

Fig. 10 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium monohydrobromide obtained in Example 12 of the present invention;

Fig. 11 shows the X-ray diffraction pattern of 2-amino-1-

((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium sodium salt obtained in Example 13 of the present invention;

Fig. 12 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium hemicalcium salt obtained in Example 14 of the present invention;

Fig. 13 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium lithium salt obtained in Example 15 of the present invention;

Fig. 14 shows the X-ray diffraction pattern of 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium potassium salt obtained in Example 16 of the present invention;

FIG. 15 shows a drawing of results of measuring the plasma concentration of the compound of Example 1 and the active form (the parent compound of the compound of Example 1) measured by pharmacokinetic evaluation in mice in one aspect of the present invention; and

FIG. 16 shows a drawing of results of measuring the plasma concentration of the compound of Example 1 and the active form (the parent compound of the compound of Example 1) measured by pharmacokinetic evaluation in monkeys in other aspect of the present invention.

Best Mode for Carrying Out the Invention

The present invention is explained below in more detail by reference to the symbols and the terms used herein being defined and the following examples. The present invention is not limited to the following embodiments, however, and various modifications can be made without departing from the gist thereof.

Herein, a structural formula of a compound sometimes represents a certain isomer for convenience of description. However, compounds according to the present invention may include all possible isomers, such as structurally possible geometric isomers, optical isomers generated due to the presence of asymmetric carbons, stereoisomers, tautomers, and mixtures of isomers, and are not limited to formulae being used for the convenience of description, and may be either one of two isomers or a mixture of both isomers. Thus, the compounds according to the present invention may be either optically active compounds having an asymmetric carbon atom in their molecules or their racemates, and are not restricted to either of them but include both. Furthermore, the compounds according to the present invention may exhibit crystalline polymorphism, but likewise are not restricted to any one of these, but may be in any one of these crystal forms or exist as a mixture of two or more crystal forms. The compounds according to the present invention also include both anhydrous and solvates such as hydrated forms.

The term "C_{1-6} alkyl group" used in the present specification refers to a straight-chain or branched-chain alkyl group with 1 to 6 carbon atoms which is a monovalent group induced by removal of any one hydrogen atom from an aliphatic hydrocarbon with 1 to 6 carbon atoms. Specifically, examples of "C_{1-6} alkyl group" may include a methyl group, an ethyl group, a *n*-propyl group, an isopropyl group, a *n*-butyl group, an isobutyl group, a *sec*-butyl group, a *tert*-butyl group, a *n*-pentyl group, an isopentyl group, a *sec*-pentyl group, a neopentyl group, a 1-methylbutyl group, a 2-methylbutyl group, a 1,1-dimethylpropyl group, a 1,2-dimethylpropyl group, a *n*-hexyl group, an isohexyl group, a 1-methylpentyl group, a 2-methylpentyl group, a 3-methylpentyl group, a 1,1-dimethylbutyl group, a 1,2-dimethylbutyl group, a 2,2-dimethylbutyl group, a 1,3-dimethylbutyl group, a 2,3-dimethylbutyl group, a 3,3-

dimethylbutyl group, a 1-ethylbutyl group, a 2-ethylbutyl group, a 1,1,2,-trimethylpropyl group, a 1,2,2-trimethylpropyl group, a 1-ethyl-1-methylpropyl group, a 1-ethyl-2-methylpropyl group or the like, preferably a methyl group, an ethyl group, a *n*-propyl group, an isopropyl group, a *n*-butyl group, an isobutyl group, a *sec*-butyl group or a *tert*-butyl group or the like.

The term "C_{2-6} alkenyl group" used in the present specification refers to a straight-chain or branched-chain alkenyl group with 2 to 6 carbon atoms which may contain 1 or 2 double bonds. Specifically, examples of "C_{2-6} alkenyl group" may include an ethenyl group, a 1-propenyl group, a 2-propenyl group, a 1-butenyl group, a 2-butenyl group, a 3-butenyl group, a 2-methyl-1-propenyl group, a pentenyl group, a 3-methyl-2-butenyl group, a hexenyl group, a hexanedienyl group or the like, preferably an ethenyl group, a 1-propenyl group, a 2-propenyl group, a 1-butenyl group, a 2-butenyl group, a 3-butenyl group, a 2-methyl-1-propenyl group, a 3-methyl-2-butenyl group or the like.

The term "C_{2-6} alkynyl group" used in the present specification refers to a straight-chain or branched-chain alkynyl chain with 2 to 6 carbon atoms which may contain 1 or 2 triple bonds. Specifically, examples of "C_{2-6} alkynyl group" may include an ethynyl group, a 1-propynyl group, a 2-propynyl group, a 1-butynyl group, a 2-butynyl group, a 3-butynyl group, a pentynyl group, a hexynyl group, a hexanediynyl group or the like, preferably an ethynyl group, a 1-propynyl group, a 2-propynyl group, a 1-butynyl group, a 2-butynyl group, a 3-butynyl group or the like.

The term "C_{3-8} cycloalkyl group" used in the present specification refers to a cyclic aliphatic hydrocarbon group with 3 to 8 carbon atoms. Specifically, examples of "C_{3-8} cycloalkyl group" may include a cyclopropyl group, a cyclobutyl group, a cyclopentyl group, a cyclohexyl group, a cycloheptyl group, a cyclooctyl group or the

like, preferably a cyclopropyl group, a cyclobutyl group, a cyclopentyl group, a cyclohexyl group or the like.

The term "C_{1-6} alkoxy group" used in the present specification refers to a group in which an oxygen atom is bonded to terminus of the "C_{1-6} alkyl group" defined above. Specifically, examples of "C_{1-6} alkoxy group" may include a methoxy group, an ethoxy group, a *n*-propoxy group, an isopropoxy group, a *n*-butoxy group, an isobutoxy group, a *sec*-butoxy group, a *tert*-butoxy group, a *n*-pentyloxy group, an isopentyloxy group, a *sec*-pentyloxy group, a neopentyloxy group, a 1-methylbutoxy group, a 2-methylbutoxy group, a 1,1-dimethylpropoxy group, a 1,2-dimethylpropoxy group, a *n*-hexyloxy group, an isohexyloxy group, a 1-methylpentyloxy group, a 2-methylpentyloxy group, a 3-methylpentyloxy group, a 1,1-dimethylbutoxy group, a 1,2-dimethylbutoxy group, a 2,2-dimethylbutoxy group, a 1,3-dimethylbutoxy group, a 2,3-dimethylbutoxy group, a 3,3-dimethylbutoxy group, a 1-ethylbutoxy group, a 2-ethylbutoxy group, a 1,1,2-trimethylpropoxy group, a 1,2,2-trimethylpropoxy group, a 1-ethyl-1-methylpropxy group, a 1-ethyl-2-methylpropoxy group or the like, preferably a methoxy group, an ethoxy group , a *n*-propoxy group, an isopropoxy group, a *n*-butoxy group, an isobutoxy group, a *sec*-butoxy group, a *tert*-butoxy group or the like.

The term "hydroxyl C_{1-6} alkyl group" used in the present specification refers to a group in which any of the hydrogen atoms in a "C_{1-6} alkyl group" as defined above has been replaced with a hydroxyl group. Specifically, examples of "hydroxyl C_{1-6} alkyl group" may include a hydroxymethyl group, a 1-hydroxyethyl group, a 2-hydroxyethyl group, a 1-hydroxy-*n*-propyl group, a 2-hydroxy-*n*-propyl group, a 3-hydroxy-*n*-propyl group, a 1-hydroxy-isopropyl group, a 2-hydroxy-isopropyl group, a 3-hydroxy-isopropyl group, a 1-hydroxy-*tert*-butyl group or the like, preferably a hydroxymethyl group , a 1-hydroxyethyl group, a 2-hydroxyethyl group or the like.

The term "cyano C_1 to C_6 alkyl group" used in the present specification refers to a group in which any hydrogen atom in the "C_{1-6} alkyl group" defined above has been replaced with a cyano group. Specific examples of "cyano C_1 to C_6 alkyl group" include a cyanomethyl group, a 1-cyanoethyl group, a 2-cyanoethyl group, a 1-cyano-n-propyl group, a 2-cyano-n-propyl group, a 3-cyano-n-propyl group, a 1-cyano-isopropyl group, a 2-cyano-isopropyl group, a 3-cyano-isopropyl group, and a 1-cyano-tert-butyl group, preferably a cyanomethyl group, a 1-cyanoethyl group, a 2-cyanoethyl group, or the like.

The term "C_{1-6} alkoxycarbonyl group" used in the present specification refers to a group in which a carbonyl group is bonded to terminus of the "C_{1-6} alkoxy group" defined above. Specifically, examples of "C_{1-6} alkoxycarbonyl group" may include a methoxycarbonyl group, an ethoxycarbonyl group, a *n*-propoxycarbonyl group, an isopropoxycarbonyl group or the like.

The term "C_{1-6} alkoxycarbonyl C_{1-6} alkyl group" used in the present specification refers to a group in which the "C_{1-6} alkyl group" defined above is bonded to terminus of the "C_{1-6} alkoxycarbonyl group" defined above. Specifically, examples of the "C_{1-6} alkoxycarbonyl C_{1-6} alkyl group" may include a methoxycarbonyl methyl group, a methoxycarbonyl ethyl group, an ethoxycarbonyl methyl group, an ethoxycarbonyl ethyl group or the like.

The term "C_{6-10} aryl group" used in the present specification refers to an aromatic hydrocarbon cyclic group with 6 to 10 carbon atoms. Specifically, examples of "C_{6-10} aryl group" may include a phenyl group, a 1-naphthyl group, a 2-naphthyl group, an indenyl group, an azulenyl group, a heptalenyl group or the like, preferably a phenyl group, a 1-naphthyl group, 2-naphthyl group or the like.

The term "C_{1-6} alkoxy C_{1-6} alkyl group" used in the present specification refers to a group in which any of the hydrogen atoms in a "C_{1-6} alkyl group" as defined above has been replaced with a "C_{1-6} alkoxy group" as defined above. Specifically, examples of "C_{1-6} alkoxy C_{1-6} alkyl group" may include a methoxymethyl group, an ethoxymethyl group, a *n*-propoxymethyl group, a methoxyethyl group, an ethoxyethyl group or the like.

The term "halogen atom" used in the present specification refers a fluorine atom, a chlorine atom, a bromine atom or an iodine atom.

The term "hetero atom" used in the present specification refers to a nitrogen atom, a sulfur atom or an oxygen atom.

The term "5- or 6-member heteroaryl ring" used in the present specification refers to an aromatic ring in which the number of atoms making up the ring is 5 or 6, and 1 or more hetero atoms are included in the atoms making up the ring. Specifically, examples of "5- or 6-member heteroaryl ring" may include a furan ring, a thiophene ring, a pyrrole ring, a pyridine ring, a pyrazine ring, a pyridazine ring, a pyrimidine ring, a triazole ring (a 1,2,3-triazole ring, a 1,2,4-triazole ring, etc.), a tetrazole ring (a 1*H*-tetrazole ring, a 2*H*-tetrazole ring, etc.), a thiazole ring, a pyrazole ring, an oxazole ring, an isoxazole ring, an isothiazole ring, an oxadiazole ring, a thiadiazole ring or the like.

The term "5- or 6-member heteroaryl group" used in the present specification refers to a monovalent group induced by removing 1 hydrogen atom from any position in an aromatic ring in which the number of atoms making up the ring is 5 or 6 and one or more hetero atoms are included in the atoms making up the ring. Specifically, examples of "5- or 6-member heteroaryl group" may include a furyl group (a 2-furyl group or a 3-furyl group, etc.), a thienyl group (a 2-thienyl group or a

3-thienyl group, etc.), a pyrrolyl group (a 1-pyrrolyl group, a 2-pyrrolyl group or a 3-pyrrolylgroup, etc.), a pyridyl group (a 2-pyridyl group, a 3-pyridyl group, a 4-pyridyl group, etc.), a pyrazinyl group, a pyridazinyl group (a 3-pyridazinyl group or a 4-pyridazinyl group, etc.), a pyrimidinyl group (a 2-pyrimidinyl group, a 4-pyrimidinyl group or a 5-pyrimidinyl group, etc.), a triazolyl froup (a 1,2,3-triazolyl group or a 1,2,4-triazolyl group, etc.), a tetrazolyl group (a 1*H*-tetrazolyl group or a 2*H*-tetrazolyl group, etc.), a thiazolyl group (a 2-thiazolyl group, a 4-thiazolyl group or a 5-thiazolyl group, etc.), a pyrazolyl group (a 3-pyrazolyl group or a 4-pyrazolyl group, etc.), an oxazolyl group (a 2-oxazolyl group, a 4-oxazolyl group or a 5-oxazolyl group, etc.), an isoxazolyl group (a 3-isoxazolyl group, a 4-isoxazolyl group or a 5-isoxazolyl group, etc.), an isothiazolyl group (a 3-isothiazolyl group, a 4-isothiazolyl group or a 5-isothiazolyl group, etc.), an oxadiazolyl group ,a thiadiazolyl group or the like.

The term "5- or 6-member non-aromatic heterocyclic group" used in the present specification refers to a monovalent group induced by removing 1 hydrogen atom from any position in a non-aromatic ring in which the number of atoms making up the ring is 5 or 6 and 1 or more hetero atoms are included in the atoms making up the ring. Specifically, examples of "5- or 6-member non-aromatic heterocyclic group" may include a pyrrolidinyl group, a piperadinyl group, a piperidinyl group, a morpholinyl group, a tetrahydrofuryl group, a tetrahydropyranyl group or the like.

The term "di C_{1-6} alkylamino group" used in the present specification refers to a group in which 2 hydrogen atoms of the amino group are replaced with the "C_{1-6} alkyl groups" defined above being the same as or different from each other. Specifically, examples of the term "di C_{1-6} alkylamino group" may include a N, N-dimethylamino group, a N, N-diethylamino group, a N, N-di-*n*-propylamino group, a N, N-di-isopropylamino group, a N, N-di-*n*-butylamino group, a N, N-isobutylamino group,

a N, N-di-*sec*-butylamino group, a N, N-di-*tert*-butylamino group, a N-ethyl-N-methylamino group, a N-*n*-propylamino-N-methylamino group, a N-isopropyl-N-methylamino group, a N-*n*-butyl-N-methylamino group, a N-isobutyl-N-methylamino group, a N-*sec*-butyl-N-methylamino group, a N-*tert*-butyl-N-methlamino group or the like, preferably a N, N-dimethylamino group, a N, N-diethylamino group, N-ethyl-N-methylamino group or the like.

The term "may have 1 or 2 substituents" used in the specification means that there may be 1 or 2 substituents in any combination in sites capable of substituting.

The term "Q^-" used in the present specification refers to a pharmaceutically acceptable anion, and as long as it originates from a pharmaceutically acceptable organic acid or inorganic acid, it is not limited to specific anions. Examples of these organic acids or inorganic acids may include the following. Specifically examples of the organic acids may include aliphatic, aromatic, and other such acids, such as methanesulfonic acid, benzenesulfonic acid, maleic acid, citric acid, succinic acid, fumaric acid, toluenesulfonic acid, trifluoromethanesulfonic acid, and the like. Examples of the inorganic acids may include hydrochloric acid, sulfuric acid, phosphoric acid, hydrofluoric acid, hydrobromic acid, hydriodic acid, and the like.

The term "J^+" used in the present specification refers to a pharmaceutically acceptable cation, and while not limited to the following cations. Specific examples thereof may include alkali metal ions such as sodium ions, potassium ions, lithium ions, and the like; alkali earth ions such as calcium ions, magnesium ions, and the like; alkylamines such as methylamine, ethylamine, cyclohexylamine, and the like; alkylamines that have been substituted with a hydroxyl group, such as diethanolamine, triethanolamine, tris(hydroxymethyl)-aminomethane and the like; amino acids such as lysine, arginine, histidine, and the like; and organic base-derived

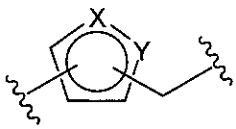
ammonium ions such as piperidine, morpholine, and the like.

R^1 represents a hydrogen atom, a halogen atom, an amino group, a C_{1-6} alkyl group, a C_{1-6} alkoxy group, a C_{1-6} alkylamino group, a hydroxy C_{1-6} alkylamino group, or a C_{1-6} alkoxy C_{1-6} alkyl group, with a hydrogen atom, an amino group, or a C_{1-6} alkoxy C_{1-6} alkyl group being particularly favorable, and with a methoxymethyl group being preferable as the C_{1-6} alkoxy C_{1-6} alkyl group.

R^2 represents a hydrogen atom, an amino group, or a di-C_{1-6} alkylamino group, with a hydrogen atom or an amino group being preferable.

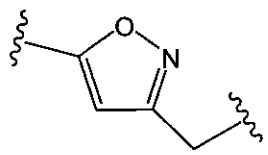
One of X and Y represents a nitrogen atom, and the other represents a nitrogen atom or an oxygen atom.

The partial structure represented by the following formula (II) including X and Y:

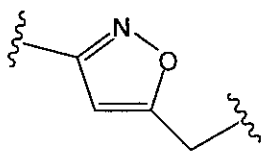


(II)

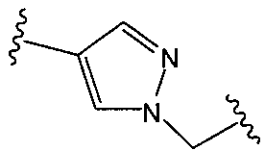
preferably has the structure represented below, in which each left end is bonded to the 3-position of a pyridine ring via a single bond, and the right end is bonded to an A ring via a methylene group:



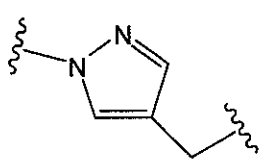
(III)



(IV)



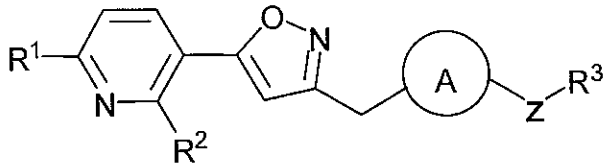
(V)



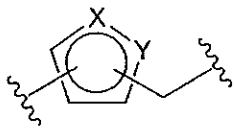
(VI)

For example, in the case of the partial structure of formula (III), the structure of

the parent compound of the compound according to the present invention, that is, a compound prior to the introduction of a phosphonoxymethyl group, is represented by the following formula:

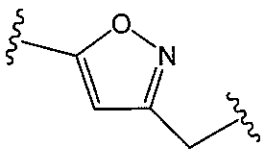


It is preferable when one of X and Y represents a nitrogen atom and the other represents an oxygen atom, or when both of X and Y represents nitrogen atoms, and when one of X and Y represents a nitrogen atom and the other represents an oxygen atom, the partial structure represented by the following formula (II) including X and Y



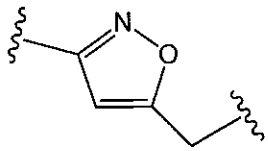
(II)

preferably has the structure represents the following formula (III) or (IV), in which each left end is bonded to the 3-position of a pyridine ring via a single bond, and the right end is bonded to an A ring via a methylene group:



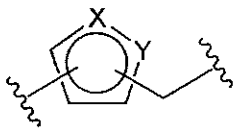
(III)

or



(IV)

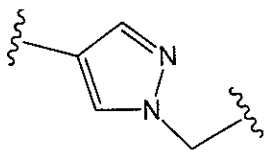
Also, when both of X and Y represent nitrogen atoms, the partial structure represented by the following formula (II) including X and Y



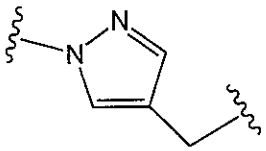
(II)

preferably has the structure represented the following formula (V) or (VI), in which each left end is bonded to the 3-position of a pyridine ring via a single bond, and the

right end is bonded to an A ring via a methylene group:


(V)

or


(VI)

The ring A represents a 5- or 6-member heteroaryl ring or a benzene ring, which may have one or two halogen atoms or C_{1-6} alkyl groups, and preferably represents a pyridine ring, a benzene ring, a furan ring, a thiophene ring, or a pyrrole ring, with a pyridine ring, a benzene ring, or a thiophene ring being more preferable, and a pyridine ring or a benzene ring being particularly preferable.

Z represents a single bond, a methylene group, an ethylene group, an oxygen atom, a sulfur atom, $-CH_2O-$, $-OCH_2-$, -NH-, $-NHCH_2-$, $-CH_2NH-$, $-CH_2S-$, or $-SCH_2-$, of which a methylene group, an oxygen atom, $-CH_2O-$, or $-OCH_2-$ is preferred, with an oxygen atom, $-CH_2O-$, or $-OCH_2-$ being particularly preferable.

R^3 represents a hydrogen atom or a halogen atom, or represents a C_{1-6} alkyl group, a C_{3-8} cycloalkyl group, a C_{6-10} aryl group, or a 5- or 6-member heteroaryl group, each of which may have one or two substituents selected from a substituent group α.

[Substituent Group α]

a halogen atom, a cyano group, a C_{1-6} alkyl group, a C_{1-6} alkoxy group, a C_{1-6} alkoxycarbonyl, a C_{3-8} cycloalkyl group, a C_{2-6} alkenyl group, and a C_{2-6} alkynyl group.

Preferable examples of R^3 may include an *n*-butyl group, a cyclopropyl group, a phenyl group, a fluorophenyl group, a furyl group, a chlorofuryl group, a methylfuryl group, a thienyl group, a bromothienyl group, a methylthienyl group, a pyridyl group, and a methylpyridyl group, with an *n*-butyl group, a cyclopropyl group, a phenyl group,

a fluorophenyl group, a pyridyl group, and a methylpyridyl group being particularly preferable.

Z and R^3 can be combined as necessary to configure substituents for the ring A. Preferable examples of R^3-Z- as substituents of the ring A thus configured may include a phenoxy group, a benzyloxy group, a 2-fluoro-benzyloxy group, a 3-fluoro-benzyloxy group, a 4-fluoro-benzyloxy group, a pyridin-2-yloxymethyl group, a 6-methyl-pyridin-2-yloxymethyl group, a pyridin-2-ylmethoxy group, a 6-methyl-pyridin-2-ylmethoxy group, a 4-methyl-pyridin-2-ylmethoxy group, a butoxymethyl group, and a cyclopropylmethoxy group.

The term "salt" used in the present specification refers to a salt with a compound or an atom capable of forming a monovalent counter ion or a divalent counter ion. Examples thereof may include salts of inorganic acid (for example, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, nitric acid and the like), and salts of organic acid (for example, methanesufonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesufonic acid, fumaric acid, maleic acid, succinic acid, citric acid, malic acid, trifluoroacetic acid and the like), or salts of inorganic bases (for example, sodium salt, potassium salt, calcium salt, lithium salt and the like), and salts of organic bases (for example, methylamine salt, ethylamine salt, tert-butylamine salt, cyclohexlamine salt, N-methyl-D-glucamine salt, lysine salt, piperidine salt, morpholine salt and the like). The term "salt" includes mono- and bis-salts. The salt of the compound according to the present invention includes solvates of this salt such as anhydrous form and hydrate of this salt.

The term "antifungal agent" used in the present specification refers to a preventive agent or a therapeutic agent for fungal infection.

The compounds according to the present invention can be formulated into tablets, powders, fine granules, granules, coated tablets, capsulates, syrups, troches, inhalants, suppositories, injections, ointments, eye ointments, tapes, eye drops, nose drops, ear drops, cataplasms, lotions or the like, by the conventional methods.

Such formulation can be achieved by using typical diluents, binders, lubricants, colorants, flavorants, and, as necessary, stabilizers, emulsifiers, absorbefacients, surfactants, pH modulators, preservatives, antioxidants or the like, and materials commonly used as ingredients of pharmaceutical preparations according to the conventional methods. For example, an oral preparation can be produced by combining a compound of the present invention or a pharmaceutically acceptable salt thereof with a diluent, and if required, a binder, a disintegrating agent, a lubricant, a colorant, a flavorant or the like, and formulating the mixture into powders, fine granules, granules, tablets, coated tablets, capsules or the like according to the conventional methods.

Examples of these materials may include animal and vegetable oils such as soy bean oil, beef tallow, and synthetic glyceride; hydrocarbons such as liquid paraffin, squalane, and solid paraffin; ester oils such as octyldodecyl myristate and *iso*-propyl myristate; higher alcohols such as cetostearyl alcohol and behenyl alcohol; silicone resins; silicone oils; surfactants such as polyoxyethylene fatty acids ester, sorbitan fatty acid ester, polyoxyethylene sorbitan fatty acid ester, polyoxyethylene hydrogenated castor oil, and polyoxyethylene polyoxypropylene block co-polymer; water-soluble polymers such as hydroxyethyl cellulose, polyacrylic acid, carboxyvinyl polymer, polyethylene glycol, polyvinylpyrrolidone, and methytl cellulose; lower alcohols such as ethanol and isopropanol; polyhydric alcohols such as glycerol, propylene glycol, dipropylene glycol, and sorbitol; sugars such as glucose and

sucrose; inorganic powder such as anhydrous silicic acid, magnesium aluminum silicate, and aluminum silicate; and pure water. Examples of the diluents may include lactose, corn starch, white sugar, glucose, mannitol, sorbitol, crystalline cellulose, silicon dioxide or the like. Examples of the binders may include polyvinyl alcohol, polyvinyl ether, methylcellulose, ethylcellulose, gum Arabic, tragacanth, gelatin, shellac, hydroxypropyl methylcellulose, hydroxypropyl cellulose, polyvinylpyrrolidone, polypropylene glycol-polyoxyethylene block co-polymer, meglumine or the like. Examples of disintegrating agents may include starch, agar, gelatin powder, crystalline cellulose, calcium carbonate, sodium hydrogencarbonate, calcium citrate, dextrin, pectin, calcium carboxymethyl cellulose or the like. Examples of lubricants may include magnesium stearate, talc, polyethylene glycol, silica, hydrogenated vegetable oil or the like. Examples of colorants may include those pharmaceutically acceptable. Examples of flavorants may include cocoa powder, peppermint camphor, aromatic powder peppermint oil, Borneo camphor, cinnamon powder or the like. Tablets and granules may be coated with sugar, or if required, other appropriate coatings can be made. Solutions, such as syrups or injectable preparations, to be administered can be formulated by combining a compound according to the present invention or a pharmaceutically acceptable salt thereof with a pH modulator, a solubilizing agent, an isotonizing agent or the like, and if required, with an auxiliary solubilizing agent, a stabilizer or the like, according to the conventional methods. Methods for manufacturing external preparations are not limited and such preparations can be manufactured by the conventional methods. Specifically, various materials typically used for manufacturing pharmaceuticals, quasi drugs, cosmetics or the like can be used as base materials for the external formulation. More specifically, examples of base materials to be used may include animal and

vegetable oils, minerals oils, ester oils, wax, higher alcohols, fatty acids, silicone oil, surfactants, phospholipids, alcohols, polyhydric alcohols, water-soluble polymers, clay minerals, pure water or the like. Furthermore, external preparations of the present invention can contain, as required, pH modulators, antioxidants, chelating agents, antibacterial/antifungal agents, colorants, odoriferous substances or the like. But this does not limit the type of base materials that are to be used in the external preparations of the present invention. If required, the preparation may contain differentiation inducers, blood flow improving agents, antimicrobial agents, antiphologistics, cell activators, vitamins, amino acids, humectants, keratolytic agents or the like. The amount of the base materials listed above is adjusted within a concentration range used for producing typical external preparations.

When administering the compound according to the present invention, the forms of the compounds are not limited in particular, and the compound can be given orally or parenterally by the conventional method. For instance, the compound can be administered as a dosage form such as tablets, powders, granules, capsules, syrups, troches, inhalants, suppositories, injections, ointments, eye ointments, tapes, eye drops, nasal drops, ear drops, cataplasms and lotions.

Dose of a medicament according to the present invention can be selected appropriately according to symptom severity, age, sex, body weight, forms of administration, type of salts, specific type of disease or the like.

The does varies remarkably depending on the patient's disease, symptom severity, age and sex, drug susceptibility or the like. An oral preparation according to the present invention can be generally administered once or several time at a dose of from 1 to 10000 mg/adult/day, preferably from 10 to 2000 mg/adult/day. An injection according to the present invention can be generally administered at a dose of from

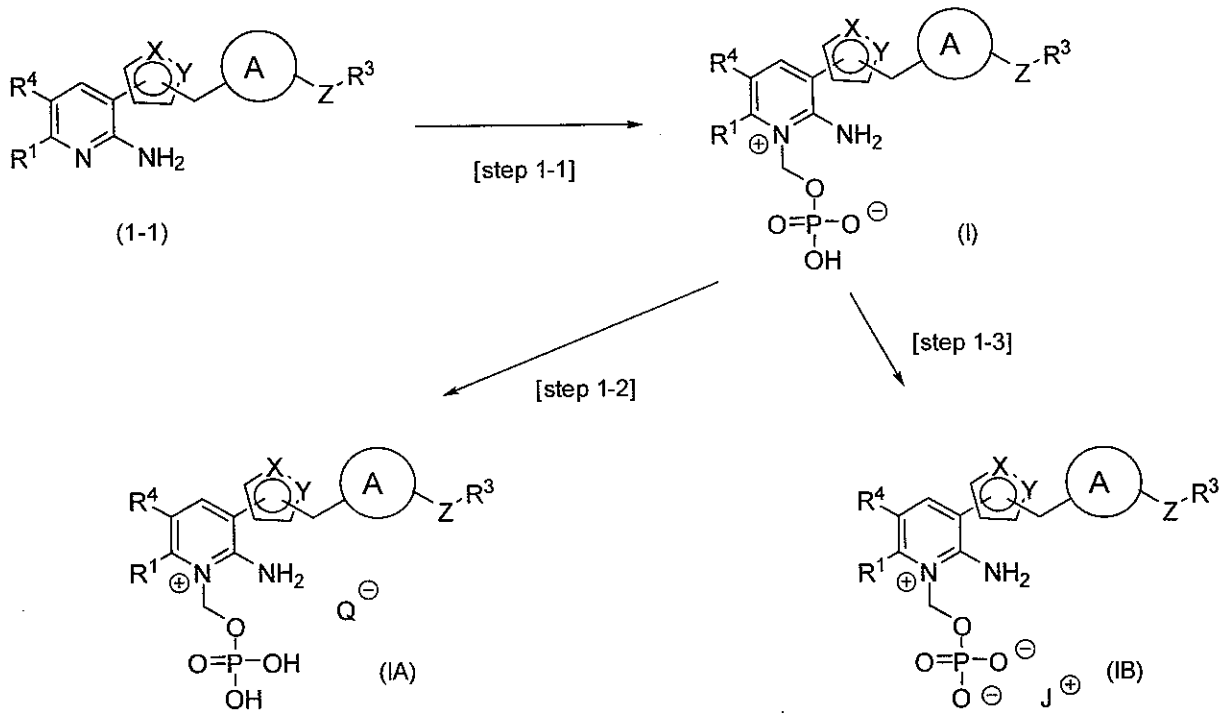
0.1 to 10000 mg/adult/day, preferably from 1 to 2000 mg/adult/day.

Typical Manufacture Method

The method for manufacturing the compounds represented by formulas (I), (IA), and (IB) (hereinafter referred to as compound (I), compound (IA), and compound (IB)) will now be described.

Typical Method for Manufacturing Phosphoric Ester

Manufacturing Method 1 – Method for Manufacturing Compounds (I), (IA), and (IB)



wherein ring A, R^1, R^3, R^4, X, Y, Z, Q^- and J^+ have the same meanings as defined above.

Compound (1-1) can be manufactured by the methods described in the following reference examples, etc. Compound (1-1) can also be manufactured by the method described in U.S. Patent 2007/0105904 (A1).

[Step 1-1]

This step is a step wherein after compound (1-1) and phosphoric acid di-*tert*-butyl ester chloromethyl ester are reacted in the presence of sodium iodide, compound (I) is then obtained by acid treatment.

There are no particular restrictions on the solvent used when compound (1-1) and phosphoric acid di-*tert*-butyl ester chloromethyl ester are reacted in the presence of sodium iodide, as long as it can dissolve the starting materials to a certain extent without impeding the reaction. Examples of the solvents that can be used may include halogenated hydrocarbon-based solvents such as methylene chloride, chloroform, and the like; ether-based solvents such as tetrahydrofuran, diethyl ether, and the like; ester-based solvents such as ethyl acetate and the like; acetonitrile; tetramethylene sulfolane; and mixtures of these solvents. The use of ethyl acetate, tetrahydrofuran or acetonitrile is preferred. The phosphoric acid di-*tert*-butyl ester chloromethyl ester can be used in an amount of from 1 to 10 equivalents, and preferably from 1 to 2 equivalents, based on compound (1-1). The sodium iodide can be used in an amount of from 1 to 10 equivalents, and preferably from 1 to 2 equivalents, based on compound (1-1). The reaction temperature is from 0 to 60°C, and preferably from 4°C to room temperature. The reaction time is from 4 to 216 hours.

The acid used in the acid treatment may be organic acids such as trifluoroacetic acid or the like; or mineral acids such as hydrochloric acid or the like, with the use of hydrochloric acid, or trifluoroacetic acid being preferred. In the acid treatment, the acid may be added as is to the reaction solvent at a previous stage, or the solvent may first be concentrated under a reduced pressure, and the acid is then added after changing to a suitable solvent such as dichloromethane. The reaction temperature is from -10°C to room temperature, and the reaction time is from 5

minutes to 2 hours.

[Step 1-2]

This step is a step wherein compound (I) is reacted with an acid to obtain compound (IA). There are no particular restrictions on the acid used in this reaction, so long as it is pharmaceutically acceptable. Examples thereof may include monobasic acids such as hydrochloric acid, hydrobromic acid, nitric acid, and the like; and dibasic acids such as sulfuric acid and the like. There are no particular restrictions on the solvent used in this reaction, so long as it can dissolve the starting materials to a certain extent without impeding the reaction. Examples of the solvents that can be used may include amide-based solvents such as N,N-dimethylformamide, N-methylpyrrolidinone, and the like; alcohol-based solvents such as methanol, ethanol, and the like; tetrahydrofuran; acetonitrile; water; and mixtures of these solvents. The monobasic acid is used in an amount of 1 equivalent based on compound (I), and a dibasic acid is used in an amount of from 1 or 0.5 equivalent based on compound (I). The reaction temperature is from 0°C to room temperature, and the reaction time is from 5 minutes to 4 hours.

[Step 1-3]

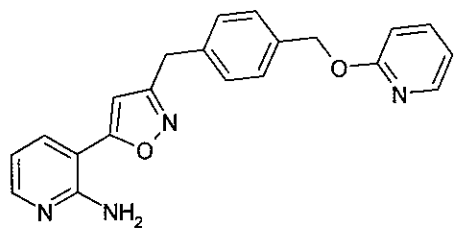
This step is a step wherein compound (I) and a base are reacted to obtain compound (IB). There are no particular restrictions on the base used in this reaction, so long as it is pharmaceutically acceptable. Examples thereof may include inorganic bases such as sodium hydroxide, potassium hydroxide, and the like; and organic bases such as lysine and the like. There are no particular restrictions on the solvent used in this reaction, so long as it can dissolve the starting materials to a certain extent without impeding the reaction. Examples of the solvents that can be used may include amide-based solvents such as N,N-dimethylformamide, N-

methylpyrrolidinone, and the like, alcohol-based solvents such as methanol, ethanol, and the like; tetrahydrofuran; acetonitrile; water; and mixtures of these solvents. The monoacid base is used in an amount of 1 equivalent based on compound (I), and a diacid base acid is used in an amount of 1 or 0.5 equivalent based on compound (I). The reaction temperature is from 0°C to room temperature, and the reaction time is from 5 minutes to 4 hours.

Examples

The compounds according to the present invention can be manufactured, for example, by the methods described in the following examples, reference examples, and manufacturing examples. However, these are used for illustrative purposes, and the compounds according to the present invention are not in any case limited to the following specific examples.

[Reference Example 1] 3-(3-(4-(Pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine



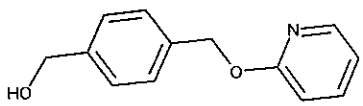
To a tetrahydrofuran solution (5 mL) of (4-(pyridin-2-yloxymethyl)-phenyl)-acetohydroxymoyl chloride (510 mg, 1.84 mmol) described in Manufacturing Example 1-1-5 and 3-ethynyl-pyridin-2-ylamine (150 mg, 1.27 mmol) described in Manufacturing Example 1-2-3 of International Publication WO 07/052615 was added triethylamine (708 μL, 5.08 mmol) at room temperature, which was stirred for 95 minutes at room temperature. Water was added to the reaction solution at room temperature, and extracted with ethyl acetate. The organic layer was washed with a

saturated saline and dried over anhydrous magnesium sulfate, and the solvent was then evaporated under a reduced pressure. The residue was purified by NH silica gel column chromatography (heptane : ethyl acetate = 2 : 1) to obtain the title compound (120 mg, 26%).

^{1}H-NMR spectrum ($CDCl_3$) δ (ppm): 4.08 (2H, s), 5.37 (2H, s), 6.33 (1H, s), 6.45 (2H, brs), 6.79-6.82 (2H, m), 6.88-6.91 (1H, m), 7.30 (2H, d, J = 8.1 Hz), 7.45 (2H, d, J = 8.1 Hz), 7.57-7.61 (1H, m), 7.85 (1H, d, J = 7.3 Hz), 8.03 (1H, d, J = 5.5 Hz), 8.17 (1H, m).

The starting material, (4-(pyridin-2-yloxymethyl)-phenyl)-acetohydroxymoyl chloride was synthesized by the following method.

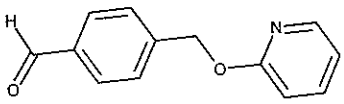
Manufacturing Example 1-1-1: (4-(Pyridin-2-yloxymethyl)-phenyl)-methanol



To a mixture of 1,4-benzenedimethanol (5.5 g, 40 mmol), 2-fluoropyridine (1.3 g, 13 mmol) and N,N-dimethylformamide (15 mL) was added sodium hydride (1.4 g, 40 mmol, 66% in oil) at 0°C, which was stirred for 20 minutes at room temperature and for 1 hour at 70°C. Water was added to the reaction mixture, and extracted with ethyl acetate. The organic layer was washed with a saturated saline, and the solvent was evaporated under a reduced pressure. The residue was purified by NH silica gel column chromatography (ethyl acetate : heptane = 1 : 1) to obtain the title compound (1.9 g, 66%).

^{1}H-NMR spectrum ($CDCl_3$) δ (ppm): 4.71 (2H, s), 5.38 (2H, s), 6.81 (1H, td, J = 0.9, 8.4 Hz), 6.89 (1H, ddd, J = 0.9, 5.1, 7.1 Hz), 7.37-7.47 (4H, m), 7.59 (1H, ddd, J = 2.0, 7.1, 8.3 Hz), 8.17 (1H, ddd, J = 0.7, 2.0, 5.1 Hz).

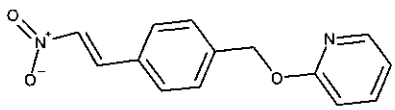
Manufacturing Example 1-1-2: 4-(Pyridin-2-yloxymethyl)-benzaldehyde



To a mixture of (4-(pyridin-2-yloxymethyl)-phenyl)-methanol (1.9 g, 8.6 mmol) described in Manufacturing Example 1-1-1 and methylene chloride (30 mL) was added manganese dioxide (15 g, 17 mmol) at room temperature, which was stirred overnight at this temperature. The reaction mixture was filtered through a Celite pad, and the solvent was evaporated under a reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate : heptane = 1 : 4), to obtain the title compound (770 mg, 42%).

^{1}H-NMR spectrum ($CDCl_3$) δ (ppm): 5.48 (2H, s), 6.85 (1H, d, J = 8.2 Hz), 6.90-6.93 (1H, m), 7.60-7.64 (3H, m), 7.89 (2H, d, J = 8.1 Hz), 8.16 (1H, dd, J = 1.3, 4.9 Hz), 10.0 (1H, s).

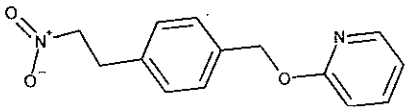
Manufacturing Example 1-1-3: 2-(4-((*E*)-2-Nitro-vinyl)-benzyloxy)-pyridine



A mixture of 4-(pyridin-2-yloxymethyl)-benzaldehyde (23.4 g, 110 mmol) described in Manufacturing Example 1-1-2, nitromethane (33.6 g, 550 mmol), ammonium acetate (17.0 g, 220 mmol), and acetic acid (200 mL) was stirred for 1 hour and 45 minutes at 100°C. A small amount of water was added thereto while the reaction solution was stirred under an ice cooling, and the precipitated solids were collected by filtration, so as to obtain title compound (21.0 g, 74.5%).

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 5.41 (2H, s), 6.91 (1H, dd, J = 0.8, 8.4 Hz), 6.99-7.10 (1H, m), 7.53 (2H, d, J = 8.0 Hz), 7.72-7.79 (1H, m), 7.86 (2H, d, J = 8.0 Hz), 8.13 (1H, d, J = 10 Hz), 8.15-8.20 (1H, m), 8.23 (1H, d, J = 10 Hz).

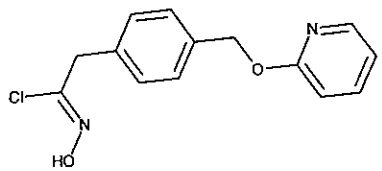
Manufacturing Example 1-1-4: 2-(4-(2-Nitro-ethyl)-benzyloxy)-pyridine



To a solution of 2-(4-((*E*)-2-nitro-vinyl)-benzyloxy)-pyridine (21.0 g, 81.9 mmol) described in Manufacturing Example 1-1-3, acetic acid (21 mL), and dimethyl sulfoxide (200 mL) was added sodium borohydride (4.96 g, 131 mmol) at room temperature while cooling suitably. After the addition of the sodium borohydride, the ice bath was removed, followed by stirring for 15 minutes at room temperature. The reaction solution was separated into water and ethyl acetate. The ethyl acetate layer was washed with water twice and with saline once, and this product was dried over anhydrous magnesium sulfate and the solvent was evaporated under a reduced pressure. The residue was purified by NH silica gel column chromatography (ethyl acetate : heptane = 1 : 3) to obtain the title compound (16.3 g,77.1%).

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 3.23 (2H, t, J = 6.8 Hz), 4.85 (2H, t, J = 6.8 Hz), 5.32 (2H, s), 6.82-6.88 (1H, m), 6.96-7.01 (1H, m), 7.28 (2H, d, J = 8.0 Hz), 7.38 (2H, d, J = 8.0 Hz), 7.69-7.74 (1H, m), 8.15-8.19 (1H, m).

Manufacturing Example 1-1-5: 4-(Pyridin-2-yloxymethyl)-phenyl-acetohydroxymoyl chloride



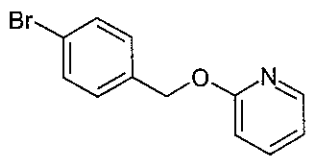
Lithium wire (323 mg, 46.6 mmol) was added to and dissolved in methanol (75 mL). To this solution was added 2-(4-(2-nitro-ethyl)-benzyloxy)-pyridine (6.0 g, 23.3 mmol)) described in Manufacturing Example 1-1-4, and the reaction solution was concentrated under a reduced pressure. Toluene was added to the residue, and this solvent was concentrated under a reduced pressure. A solution of the resulting residue in methylene chloride (90 mL) and tetrahydrofuran (45 mL) was cooled to -

78°C, and titanium(IV) chloride (8.15 mL, 74.4 mmol) was added thereto. As soon as the addition of the titanium(IV) chloride was complete, the reaction solution was stirred for 10 minutes at 0°C, and then for 30 minutes at room temperature. The reaction solution was put into an ice water and extracted with ethyl acetate. The organic layer was dried over anhydrous magnesium sulfate, and the magnesium sulfate was filtered off. The filtrate was passed through a glass filter (eluted with ethyl acetate) spread with neutral silica gel. The eluate thus obtained was concentrated under a reduced pressure. A small amount of ethyl acetate was added to the residue, and the precipitated solids were collected by filtration, so as to obtain the title compound (1.86 g, 28.8%).

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 3.82 (2H, s), 5.33 (2H, s), 6.84-6.89 (1H, m), 6.97-7.01 (1H, m), 7.25 (2H, d, J = 8.4 Hz), 7.41 (2H, d, J = 8.4 Hz), 7.70-7.76 (1H, m), 8.15-8.18 (1H, m), 11.7 (1H, s).

The title compound of Manufacturing Example 1-1-5 can also be synthesized by another method.

Manufacturing Example 1-2-1: 2-(4-Bromo-benzyloxy)-pyridine

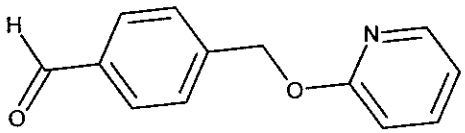


To a N,N-dimethylformamide solution (125 mL) of 4-bromobenzyl alcohol (25 g, 130 mmol) was added potassium *tert*-butoxide (15.8 g, 141 mmol) at room temperature, which was stirred for 10 minutes at 54°C. To this reaction solution was added 2-fluoropyridine (15 mL, 154 mmol) at a temperature ranging from 40°C to 58°C, which was stirred for another 30 minutes at 65°C. The reaction solution was cooled to room temperature, water and ethyl acetate were added thereto, which was

separated. The aqueous layer was further extracted with ethyl acetate twice. The ethyl acetate layers were combined,, washed with water three times and saline once, and was then dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under a reduced pressure. Diethyl ether was added to the residue, and this was concentrated under a reduced pressure, so as to obtain a crude product of the title compound (34 g).

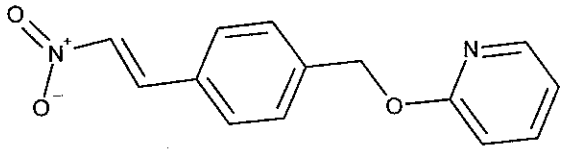
^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 5.33 (2H, s), 6.87-6.90 (1H, m), 6.98-7.02 (1H, m), 7.38-7.44 (2H, m), 7.55-7.60 (2H, m), 7.71-7.76 (1H, m), 8.15-8.18 (1H, m)

Manufacturing Example 1-2-2: 4-(Pyridin-2-yloxymethyl)-benzaldehyde



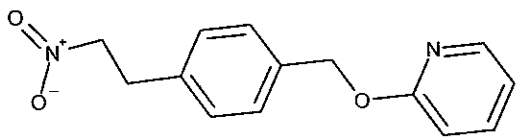
To a tetrahydrofuran solution (120 mL) of 2-(4-bromo-benzyloxy)pyridine (34 g, 128 mmol) described in Manufacturing Example 1-2-1 was added n-butyl lithium (50 mL, 2.6 M, hexane solution 134 mmol) dropwise at -78°C. After stirring for 30 minutes, N,N-dimethylformamide (10 mL, 134 mmol)) was added dropwise at -78°C to the reaction solution, which was stirred at room temperature. Water and ethyl acetate were added thereto, which was separated. The ethyl acetate layer was washed with water twice and saline once. The aqueous layers were combined and extracted with ethyl acetate. The ethyl acetate layer thus obtained was washed with water twice and saline once. The previously obtained ethyl acetate layer and the ethyl acetate layer obtained this time were combined, dried over anhydrous magnesium sulfate, and filtered. This filtrate was concentrated under a reduced pressure, so as to obtain a crude product of the title compound (26.8 g).

Manufacturing Example 1-2-3: 2-(4-((*E*)-2-Nitro-vinyl)-benzyloxy)-pyridine



A mixture of 4-(pyridin-2-yloxymethyl)-benzaldehyde (26.8 g, 126 mmol)) described in Manufacturing Example 1-2-2, nitromethane (34 mL, 630 mmol), ammonium acetate (19 g, 252 mmol), and acetic acid (90 mL) was stirred for 1 hour and 30 minutes at 100°C. Ethyl acetate and water were added thereto, which was separated. The organic layer was washed with water five times and saturated sodium bicarbonate water once, dried over anhydrous magnesium sulfate, and filtered. This filtrate was concentrated under a reduced pressure, so as to obtain a crude product of the title compound (31 g).

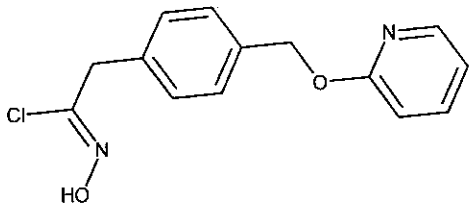
Manufacturing Example 1-2-4: 2-(4-(2-Nitro-ethyl)-benzyloxy)-pyridine



To a dimethyl sulfoxide solution (150 mL) of acetic acid (7.4 mL) and 2-(4-((E)-2-nitro-vinyl)-benzyloxy)-pyridine (30.8 g, 120 mmol)) described in Manufacturing Example 1-2-3 was added sodium borohydride (2.45 g, 64.8 mmol) at 30°C or lower. The reaction solution was stirred for 40 minutes at room temperature. Water, ethyl acetate, and diethyl ether were added at 30°C or lower to the reaction solution, to separate the reaction solution into water and an organic layer. The aqueous layer was extracted with ethyl acetate. The previously obtained organic layer and the ethyl acetate layer obtained this time were combined, washed with water three times and saline once, dried over anhydrous magnesium sulfate, and filtered. This filtrate was concentrated under a reduced pressure. The residue was purified by NH silica gel

column chromatography (ethyl acetate : heptane = 1 : 4) to obtain the title compound (15.2 g).

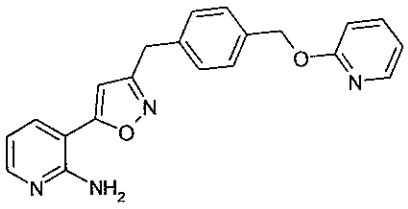
Manufacturing Example 1-2-5: 4-(Pyridin-2-yloxymethyl)-phenyl-acetohydroxymoyl chloride



To a methanol solution (80 mL) of 2-(4-(2-nitro-ethyl)-benzyloxy)-pyridine (15.2 g, 59 mmol)) described in Manufacturing Example 1-2-4 was added lithium methoxide (4.49 g, 118 mmol), which was stirred for 3 minutes. The reaction solution was concentrated under a reduced pressure. Toluene was added to the residue, and the solvent was concentrated under a reduced pressure. A solution of methylene chloride (100 mL) and tetrahydrofuran (50 mL) of the residue thus obtained was cooled to -66°C, and titanium(IV) chloride (20.8 mL, 189 mmol) was added thereto while stirring. The reaction solution was stirred for 10 minutes at 0°C, and then stirred for 30 minutes at room temperature. The reaction solution was poured into an ice water and stirred for 30 minutes at room temperature. Ethyl acetate and diethyl ether were added to the reaction solution, which was separated. The organic layer was washed with water three times and saline once. The aqueous layers were combined, and extracted with ethyl acetate twice. The ethyl acetate layers were combined and washed with water three times and saline once. The previously obtained organic layer and the ethyl acetate layer obtained this time were combined, dried over anhydrous magnesium sulfate and sodium sulfate, and filtered. This filtrate was concentrated under a reduced pressure, so as to obtain a crude product of the title compound (11.5 g).

The title compound of Reference Example 1 can also be synthesized by the following other methods 1 to 3.

Other Method 1 of Reference Example 1: 3-(3-(4-(Pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine



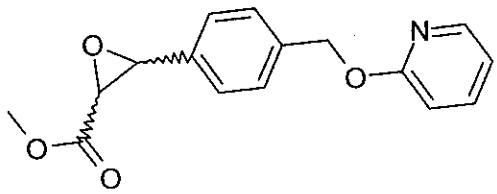
To a mixture of zinc chloride (8.82 g) and tetrahydrofuran (130 mL) were added 3-ethynyl-pyridin-2-ylamine (3.00 g, 98% purity) described in Manufacturing Example 1-2-3 of International Publication WO 07/052615 and 4-(pyridin-2-yloxymethyl)-phenyl-acetohydroxymoyl chloride (17.4 g, 94% purity) described in Manufacturing Example 1-2-5 at 0°C. The reaction mixture was warmed to room temperature, and triethylamine (9.02 mL) was added dropwise while maintaining the internal temperature to 28°C or lower by using a water bath. The reaction mixture was stirred for 20 minutes at room temperature, and then stirred for 1 hour at 35°C. The reaction mixture was brought to room temperature, an ammonium chloride aqueous solution and ethyl acetate were added to the reaction mixture, and then an ammonia aqueous solution was added to adjust the pH to approximately 8, and extraction was performed. The organic layer was washed with saturated saline and dried over anhydrous magnesium sulfate, and the solvent was evaporated under a reduced pressure. The residue was purified by NH silica gel column chromatography (heptane : ethyl acetate = 3 : 2), then crystallization was performed using a mixed solvent of *tert*-butyl methyl ether and heptane, so as to obtain the title compound (5.32 g).

^{1}H-NMR spectrum ($CDCl_3$) δ (ppm): 4.07 (2H, s), 5.37 (4H, brs), 6.25 (1H, s),

6.71 (1H, dd, J = 4.8, 7.7 Hz), 6.79-6.81 (1H, m), 6.89 (1H, ddd, J = 0.8, 5.0, 7.0 Hz), 7.30 (2H, d, J = 7.9 Hz), 7.44 (2H, d, J = 8.1 Hz), 7.58 (1H, ddd, J = 2.0, 7.1, 8.4 Hz), 7.70 (1H, dd, J = 1.8, 7.7 Hz), 8.14 (1H, dd, J = 1.8, 4.9 Hz), 8.17-8.18 (1H, m).

In the Other Method 1 of Reference Example 1, the starting material, 4-(pyridin-2-yloxymethyl)-phenyl-acetohydroxymoyl chloride, was synthesized by the following method.

Manufacturing Example 1-3-1: Methyl 3-(4-(pyridin-2-yloxymethyl)-phenyl)-oxiran-2-carboxylate

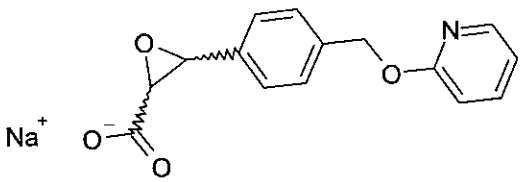


To a mixture of 4-(pyridin-2-yloxymethyl)-benzaldehyde (24.8 g) described in Manufacturing Example 1-1-2 and tetrahydrofuran (160 mL) was added methyl chloroacetate (10.2 mL) at -15°C, and then sodium methoxide (23.7 mL, 28% methanol solution) was added thereto at the same temperature. The reaction mixture was stirred for 1 hour at 0°C, and then stirred for 2 hours at room temperature. The reaction mixture was added to 800 mL of an ice water containing acetic acid (6 mL), and the reaction mixture was warmed to room temperature. Ethyl acetate was added to the reaction mixture for extraction, and then the organic layer was separated, washed with saturated saline, and dried over anhydrous magnesium sulfate. The solvent was evaporated under a reduced pressure to obtain the title compound (30.2 g).

^{1}H-NMR spectrum ($CDCl_3$) δ (ppm): 3.51 (1H, d, J = 1.8 Hz), 3.83 (3H, s), 4.11 (1H, d, J = 1.8 Hz), 5.38 (2H, s), 6.81 (1H, td, J = 0.9, 8.4 Hz), 6.89 (1H, ddd, J = 0.9, 5.1, 7.1 Hz), 7.29-7.31 (2H, m), 7.47 (2H, d, J = 8.2 Hz), 7.59 (1H, ddd, J = 2.0, 7.1,

8.4 Hz), 8.17 (1H, ddd, J = 0.8, 2.0, 5.1 Hz).

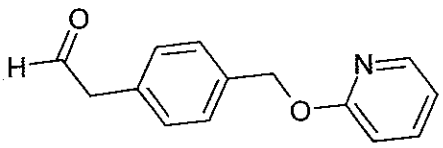
Manufacturing Example 1-3-2: Sodium 3-(4-(pyridin-2-yloxymethyl)-phenyl)-oxiran-2-carboxylate



To a mixture of methyl 3-(4-(pyridin-2-yloxymethyl)-phenyl)-oxiran-2-carboxylate (19.9 g) described in Manufacturing Example 1-3-1 and ethanol (300 mL) were added sodium methoxide (14.2 mL, 28% methanol solution), water (1.3 mL), and tetrahydrofuran (100 mL) in that order at 0°C, which was stirred for 1 hour at room temperature. Diethyl ether (200 mL) was added to the reaction mixture, and the precipitated solids were collected by filtration, so as to obtain the title compound (14.3 g).

^{1}H-NMR spectrum (CD_3OD) δ (ppm): 3.31 (1H, d, J = 1.8 Hz), 3.88 (1H, d, J = 1.8 Hz), 5.33 (2H, s), 6.84 (1H, td, J = 0.9, 8.2 Hz), 6.94 (1H, ddd, J = 0.9, 5.1, 7.1 Hz), 7.29-7.31 (2H, m), 7.42 (2H, d, J = 8.2 Hz), 7.68 (1H, ddd, J = 2.0, 7.1, 8.4 Hz), 8.12 (1H, ddd, J = 0.7, 2.0, 5.1 Hz).

Manufacturing Example 1-3-3: 4-(Pyridin-2-yloxymethyl)-phenyl-acetaldehyde

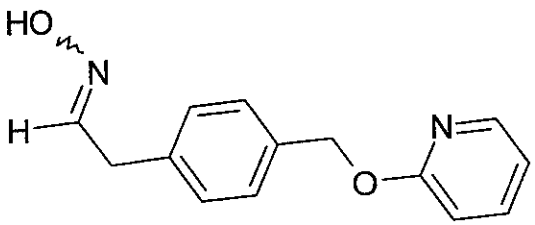


A mixture of sodium 3-(4-(pyridin-2-yloxymethyl)-phenyl)-oxiran-2-carboxylate (9.95 g) described in Manufacturing Example 1-3-2, toluene (200 mL), water (120 mL), and acetic acid (16 mL) was stirred for 90 minutes at 73°C. The reaction mixture was cooled to room temperature, ethyl acetate was added to the reaction mixture for extraction, and the organic layer was separated, washed with saturated

saline, and dried over anhydrous magnesium sulfate. The solvent was evaporated under a reduced pressure to obtain the title compound (6.82 g).

^{1}H-NMR spectrum ($CDCl_3$) δ (ppm): 3.70 (2H, d, J = 2.2 Hz), 5.38 (2H, s), 6.81 (1H, td, J = 0.8, 8.2 Hz), 6.89 (1H, ddd, J = 0.9, 5.1, 7.1 Hz), 7.24 (2H, d, J = 8.1), 7.48 (2H, d, J = 8.1 Hz), 7.59 (1H, ddd, J = 2.0, 7.1, 8.4 Hz), 8.18 (1H, ddd, J = 0.6, 2.0, 5.0 Hz), 9.75 (1H, t, J = 2.4).

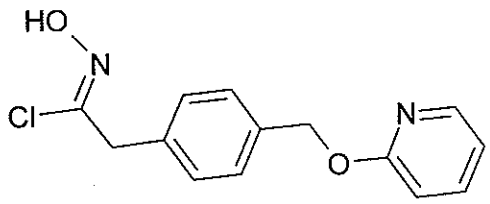
Manufacturing Example 1-3-4: 4-(Pyridin-2-yloxymethyl)-phenyl-acetaldehyde oxime (*E*/*Z* mixture)



To a mixture of hydroxylamine sulfate (19.7 g) and water (250 mL) was added a 1N aqueous sodium hydroxide (240 mL) at 0°C, which was stirred for 15 minutes at the same temperature. To the reaction mixture was then added a mixture of 4-(pyridin-2-yloxymethyl)-phenyl)-acetaldehyde (27.3 g) described in Manufacturing Example 1-3-3 and methanol (250 mL) dropwise, which was stirred overnight at room temperature. The precipitated solids were collected by filtration, so as to obtain the title compound (20.3 g) as a mixture of *E* and *Z* isomers.

^{1}H-NMR spectrum ($CDCl_3$) δ (ppm): 3.54 (2H, d, J = 6.2 Hz), 3.74 (2H, d, J = 5.3 Hz), 5.36 (2H+2H, s), 6.79-6.81 (1H+1H, m), 6.87-6.90 (1H+2H, m), 7.22-7.24 (2H+2H, m), 7.42-7.44 (2H+2H, m), 7.53 (1H, t, J = 6.3 Hz), 7.56-7.61 (1H+1H, m), 8.17-8.18 (1H+1H, m) (underbar = E or Z).

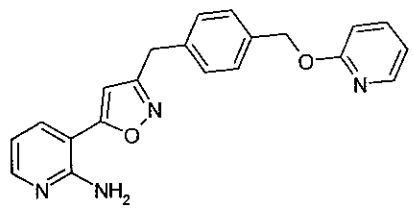
Manufacturing Example 1-3-5: 4-(Pyridin-2-yloxymethyl)-phenyl-acetohydroxymoyl chloride



To a mixture of 4-(pyridin-2-yloxymethyl)-phenyl-acetaldehyde oxime (*E/Z* mixture) (132 mg) described in Manufacturing Example 1-3-4 and N,N-dimethylformamide (2 mL) was added N-chlorosuccinimide (72.8 mg) at room temperature. Hydrochloric acid gas was then blown into the reaction mixture at the same temperature, which was stirred for 90 minutes at the same temperature. Ethyl acetate and water were added to the reaction mixture for extraction, and then the organic layer was separated, washed with saturated saline, and dried over anhydrous magnesium sulfate. The solvent was evaporated under a reduced pressure, and the resulting residue was washed with a mixed solvent of diethyl ether and heptane, so as to obtain the title compound (123 mg).

^{1}H-NMR spectrum ($CDCl_3$) δ (ppm): 3.81 (2H, s), 5.36 (2H, s), 6.81 (1H, d, J = 8.2 Hz), 6.88-6.91 (1H, m), 7.28 (2H, d, J = 8.1), 7.43 (2H, d, J = 8.1 Hz), 7.57-7.62 (1H, m), 8.17-8.19 (1H, m).

Other Method 2 of Reference Example 1: 3-(3-(4-(Pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine



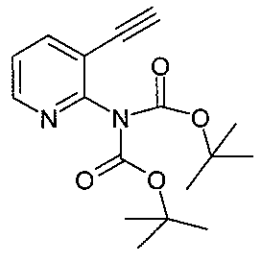
To a dichloromethane solution (120 mL) of di-*tert*-butyl (3-(3-(4-((pyridin-2-yloxy)methyl)benzyl)isoxazol-5-yl)pyridin-2-yl)imide dicarbonate (11.8 g, approximately 70% purity) described in Manufacturing Example 1-4-2 was added

trifluoroacetic acid (40 mL) at 0°C, which was stirred for 14 hours at room temperature. Saturated sodium bicarbonate water was added at 20°C or lower to the reaction solution, extraction was performed with ethyl acetate, and then this product was purified by NH silica gel column chromatography (heptane : ethyl acetate = 1 : 1). The solvent was concentrated under a reduced pressure and to the resulting residue was added *tert*-butyl methyl ether, followed by filtering solids, and the title compound (7.29 g) was obtained.

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 4.04 (2H, s), 5.32 (2H, s), 6.26 (2H, brs), 6.69 (1H, dd, J = 4.8, 8.0 Hz), 6.81 (1H, s), 6.83-6.87 (1H, m), 6.97-7.00 (1H, m), 7.33 (2H, d, J = 8.0 Hz), 7.40 (2H, d, J = 8.0 Hz), 7.69-7.74 (1H, m), 7.87 (1H, dd, J = 2.0, 7.6 Hz), 8.08 (1H, dd, J = 2.0, 7.6 Hz), 8.15-8.17 (1H, m).

The starting material, di-*tert*-butyl (3-(3-(4-((pyridin-2-yloxy)methyl)benzyl) isoxazol-5-yl)pyridin-2-yl)imide dicarbonate was synthesized by the following method.

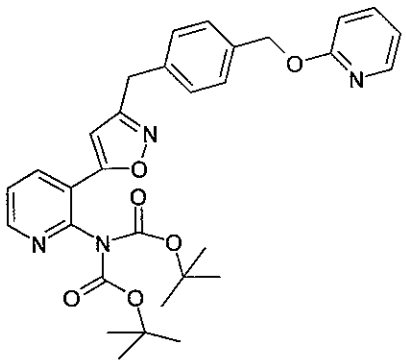
Manufacturing Example 1-4-1: Di-*tert*-butyl (3-ethynylpyridin-2-yl)imide dicarbonate



3-Ethynyl-pyridin-2-ylamine (6.34 g) described in Manufacturing Example 1-2-3 of International Publication WO 07/052615, di-*tert*-butyl dicarbonate (58.5 g), triethylamine (27.1 g), 4-dimethylaminopyridine (655 mg), and tetrahydrofuran (254 mL) were stirred for 18 hours at room temperature. Silica gel was added to the reaction solution, and the solvent was concentrated under a reduced pressure. The silica gel thus obtained was purified by silica gel chromatography (heptane : ethyl acetate = 3 : 1) to obtain the title compound (15 g) as a white solid.

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 1.32 (18H, s), 4.59 (1H, s), 7.39-7.44 (1H, m), 7.99-8.03 (1H, m), 8.46-8.48 (1H, m).

Manufacturing Example 1-4-2: Di-*tert*-butyl (3-(3-(4-((pyridin-2-yloxy)methyl)benzyl)isoxazol-5-yl)pyridin-2-yl)imide dicarbonate

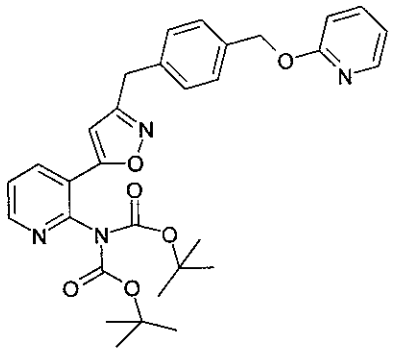


To a tetrahydrofuran solution (200 mL) of di-*tert*-butyl (3-ethynylpyridin-2-yl)imide dicarbonate (12 g) described in Manufacturing Example 1-4-1, 2-(4-(2-nitro-ethyl)-benzyloxy)-pyridine (19.4 g) described in Manufacturing Example 1-1-4, and 4-dimethylaminopyridine (230 mg) was added di-*tert*-butyl dicarbonate (28.8 g) over a period of 8 hours and in four batches, at room temperature while stirring. Upon completion of the addition, the reaction mixture was stirred for another 22 hours at room temperature. Silica gel was added to the reaction solution and the solvent was concentrated under a reduced pressure. The silica gel thus obtained was purified by silica gel chromatography (heptane : ethyl acetate = 3 : 1, and then 2 : 1) to obtain an oily substance (11.8 g containing approximately 70% the target substance) containing the title compound.

Di-*tert*-butyl (3-(3-(4-((pyridin-2-yloxy)methyl)benzyl)isoxazol-5-yl)pyridin-2-yl)imide dicarbonate of Manufacturing Example 1-4-2 can also be synthesized by the following Other Method 1 or 2.

Manufacturing Example 1-5-1: Di-*tert*-butyl (3-(3-(4-((pyridin-2-yloxy)methyl)benzyl)isoxazol-5-yl)pyridin-2-yl)imide dicarbonate (Other Method 1 of

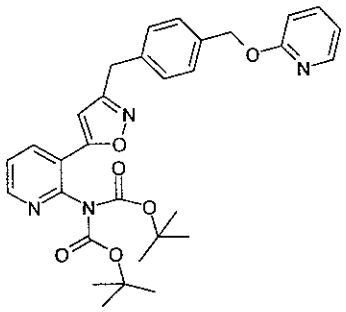
Manufacturing Example 1-4-2)



To a tetrahydrofuran solution (20 mL) of di-*tert*-butyl(3-ethynylpyridin-2-yl)imide dicarbonate (2.0 g) described in Manufacturing Example 1-4-1, 2-(4-(2-nitro-ethyl)-benzyloxy)-pyridine (2.44 g) described in Manufacturing Example 1-1-4, and triethylamine (0.086 μL) was added phenyl isocyanate (2.8 mL) over a period of 5.5 hours and in four batches, at 50°C while stirring. Upon completion of the addition, the reaction mixture was stirred for another 2 hours at 50°C. NH-silica gel was added to the reaction solution and the solvent was concentrated under a reduced pressure. The NH-silica gel thus obtained was purified by NH-silica gel chromatography (heptane : ethyl acetate = 3 : 1). The solution thus obtained was concentrated under a reduced pressure and purified by silica gel chromatography (heptane : ethyl acetate = 3 : 1, and then 2 : 1) to obtain the title compound (2.2 g) as an oily substance.

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 1.18 (18H, s), 4.07 (2H, s), 5.32 (2H, s), 6.58 (1H, s), 6.83-6.86 (1H, m), 6.96-7.01 (1H, m), 7.29 (2H, d, J = 8.0 Hz), 7.40 (2H, d, J = 8.0 Hz), 7.58 (1H, dd, J = 4.8, 7.6 Hz), 7.69-7.74 (1H, m), 8.15-8.18 (1H, m), 8.34 (1H, dd, J = 2.0, 7.6 Hz), 8.59 (1H, dd, J = 2.0, 5.2 Hz).

Manufacturing Example 1-6-1: Di-*tert*-butyl (3-(3-(4-((pyridin-2-yloxy)methyl)benzyl)isoxazol-5-yl)pyridin-2-yl)imide dicarbonate (Other Method 2 of Manufacturing Example 1-4-2)

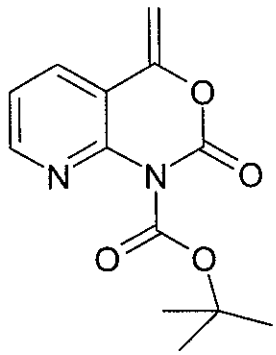


4-Methylen-2-oxo-4*H*-pyrido[2,3-d][1,3]oxazin-1-carboxylic acid *tert*-butyl ester (1.48 g) described in Manufacturing Example 1-6-2, 2-(4-(2-nitro-ethyl)-benzyloxy)-pyridine (2.9 g) described in Manufacturing Example 1-1-4, di-*tert*-butyl dicarbonate (6.14 g), 4-dimethylaminopyridine (68.6 mg), and tetrahydrofuran (50 mL) were stirred for 2 hours at room temperature. Silica gel was added to the reaction solution, and the solvent was concentrated under a reduced pressure. The silica gel thus obtained was purified by silica gel chromatography (heptane : ethyl acetate = 3 : 1, then 1 : 1, and then 1 : 2) to obtain the title compound (2.1 g) as an oily substance.

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 1.18 (18H, s), 4.07 (2H, s), 5.32 (2H, s), 6.58 (1H, s), 6.83-6.86 (1H, m), 6.96-7.01 (1H, m), 7.29 (2H, d, J = 8.0 Hz), 7.40 (2H, d, J = 8.0 Hz), 7.58 (1H, dd, J = 4.8, 7.6 Hz), 7.69-7.74 (1H, m), 8.15-8.18 (1H, m), 8.34 (1H, dd, J = 2.0, 7.6 Hz), 8.59 (1H, dd, J = 2.0, 5.2 Hz).

The starting material, 4-methylen-2-oxo-4*H*-pyrido[2,3-d][1,3]oxazin-1-carboxylic acid *tert*-butyl ester, was synthesized by the following method.

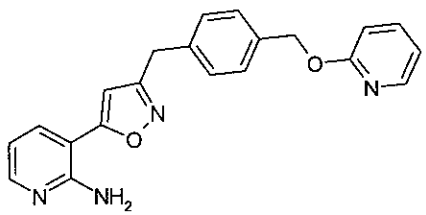
Manufacturing Example 1-6-2: 4-Methylen-2-oxo-4*H*-pyrido[2,3-d][1,3]oxazin-1-carboxylic acid *tert*-butyl ester



1-(2-Amino-pyridin-3-yl)-ethanone (990 mg), di-*tert*-butyl dicarbonate (7.92 g), 4-dimethylaminopyridine (88.8 mg), triethylamine (4.95 mL), and tetrahydrofuran (16.5 mL) were stirred for 24 hours at room temperature. Silica gel was added to the reaction solution, and the solvent was concentrated under a reduced pressure. The silica gel thus obtained was purified by silica gel chromatography (heptane : ethyl acetate = 2 : 1) to obtain the title compound (1.48 g) as an oily substance.

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 1.56 (9H, s), 5.01 (1H, d, J = 3.6 Hz), 5.45 (1H, d, J = 3.6 Hz), 7.28 (1H, dd, J = 4.8, 8.0 Hz), 8.25 (1H, dd, J = 1.6, 8.0 Hz), 8.36 (1H, dd, J = 1.6, 4.8 Hz).

Other Method 3 of Reference Example 1: 3-(3-(4-(Pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine

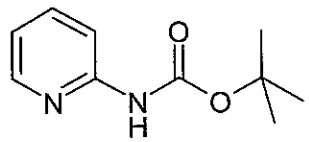


A mixture of 2-(4-(5-iodo-isoxazol-3-ylmethyl)-benzyloxy)-pyridine (200 mg) described in Manufacturing Example 1-8-2, 2-N-butoxycarbonyl-3-pyridineboronic acid (134 mg) described in Manufacturing Example 1-7-2, sodium carbonate (82 mg), tetrakis(triphenylphosphine) palladium (59 mg), 1,2-dimethoxyethane (6 mL), and water (1 mL) was stirred under a nitrogen atmosphere for 2 hours at 80°C. This

mixture was cooled to room temperature, and ethyl acetate and water were added. The organic layer was separated, washed with water and saturated saline, dried over anhydrous magnesium sulfate, and then filtered. After the filtrate was adsorbed to silica gel, it was purified by silica gel column chromatography (heptane : ethyl acetate = 4 : 1 to 1 : 1 to ethyl acetate) to obtain the title compound (116 mg).

The starting material, 2-N-butoxycarbonyl-3-pyridineboronic acid, was synthesized by the following method.

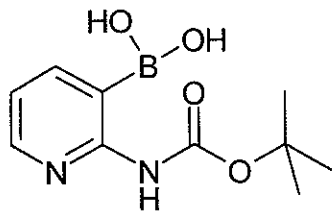
Manufacturing Example 1-7-1: Pyridin-2-yl-carbamic acid *tert*-butyl ester



To a solution of *tert*-butyl alcohol (650 mL) and di-*tert*-butyl carbonate (24 g) was added 2-aminopyridine (9.4 g) slowly. This mixture was stirred for 24 hours at room temperature. This reaction solution was concentrated under a reduced pressure, and the residue was purified by silica gel chromatography (heptane : ethyl acetate = 1:1) to obtain the title compound (18 g).

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 1.47 (9H, s), 6.99-7.03 (1H, m), 7.70-7.74 (1H, m), 7.77-7.80 (1H, m), 8.23-8.24 (1H, m), 9.72 (1H, brs).

Manufacturing Example 1-7-2: 2-N-butoxycarbonyl-3-pyridineboronic acid



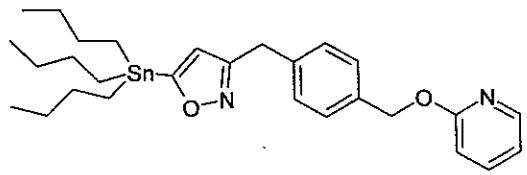
A tetrahydrofuran solution (400 mL) of pyridin-2-yl-carbamic acid *tert*-butyl ester (16 g) described in Manufacturing Example 1-7-1 and N,N,N',N'-tetramethylethylenediamine (25 g) was cooled to -70°C, n-butyl lithium (78 mL, 2.64M

heptane solution) was then added dropwise over a period of 1 hour, which was stirred for 10 minutes. This mixture was warmed to a temperature between -10°C and -6°C, which was stirred for 2 hours at that temperature. The solution was again cooled to -70°C, and triisobutyl borate (58 g) was added dropwise over a period of 1 hour. This mixture was warmed to 0°C, and then a saturated ammonium chloride aqueous solution was added thereto. Ether was added to the yellow solid thus produced, which was stirred, and the solid was then collected by filtration and washed with ether and water. This solid was dried under a reduced pressure, so as to obtain the title compound (14 g).

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 1.32-1.41 (9H, m), 6.80-6.84 (1H, m), 7.95-8.13 (2H, m).

In the Other Method 3 of Reference Example 1, the starting material, 2-(4-(5-iodo-isoxazol-3-ylmethyl)-benzyloxy)-pyridine, was synthesized by the following method.

Manufacturing Example 1-8-1: 2-(4-(5-Tri-n-butylstannyl-isoxazol-3-ylmethyl)-benzyloxy)-pyridine

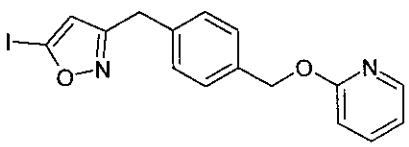


To a tetrahydrofuran solution (90 mL) of tri-n-butylethynyltin (3 g), 2-(4-(2-nitro-ethyl)-benzyloxy)-pyridine (4.9 g) described in Manufacturing Example 1-1-4, and 4-dimethylaminopyridine (116 mg) was added a tetrahydrofuran solution (30 mL) of di-*tert*-butyl dicarbonate (7.3 g), which was stirred for 15 hours at room temperature. Ethyl acetate and water were added to this mixture. The organic layer was separated, washed with water and saturated saline, dried over anhydrous magnesium sulfate,

and filtered. The filtrate was adsorbed to silica gel, and then purified by silica gel column chromatography (heptane : ethyl acetate = 4 : 1) to obtain the title compound (5.3 g).

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 0.81-0.85 (9H, m), 1.08-1.12 (6H, m), 1.23-1.30 (6H, m), 1.46-1.54 (6H, m), 4.00 (2H, s), 5.30 (2H, s), 6.40 (1H, s), 6.83-6.86 (1H, m), 6.97-7.00 (1H, m), 7.25-7.26 (2H, m), 7.36-7.38 (2H, m), 7.69-7.74 (1H, m), 8.15-8.17 (1H, m).

Manufacturing Example 1-8-2: 2-(4-(5-Iodo-isoxazol-3-ylmethyl)-benzyloxy)-pyridine

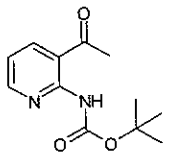


To a tetrahydrofuran solution (15 mL) of 2-(4-(5-tri-n-butylstannyl-isoxazol-3-ylmethyl)-benzyloxy)-pyridine (5.1 g) described in Manufacturing Example 1-8-1 was added iodine (2.5 g) at 0°C. This mixture was stirred at this temperature for 20 minutes. A 10% sodium thiosulfate aqueous solution and ethyl acetate were added to this mixture. The organic layer was separated, washed with saturated saline, dried over anhydrous magnesium sulfate, and then filtered. The filtrate was concentrated, and the residue was purified by silica gel column chromatography (heptane : ethyl acetate = 10 : 1 to 4 : 1) to obtain the title compound (2.4 g).

^{1}H-NMR spectrum (DMSO-d_6) δ (ppm): 3.99 (2H, s), 5.31 (2H, s), 6.66 (1H, s), 6.84-6.87 (1H, m), 6.97-7.00 (1H, m), 7.26 (2H, d, J = 8 Hz), 7.39 (2H, d, J = 8 Hz), 7.70-7.74 (1H, m), 8.16-8.17 (1H, m).

Another method for the manufacturing example of 3-(3-(4-(pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine described in Reference Example 1 will now be described.

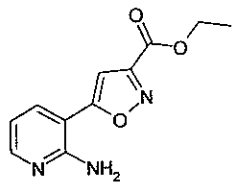
[Reference Example 2] Synthesis of *tert*-butyl (3-acetylpyridin-2-yl)carbamate



A mixture of 1-(2-aminopyridin-3-yl)ethanone (50 g, 368 mmol), di-*tert*-butyldicarbonate (120 g, 552 mmol) and *tert*-butanol (200 mL) was stirred at 90°C for three hours under a nitrogen atmosphere. After cooling, the solvent was evaporated under a reduced pressure, n-heptane (500 mL) was added to the residue and the precipitated solid was filtered to obtain the title compound (77 g) as a yellow solid.

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.54(9H,s), 2.64(3H,s), 7.03(1H,dd,J=4.8,8.0Hz), 8.16(1H,dd,J=2.0,8.0Hz), 8.63(1H,dd,J=2.0,4.8Hz), 10.82(1H,brs).

[Reference Example 3] Synthesis of ethyl 5-(2-aminopyridin-3-yl)isoxazol-3-carboxylate

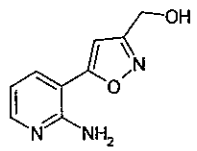


To a solution of *tert*-butyl (3-acetyl pyridin-2-yl)carbamate (600 mg, 2.29 mmol) and diethyl oxalate (669 mg, 4.58 mmol) in toluene (5.0 mL) was added potassium *tert*-butoxide (514 mg, 4.58 mmol) at room temperature under a nitrogen atmosphere, which was stirred for two hours. After addition of toluene (5.0 mL) and stirring for one hour, potassium *tert*-butoxide (257 mg, 2.29 mmol) was added thereto and the solution was stirred for two hours. To the reaction mixture were added hydroxylamine hydrochloride (477 mg, 6.87 mmol) and ethanol (10 mL), which was stirred for one hour, water (1.0 mL) was added thereto and the solution was stirred overnight at room temperature. Water (30 mL) was added thereto and the solution

was extracted with ethyl acetate. After the organic layer was washed with saturated sodium chloride water and dried over anhydrous magnesium sulfate, the solution was concentrated. The concentrated residue was dissolved in N,N-dimethyl formamide (5 mL), triethylamine (192 mg) was added thereto, and the solution was stirred at 80°C for six hours. After cooling, water was added thereto, and the solution was extracted with ethyl acetate. After the organic layer was washed with saturated sodium chloride water and dried over anhydrous magnesium sulfate, the solvent was evaporated under a reduced pressure to obtain the title compound (443 mg) as a white solid.

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.45(3H,t,J=7.2Hz), 4.49(2H,q,J=7.2Hz), 5.40(2H,brs), 6.79(1H,dd,J=5.2,7.6Hz), 6.91(1H,s), 7.81(1H,dd,J=2.0,7.6Hz), 8.21(1H,dd,J=2.0,5.2Hz).

[Reference Example 4] Synthesis of [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol



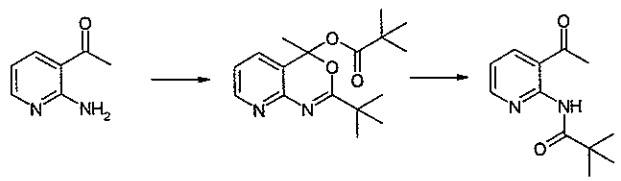
To a suspension of ethyl 5-(2-aminopyridin-3-yl)isoxazol-3-carboxylate (381 mg, 1.63 mmol) in tetrahydrofuran (3.8 mL) and ethanol (3.8 mL) was added sodium borohydride (201 mg, 4.89 mmol) at 0°C under a nitrogen atmosphere, which was stirred at 0°C for one hour and at 20°C for 21 hours. Under an ice water bath cooling, to the reaction mixture was added 2N hydrochloric acid (2.46 mL, 4.89 mmol) dropwise, which was stirred at 0°C for 10 minutes and at room temperature for 30 minutes. Under the ice water bath cooling, after an aqueous solution of 5% sodium bicarbonate was added dropwise to adjust basic, the solution was extracted with ethyl acetate. After the organic layer was dried over anhydrous magnesium sulfate,

the solvent was evaporated under a reduced pressure. The residue was suspended in tetrahydrofuran (1.4 mL), to the reaction mixture was added sodium borohydride (67 mg, 1.63 mmol) at 0°C, which was washed thereto down with methanol (1.4 mL). After stirring at room temperature for one hour, the solution was stirred at 60°C for five hours. Under an ice water bath cooling, to the reaction mixture was added 1N hydrochloric acid (1.63 mL, 1.63 mmol) dropwise, which was stirred at 0°C for 10 minutes and at room temperature for 30 minutes. Under the ice water bath cooling, after adding 1N aqueous sodium hydroxide dropwise to adjust basic, the solution was extracted with ethyl acetate. After the organic layer was dried over anhydrous magnesium sulfate, the solvent was evaporated under a reduced pressure to obtain the title compound (258 mg) as a pale yellow solid.

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm):4.56(2H,d,J=5.6Hz), 5.54(1H,t,J=5.6Hz), 6.27(2H,brs), 6.72(1H,dd,J=4.8,7.6Hz), 6.90(1H,s), 7.90(1H,dd,J=2.0,7.6Hz), 8.10(1H,dd,J=2.0,4.8Hz).

Reference Examples 5 to 10 are other synthesis methods to Reference Examples 3 and 4.

[Reference Example 5] Synthesis of N-(3-acetylpyridin-2-yl)-2,2-dimethylpropanamide



To a mixture of 1-(2-aminopyridin-3-yl)ethanone (272 mg, 2 mmol), 4-dimethylaminopyridine (24 mg, 0.2 mmol), triethylamine (0.64 mL, 4.6 mmol) and toluene (2 mL) was added pivaloyl chloride (0.52 mL, 4.2 mmol) dropwise at room temperature, which was stirred at room temperature for one hour and at 60°C for five

hours. After formation of 2-*tert*-butyl-4-methyl-4*H*-pyrido[2,3-d][1,3]oxazin-4-yl pivalate* was confirmed, to the reaction mixture were added water (2 mL) and 5N hydrochloric acid (0.8 mL), which was stirred at room temperature for 30 minutes. After the reaction mixture was separated, to the aqueous layer was added 5N aqueous sodium hydroxide (1 mL) and extracted with toluene. The solvent was evaporated under a reduced pressure and the precipitated solid was filtered to obtain the title compound (415 mg).

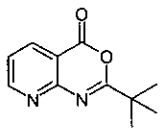
^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.33(9H,s), 2.64(3H,s), 7.10(1H,dd,J=4.8,8.0Hz), 8.17(1H,dd,J=2.0,7.6Hz), 8.64(1H,dd,J=2.0,4.8Hz).

*2-*tert*-butyl-4-methyl-4*H*-pyrido[2,3-d][1,3]oxazin-4-yl pivalate

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.09 (9H,s), 1.32 (9H,s), 2.05 (3H,s), 7.14 (1H,dd,J=4.8,7.6Hz), 7.71 (1H,dd,J=2.0,7.6Hz), 8.51 (1H,dd,J=2.0,4.8Hz).

Reference Examples 6 to 7 are other synthesis methods to Reference Example 5.

[Reference Example 6] Synthesis of 2-*tert*-butyl-4*H*-pyrido[2,3-d][1,3]oxazin-4-one

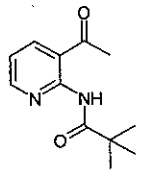


To a mixture of 2-aminonicotinic acid (13.8 g, 100 mmol), 4-dimethylaminopyridine (1.2 g, 10 mmol), triethylamine (55.8 mL, 400 mmol) and N-methylpyrrolidone (140 mL, 42 mmol) was added pivaloyl chloride (24.1 g, 200 mmol) dropwise at 0°C, and after the drop wise addition was finished, which was stirred overnight at room temperature. To the reaction mixture was added water, extracted with toluene, and the organic layer was washed with water and saturated sodium chloride water. After the solution was dried over anhydrous magnesium sulfate and

filtered, the solvent was evaporated under a reduced pressure. To the residue was added n-heptane, which was suspended and stirred at 0°C, which was filtered to obtain the title compound (16.6 g).

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.45(9H,s), 7.48(1H,dd,J=4.8,8.0Hz), 8.52(1H,dd,J=2.0,7.6Hz), 8.97(1H,dd,J=2.0,4.8Hz).

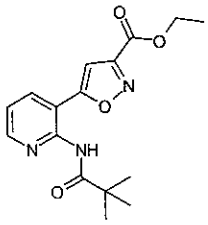
[Reference Example 7] Synthesis of N-(3-acetylpyridin-2-yl)-2,2-dimethylpropanamide



To a mixture of 2-*tert*-butyl-4*H*-pyrido[2,3-*d*][1,3]oxazin-4-one (10.2 g, 50 mmol) and tetrahydrofuran (50 mL) was added methyl magnesium bromide (0.97M tetrahydrofuran solution, 100 mL, 97 mmol) dropwise at -78°C, after the dropwise addition was finished, which was stirred at -78°C for 30 minutes. To the reaction mixture were added a saturated aqueous solution of ammonium chloride and water, which was extracted with ethyl acetate, and the organic layer was washed with an aqueous solution of ammonium chloride. The solvent was evaporated under a reduced pressure and the precipitated solid was filtered to obtain the title compound (9.1 g).

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.33(9H,s), 2.64(3H,s), 7.10(1H,dd,J=4.8,8.0Hz), 8.17(1H,dd,J=2.0,7.6Hz), 8.64(1H,dd,J=2.0,4.8Hz).

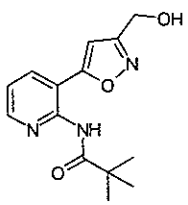
[Reference Example 8] Synthesis of ethyl 5-{2-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}isoxazol-3-carboxylate



To a mixture of N-(3-acetyl pyridin-2-yl)-2,2-dimethylpropanamide (8.08 g, 36.7 mmol), diethyl oxalate (10.0 mL, 73.4 mmol) and ethanol (36 mL) was added potassium *tert*-butoxide (8.23 g, 73.4 mmol) at -25°C, which was stirred at -25°C for one hour. The reaction mixture was added water (72 mL), which was stirred at room temperature, toluene (36 mL) was added, separated, and the obtained aqueous layer was further washed with toluene (36 mL). To the solution were added 5N hydrochloric acid (14 mL) and hydroxylamine hydrochloride (5.10 g, 73.4 mmol), which was stirred at room temperature for 30 minutes. To the reaction mixture was added 5N aqueous sodium hydroxide (14 mL), which was extracted with toluene, the solvent was evaporated under a reduced pressure. To the obtained residue were added ethanol (35 mL) and triethylamine (5 mL), which was stirred at 80°C to 85°C for six hours. To the reaction mixture was added n-heptane (105 mL) and the precipitated solid was filtered to obtain the title compound (6.90 g).

^{1}H-NMR Spectrum (DMSO-d6) δ (ppm):1.19(9H,s), 1.32(3H,t), 4.37(4H,q), 7.12(1H,s), 7.46(1H,dd,J=4.8,8.0Hz), 8.25(1H,dd,J=2.0,8.0Hz), 8.58(1H,dd,J=2.0,4.8Hz), 10.03(1H,s).

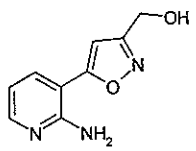
[Reference Example 9] Synthesis of N-{3-[3-(hydroxymethyl)isoxazol-5-yl]pyridin-2-yl}-2,2 dimethylpropanamide



To a mixture of ethyl 5-{2-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}isoxazol-3-carboxylate (111 g, 350 mmol), ethanol (110 mL) and tetrahydrofuran (350 mL) was added sodium borohydride (13.2 g, 350 mmol) at room temperature, which was stirred at room temperature for 6 hours. To the reaction mixture were added water (350 mL) and 5N hydrochloric acid (90 mL), which was stirred at room temperature for 30 minutes, 5N aqueous sodium hydroxide (110 mL) was added thereto, the solution was extracted with a mixture of ethyl acetate and tetrahydrofuran, and the organic layer was washed with water and saturated sodium chloride water. The solvent was evaporated under a reduced pressure to obtain the title compound (83.8 g) as a yellow solid, partially contaminated with [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol.

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm):1.20(9H,s), 4.52(2H,d,J=6.0Hz), 5.53(1H,t,J=6.0Hz), 6.70(1H,s), 7.44(1H,dd,J=4.8,8.0Hz), 8.19(1H,dd,J=5.6,7.6Hz), 8.53(1H,dd,J=2.0,4.8Hz), 9.89(1H,brs).

[Reference Example 10] Synthesis of [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol



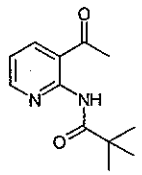
To a mixture of N-{3-[3-(hydroxymethyl)isoxazol-5-yl]pyridin-2-yl}-2,2 dimethylpropanamide (82.8 g) obtained in Reference Example 9 and methanol (350 mL) was added 5N aqueous sodium hydroxide (350 mL) at room temperature, which was stirred at 57 to 60°C for 14 hours. To the reaction mixture was added acetic acid (100 mL) and the precipitated solid was filtered to obtain the title compound (42.2 g) as a grayish white solid.

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm):4.54(2H,s), 5.57(1H,brs), 6.25(2H,brs),

6.71(1H,dd,J=4.8,8.0Hz), 6.90(1H,s), 7.90(1H,dd,J=1.6,7.6Hz), 8.09(1H,dd,J=1.6,4.8Hz).

Reference Examples 11 to 13 are other synthesis methods to Reference Examples 5 to 10.

[Reference Example 11] Synthesis of N-(3-acetylpyridin-2-yl)-2,2-dimethylpropanamide



After 1-(2-aminopyridin-3-yl)ethanone (40.0 kg, 294 mol) was added to a 1500L reactor, which was washed thereto down with toluene (approximately 15 kg). Then, after toluene was added until there was 347 kg of toluene in total, pivaloyl chloride (53.1 kg, 1.5M/M) was added thereto. Triethylamine (23.8 kg, 0.8M/M) was added thereto dropwise at an internal temperature of 30°C or lower and the solution was stirred at an internal temperature of from 20 to 30°C for one hour or longer. After triethylamine (23.8 kg, 0.8M/M) was again added dropwise at an internal temperature of 30°C or lower, the solution was stirred at an internal temperature of from 20 to 30°C for two hours or longer, it was confirmed by HPLC that the reaction had ended.

Under a brine cooling, water (100L) was added dropwise at an internal temperature of 30°C or lower, then, 35% hydrochloric acid (49.0 kg, 1.6M/M) was added thereto dropwise at an internal temperature of 30°C or lower. After the reaction solution was stirred for five minutes, the solution was left standing still for 15 minutes or longer and lower layer (a) was separated into a poly container. After water (100L) was added thereto and the solution was stirred for five minutes, the

solution was left standing still for 15 minutes or longer. After lower layer (c) was separated into a poly container and upper layer (d) was removed, lower layer (a) and lower layer (c) were returned into the 1500L reactor. Under the brine cooling, ethyl acetate (289 kg) was added thereto, then, after an aqueous solution of 48.7% sodium hydroxide (43.4 kg, 1.8M/M) was added dropwise at an internal temperature of 30°C or lower and the solution was stirred for five minutes, it was confirmed with a UNIV test paper that a pH of the lower layer was from 8 to 9. After being left standing still for 15 minutes or longer, lower layer (e) and upper layer (f) were each separated and lower layer (e) was returned into the 1500L reactor. After ethyl acetate (144 kg) was added thereto and the solution was stirred for five minutes, the solution was left standing still for 15 minutes or longer and lower layer (g) and upper layer (h) were each separated. After lower layer (g) was returned into the 1500L reactor, ethyl acetate (144 kg) was added thereto and the solution was stirred for five minutes, the solution was left standing still for 15 minutes or longer. After removing lower layer (i), upper layer (f) and upper layer (h) were returned into the 1500L reactor and which was washed thereto down with ethyl acetate (approximately15 kg).

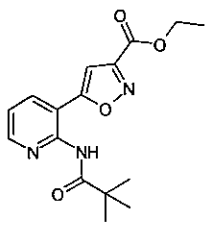
The organic layer returned into the 1500L reactor was concentrated under a reduced pressure (50°C hot water), and concentration was stopped once at the point of time when the concentrate reached approximately 200L. The concentrate was removed into SUS containers and the inside of the reactor was washed out with toluene (17 kg). Approximately half of the amount of the removed concentrate was placed in a 300L reactor and washed thereto down with toluene (9 kg). The concentrate was further concentrated under a reduced pressure (50°C hot water), and when the amount of distillation from the condenser decreased, the remaining concentrate was placed in the 300L reactor and which was washed thereto down with

toluene (9 kg). Concentration under a reduced pressure was restarted (50°C to 70°C hot water). At the point of time when there was almost no distillation, water cooling was started and toluene (52 kg) was added thereto at an internal temperature of 50°C or lower. Concentration under a reduced pressure was restarted (50 to 80°C hot water). Concentration was stopped at the point of time when distillation was no longer observed at an external temperature of 80°C and a reduced pressure level of -0.090MPa or greater, and ethanol (61 kg) was added thereto at an internal temperature of from 20 to 30°C.

Under a nitrogen atmosphere, the ethanol solution inside the can was removed into a SUS container, and the can was washed out with ethanol (13 kg). After the removed solution was added into the 1500L reactor, which was washed thereto down with ethanol (13 kg) to obtain an ethanol solution of the title compound (containing 69.4 kg of the target compound; yield: 107.3%).

HPLC conditions: column: YMC-Pack Pro C18 (5 μm, 150 × 4.6mmI.D., YMC), mobile phase: acetonitrile / water / ammonium acetate = 300 / 700 / 1 to 900 / 100 / 1 (v/v/w).

[Reference Example 12] Synthesis of ethyl 5-{2-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}isoxazol-3-carboxylate



Under a nitrogen gas stream, diethyl oxalate (64.4 kg, 1.5M/M) was added to the ethanol solution of N-(3-acetyl pyridin-2-yl)-2,2-dimethylpropanamide (294 mol, assuming that the yield of the previous step was 100%) in the 1500L reactor. Brine circulation was started and a pre-cooled ethanol solution of 22% potassium *tert*-

butoxide (212.5 kg, 1.45M/M) was added dropwise thereto at an internal temperature of 10°C or lower. After stirring at an internal temperature of from -5 to 10°C for 30 minutes or longer, it was confirmed by HPLC that the reaction had ended.

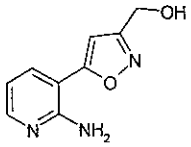
Next, hydroxylamine hydrochloride (40.8 kg, 2.0M/M) was added thereto at an internal temperature of 10°C or lower, and the solution was stirred at an internal temperature of 10°C or lower for one hour or longer. Next, prepared and cooled beforehand, hydrous ethanol (ethanol (15.3 kg)/water (5.2 kg)) was added dropwise thereto at an internal temperature of 20°C or lower while watching for heating, and water (582L) was added dropwise at an internal temperature of 30°C or lower. Switching to hot water (28°C) circulation, ethyl 4-{2-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}-2-(hydroxyimino)-4-oxobutanoate (approximately 10 g) was added thereto at an internal temperature of from 20 to 30°C. After confirming the precipitation of solid visually, the suspention was stirred overnight at an internal temperature of from 15 to 25°C. After confirming by HPLC that the reaction had ended, and an aqueous solution of 48.7% sodium hydroxide was added dropwise thereto at an internal temperature of from 10 to 25°C until a pH of the solution was from 6.50 to 7.00 (18.1 kg used). After stirring at an internal temperature of from 10 to 20°C for three hours or longer, solid-liquid separation was carried out with a centrifuge divided into six times. At each centrifugation, after washing the cake with hydrous ethanol (ethanol (2.4 kg)/water (12 kg)) prepared beforehand, the cake was washed with water until a color of the wash was colorless transparent (approximately 200L). After centrifugal separation was continued further for 30 minutes or longer, and a wet solid was removed into a poly bag. Next, in a shelf dryer, under 45 to 50°C hot water circulation, the wet solid was dried under a reduced pressure to obtain a solid (71.52 kg).

Next, the solid obtained above (71.45 kg) was added to the 1500L reactor and washed thereto down with ethanol (approximately 7 kg). Then, ethanol was added up to 226 kg in total and triethylamine (21.6 kg, 1M/M) was added thereto. Hot water (75°C) circulation was started, the solution was stirred at an internal temperature of from 70 to 75°C for 14 to 16 hours, and it was confirmed by HPLC that the reaction had ended. Next, n-heptane (488.7 kg) was added dropwise thereto at an internal temperature of from 55 to 75°C. Thereafter, ethyl 5-{2-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}isoxazol-3-carboxylate (approximately 5 g) was added thereto at an internal temperature of from 50 to 53°C, and it was confirmed visually that a solid precipitated at an internal temperature of from 45 to 50°C. Then, after the temperature of hot water was decreased gradually, cooling to an internal temperature of 15°C or lower, further, by brine or cold water cooling, the solution was stirred overnight at an internal temperature of from 0 to 10°C. Using a filtration apparatus, the suspension was filtered and washed with a mixed solution of n-heptane / ethanol (n-heptane (70 kg) / ethanol (10 kg)), followed by n-heptane (80 kg). After drying was carried out in nitrogen for 15 minutes or longer subsequent, a wet solid was removed to a SUS container. In a shelf dryer under 45 to 50°C hot water circulation, the wet solid was dried under a reduced pressure to obtain the title compound (54.55 kg; yield: 58.6%).

^{1}H-NMR Spectrum (DMSO-d6) δ (ppm):1.19(9H,s), 1.32(3H,t), 4.37(4H,q), 7.12(1H,s), 7.46(1H,dd,J=4.8,8.0Hz), 8.25(1H,dd,J=2.0,8.0Hz), 8.58(1H,dd,J=2.0,4.8Hz), 10.03(1H,s).

HPLC conditions: column: YMC-Pack Pro C18 (5 μm, 150 × 4.6mmI.D., YMC), mobile phase: acetonitrile / water / ammonium acetate=300 / 700 / 1 to 900 / 100 / 1 (v/v/w).

[Reference Example 13] Synthesis of [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol



Under a nitrogen gas stream, ethyl 5-{2-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}isoxazol-3-carboxylate (54.5 kg, 172 mol) was added to a 1500L reactor and washed thereto down with methanol (4.9 kg). Then, methanol was added thereto until there was 108 kg of methanol in total and triethylamine (8.7 kg, 0.5M/M) was added consecutively. After hot water (60°C) circulation was started, the solution was stirred at an internal temperature of from 50 to 60°C for two hours or longer, and it was confirmed by HPLC (Condition 1) that the reaction had ended.

Next, water cooling was started and tetrahydrofuran (121 kg) was added thereto at an internal temperature of 30°C or lower. Switching to brine cooling, under a nitrogen gas stream, at an internal temperature of from 0 to 10°C, sodium borohydride (7.15 kg, 1.1M/M) was added thereto, split over 5 hours or longer. After addition of sodium borohydride was finished, the jacket was switched to cold water (4.0°C) circulation, and the solution was stirred overnight at an internal temperature of from 0 to 10°C. On the next day, at an internal temperature of from 0 to 10°C, sodium borohydride (1.30 kg, 0.2M/M) was added thereto, split over one hour or longer. The jacket was switched to cold water, the internal temperature was heated up to from 20 to 30°C over three hours or longer, and further, the stirring was carried out overnight at an internal temperature of from 20 to 30°C. On the next day, how the reaction was progressing was checked by HPLC, and since the reaction almost did not progress, the solution was cooled again and sodium borohydride (1.30 kg, 0.2M/M) was split-added at an internal temperature of from 0 to 10°C. After stirring at

an internal temperature of from 0 to 10°C for one hour or longer, the jacket was switched to cold water circulation and heated up to an internal temperature of from 15 to 25°C over two hours or longer. After stirring for one hour or longer, it was confirmed by HPLC (Condition 1) that the reaction had ended, and the solution was stirred overnight.

The next day, after an aqueous solution of 48.7% sodium hydroxide (71 kg, 5M/M) was added dropwise thereto at an internal temperature of 50°C or lower, in continuation, water (133L) was added dropwise thereto at an internal temperature of 50°C or lower. Hot water (50 to 80°C) circulation was started, after stirring at an internal temperature of from 50 to 60°C for 20 hours or longer, it was confirmed by HPLC (Condition 2) that the reaction had ended.

Next, under a water cooling, water (73L) was added dropwise thereto. After switching to cold water (15°C) cooling, [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol was added thereto at an internal temperature of from 15 to 30°C, and precipitation of solid was confirmed, water (218L) was added dropwise thereto, then, under a brine cooling, 35% hydrochloric acid (115 kg) was added dropwise thereto at an internal temperature of from 15 to 30°C, and which was washed there to down with water (3L). After stirring at an internal temperature of from 15 to 30°C for five minutes or longer, it was confirmed that a pH of the reaction solution was from 4.00 to 5.00 with a pH meter, and the solution was stirred at an internal temperature of from 15 to 30°C for one hour or longer. Then, an aqueous solution of 48.7% sodium hydroxide (17.1 kg employment) was added dropwise thereto until a pH of the solution was from 7.00 to 8.00, and the solution was left standing still overnight. On the next day, after stirring and pressure reduction were started and distillation from the condenser was confirmed, hot water (40°C) circulation was started. Concentration was carried

out for one hour or longer under the conditions of hot water (35 to 45°C), 68cm Hg or greater level of reduced pressure, and 30°C or higher internal temperature. The reduced pressure was released under a nitrogen and the solid attached to the reactor wall was washed thoroughly with water (approximately 20L). The solution was stirred at an internal temperature of from 15 to 30°C for three hours or longer and was left standing still overnight. On the next day, the internal temperature was confirmed to be within a range of from 15 to 25°C, the slurry solution was solid-liquid separated with a centrifuge, divided in two stages. At each centrifugation, the solid was washed with water (approximately 200L), after draining, centrifugal separation was carried out for one hour and then the wet solid was taken out into a poly bag. Then, in a shelf dryer, under 45 to 50°C hot water circulation, the solid was dried under a reduced pressure to obtain the title compound (26.57 kg, yield: 80.9%).

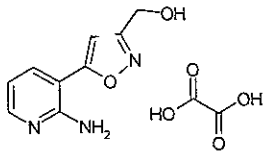
^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm):4.54(2H,s), 5.57(1H,brs), 6.25(2H,brs), 6.71(1H,dd,J=4.8,8.0Hz), 6.90(1H,s), 7.90(1H,dd,J=1.6,7.6Hz), 8.09(1H,dd,J=1.6,4.8Hz).

HPLC condition 1: column: YMC-Pack Pro C18 (5 μm, 150 × 4.6mmI.D., YMC), mobile phase: acetonitrile / water / ammonium acetate = 300 / 700 / 1 to 900 / 100 / 1 (v/v/w).

HPLC condition 2: column: YMC-Pack ODS-AQ (5 μm, 150 × 4.6mmI.D., YMC), mobile phase: acetonitrile / water / 85% phosphoric acid / sodium 1-octane sulfonate = 161.3 / 838.7 / 1 / 1.1 to 900 / 100 / 1 / 1.1 (v/v/v/w).

Reference Examples 14 to 15 are other synthesis methods to Reference Example 10.

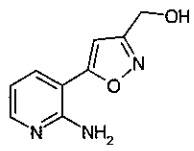
[Reference Example 14] Synthesis of [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol oxalate



To a mixture of ethyl 5-{2-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}isoxazol-3-carboxylate (3.17 g, 10 mmol), ethanol (3 mL) and tetrahydrofuran (10 mL) was added sodium borohydride (0.38 g, 10 mmol) at room temperature, which was stirred overnight under a ice cooling to room temperature. The reaction mixture was divided equally into five, among which one was added to 5N aqueous sodium hydroxide (2 mL), which was stirred overnight at 55°C. To the reaction mixture was added water, which was extracted with a mixture of methyl-*tert*-butylether and tetrahydrofuran, and oxalic acid (0.18 g, 2 mmol) was added to the organic layer. The precipitated solid was filtered to obtain the title compound (0.39 g) as a white solid.

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm):4.54(2H,s), 6.31(2H,brs), 6.72(1H,dd,J=4.8,8.0Hz), 6.89(1H,s), 7.90(1H,dd,J=2.0,8.0Hz), 8.09(1H,dd,J=2.0,4.8Hz).

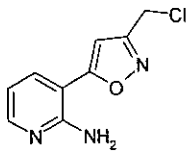
[Reference Example 15] Synthesis of [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol



To a mixture of [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol oxalate (0.39 g) and water (2 mL) was added 5N aqueous sodium hydroxide (0.5 mL) at room temperature and the precipitated solid was filtered to obtain the title compound (0.18 g) as a white solid.

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm):4.54(2H,s), 5.57(1H,brs), 6.25(2H,brs), 6.71(1H,dd,J=4.8,8.0Hz), 6.90(1H,s), 7.90(1H,dd,J=1.6,7.6Hz), 8.09(1H,dd,J=1.6,4.8Hz).

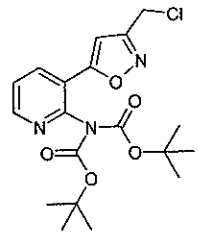
[Reference Example 16] Synthesis of 3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-amine



To a mixture of [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol (0.19 g, 1 mmol) and N,N-dimethyl acetamide (1 mL) was added a mixture of thionyl chloride (0.15 mL, 2 mmol), benzotriazole (0.26 g, 2.2 mmol) and tetrahydrofuran (1 mL) under an ice cooling, which was stirred at room temperature for 30 minutes. To the reaction mixture were added water and 5N aqueous sodium hydroxide to adjust basic, then extracted with ethyl acetate, and the organic layer was washed with saturated sodium chloride water. The solvent was evaporated under a reduced pressure to obtain the title compound (0.21 g) as a pale yellow solid.

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm):4.84(2H,s), 6.31(2H,brs), 6.72(1H,dd,J=4.8,8.0Hz), 7.04(1H,s), 7.91(1H,dd,J=1.6,7.6Hz), 8.11(1H,dd,J=1.2,4.8Hz).

[Reference Example 17] Synthesis of di-*tert*-butyl {3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate



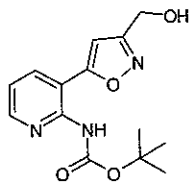
To a mixture of 3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-amine (420 mg, 2.01 mmol), 4-dimethyl aminopyridine (26.8 mg, 0.220 mmol), and tetrahydrofuran (2.1 mL) was added di-*tert*-butyldicarbonate (924 mg, 4.24 mmol) at room temperature, which was stirred. After 25 hours, to the reaction solution was added water and

extracted with toluene, then the organic layer was washed with 5% sodium chloride water and the solvent was evaporated under a reduced pressure to obtain the title compound (880 mg) as a pale yellow oily product.

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.33(18H,s), 4.63(2H,s), 6.66(1H,s), 7.45(1H,dd,J=4.8,8.0Hz), 8.30(1H,dd,J=2.0,8.0Hz), 8.62(1H,dd,J=2.0,4.8Hz).

Reference Examples 18 to 21 are other synthesis methods to Reference Examples 9 to 10 and Reference Examples 16 to 17.

[Reference Example 18] Synthesis of *tert*-butyl {3-[3-(hydroxymethyl)isoxazol-5-yl]pyridin-2-yl}carbamate

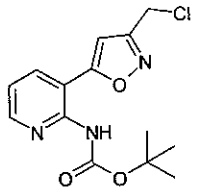


To a mixture of ethyl 5-{2-[(2,2-dimethylpropanoyl)amino]pyridin-3-yl}isoxazol-3-carboxylate (1.59 g, 5 mmol), di-*tert*-butyl dicarbonate (1.31 g, 6 mmol), and tetrahydrofuran (5 mL) was added 4-dimethylaminopyridine (61 mg, 0.5 mmol) at room temperature, which was stirred at room temperature for one hour and then stirred at 60°C for six hours. To the reaction mixture were added ethanol (2.5 mL) and sodium borohydride (0.57 g, 15 mmol), which was stirred at 0°C for 30 minutes and then stirred overnight at room temperature. To the reaction mixture was added water, extracted with ethyl acetate, and the organic layer was washed with water and saturated sodium chloride water. After drying over anhydrous magnesium sulfate and filtering, the solvent was evaporated under a reduced pressure to obtain the title compound (1.60 g).

^{1}H-NMR Spectrum (CDCl3) δ (ppm):1.47(9H,s), 4.83(2H,s), 6.63(1H,s), 7.17(1H,dd,J=4.8,8.0Hz), 7.58(1H,s), 7.97(1H,dd,J=2.0,8.0Hz),

8.51(1H,dd,J=2.0,4.8Hz).

[Reference Example 19] Synthesis of *tert*-butyl {3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-yl}carbamate

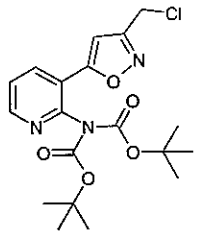


Under a nitrogen atmosphere, benzotriazole (3.55 g, 29.5 mmol) was dissolved in N,N-dimethylacetamide (10 mL), and thionyl chloride (2.06 mL, 26.8 mmol) was added dropwise thereto under an ice water cooling to prepare a solution of thionyl chloride-benzotriazole (1:1.1) in N,N-dimethylacetamide.

Under a nitrogen atmosphere, *tert*-butyl {3-[3-(hydroxymethyl)isoxazol-5-yl]pyridin-2-yl}carbamate (781 mg, 2.68 mmol) was dissolved in N,N-dimethylacetamide (2.7 mL). To the solution was added the above solution of thionyl chloride-benzotriazole (1:1.1) in N,N-dimethylacetamide (6 mL, 14.4 mmol) dropwise under an ice water cooling, which was stirred at the same temperature for one hour and then stirred at room temperature. After one hour and 20 minutes, under the ice water cooling, a solution of thionyl chloride-benzotriazole (1:1.1) in N,N-dimethylacetamide (2.2 mL, 5.12 mmol) was added dropwise thereto, and the solution was stirred at room temperature for one hour. Under the ice water cooling, to the reaction solution were added 1N aqueous sodium hydroxide and *tert*-butylmethylether to adjust basic and then extracted. The organic layer was washed sequentially with 0.5N aqueous sodium hydroxide and 5% sodium chloride water, dried over anhydrous magnesium sulfate and then the solvent was evaporated under a reduced pressure to obtain a crude body of the title compound (953 mg) as a pale yellow oily product.

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.47(9H,s), 4.65(2H,s), 6.67(1H,s), 7.20(1H,dd,J=4.8,8.0Hz), 7.44(1H,brs), 8.01(1H,dd,J=2.0,8.0Hz), 8.52(1H,dd,J=2.0,4.8Hz).

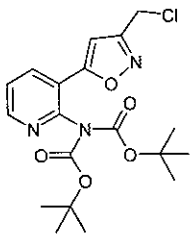
[Reference Example 20] Synthesis of di-*tert*-butyl {3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate



A crude matter of *tert*-butyl {3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-yl} carbamate (1.13 g, 3.17 mmol) was dissolved in tetrahydrofuran (7.0 mL), di-*tert*-butyl dicarbonate (761 mg, 3.49 mmol) was added thereto under an ice water cooling and washed thereto down with THF (3.0 mL). Then, after 4-dimethylaminopyridine (39.1 mg, 0.317 mmol) was added thereto, the solution was stirred at room temperature. After five hours, under the ice water cooling, to the reaction solution were added ethyl acetate and 5% sodium chloride water and extracted. After the organic layer was washed with 5% sodium chloride water and dried over anhydrous magnesium sulfate, the solvent was evaporated under a reduced pressure. The residue was purified by silica gel column chromatography to obtain the title compound (1.14 g) as a pale yellow solid.

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.33(18H,s), 4.63(2H,s), 6.66(1H,s), 7.45(1H,dd,J=4.8,8.0Hz), 8.30(1H,dd,J=2.0,8.0Hz), 8.62(1H,dd,J=2.0,4.8Hz).

[Reference Example 21] Synthesis of di-*tert*-butyl {3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate



Under a nitrogen gas stream, [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol (26.00 kg, 136.0 mol) and 1,3-dimethyl-2-imidazolidinone (143 kg, 5.5w/w, a portion was set aside for thorough wash) were added to a 500L reactor 1 and stirring was started. The solution was stirred at an internal temperature of from 35 to 45°C for one hour or longer and cooled after dissolution of [5-(2-aminopyridin-3-yl)isoxazol-3-yl]methanol. At an internal temperature of from 5 to 25°C, thionyl chloride (19.40 kg, 163.1 mol, 1.2M/M) was added dropwise thereto. After the dropwise addition was finished, thionyl chloride was thoroughly washed down with the set aside 1,3-dimethyl-2-imidazolidinone, the solution was stirred an internal temperature of from 5 to 25°C for 12 hours or longer. After the end of the reaction was confirmed by HPLC analysis, an aqueous solution of approximately 36% sodium hydroxide (mixed solution between an aqueous solution of 48% sodium hydroxide (15.9 kg; 190.8 mol, 1.4M/M as sodium hydroxide) and water (5.3 kg, 0.2w/w)) was added dropwise thereto at an internal temperature of from 0 to 25°C, and then ethyl acetate (164 kg, 6.31w/w) and water (74.2 kg, 2.85w/w) were added dropwise at an internal temperature of from 15 to 35°C. In addition, after an aqueous solution of approximately 8% sodium hydroxide (mixed solution between a solution of 48% sodium hydroxide (13.6 kg; 163.2 mol, 1.20M/M as sodium hydroxide) and water (68.0 kg, 2.6w/w)) was added dropwise thereto at an internal temperature of from 0 to 25°C and the internal temperature was adjusted to 15 to 30°C, the solution was stirred at the same temperature range for 30 minutes or longer and left standing still for 30 minutes or longer. The lower layer and the upper layer were removed

separately, each of the respective 1/2 weights was added to the 500L reactor 1 and a 500L reactor 2.

Post-processing of the 500L reactor 1 was carried out as described below. After stirring was started and water (52 kg, 2w/w) was added thereto, an aqueous solution of approximately 8% sodium hydroxide (mixed solution between an aqueous solution of 48% sodium hydroxide (11.3 kg; 135.6 mol, 1.0M/M as sodium hydroxide) and water (56.5 kg, 2.17w/w)) was added dropwise little by little at an internal temperature of from 0 to 25°C to adjust a pH of the lower layer to from 7.00 to 8.50 (actual value: pH7.84). At this time, 35.55 kg of the aqueous solution of approximately 8% sodium hydroxide was used. Then, after the internal temperature was adjusted to 15 to 30°C and the solution was stirred for 30 minutes or longer, the solution was left standing still overnight. On the next day, after the pH was confirmed again to be pH 7.59, the upper layer and the lower layer were respectively separated, only the lower layer was returned to the 500L reactor 1, and then ethyl acetate (82 kg, 3.15w/w) was added thereto. After stirring at an internal temperature of from 15 to 30°C for five minutes, the solution was left standing still for 30 minutes or longer, and the lower layer (pH7.55) was eliminated. To the upper layer that was left in the reactor were added the separated upper layer and 5% sodium chloride water (mixed solution between sodium chloride (3.3 kg, 0.13w/w) and water (618 kg, 2.38w/w)), which was stirred at an internal temperature of from 15 to 30°C for five minutes, then left standing still for 30 minutes or longer and the lower layer (pH8.23) was eliminated. Furthermore, water (65 kg, 2.5w/w) was added thereto, the solution was stirred at an internal temperature of from 15 to 30°C for five minutes and then left standing still overnight, and the lower layer (pH7.04) was eliminated.

The post-processing of the 500L reactor 2 was conducted concurrently with

the operations for the 500L reactor 1, the same procedure was carried out.

Next, the upper layer of the 500L reactor 2 was transferred to the 500L reactor 1, and the solution was concentrated under a reduced pressure at 45 to 55°C hot water and -0.070 to -0.085MPa level of the reduced pressure until the content solution was approximately 200L. Ethyl acetate (141 kg, 5.42w/w) was added therein, and the solution was concentrated again under a reduced pressure with the same conditions. After this operation was repeated further twice, before and after the 4th time addition of ethyl acetate (141 kg, 5.42w/w), the 3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-amine content in the solution was checked by HPLC analysis, the 3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-amine content (23.35 kg, 111.4 mol) in the solution and the yield thereof (81.9%) were calculated. Then, concentration under a reduced pressure was carried out once more with the same conditions until the 3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-amine content was 10.0 to 13.0% to obtain a solution of 3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-amine in ethyl acetate.

Under a nitrogen gas stream, the solution of 3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-amine in ethyl acetate (containing the total amount obtained in the previous step, 23.35 kg (111.4 mol)) inside the 500L reactor 1 was stirred, di-*tert*-butyl dicarbonate (53.47 kg, 245.0 mol, 2.2M/M) was added thereto at an internal temperature of from 15 to 25°C and which was washed thereto down with ethyl acetate (2 kg). A solution of 4-dimethyl aminopyridine in ethyl acetate (mixed solution of 4-dimethylaminopyridine (0.409 kg, 3.35 mol, 0.03M/M) and ethyl acetate (8 kg)) prepared beforehand was added therein, and after washing down with ethyl acetate (1 kg), the solution was stirred at an internal temperature of from 10 to 30°C for 22 hours or longer. After checking the end of the reaction by HPLC analysis, 1,3-dimethyl-2-imidazolidinone (50 kg, 2.12w/w) was added thereto. The solution was

concentrated under a reduced pressure at 45 to 55°C hot water circulation, until -0.092MPa or greater level of the reduced pressure and liquid distillation weakened, and after checking by GC analysis that the ethyl acetate content was 7.0%, the reactor was cooled to an internal temperature of 30°C or lower, and the solution was left standing still overnight. On the next day, to the concentration residue was added methanol (111 kg, 4.74w/w), which was stirred for 10 minutes or longer, and it was confirmed that no solid had precipitated, the solution was divided into two fractions. Next, the solutions divided into two fractions were added respectively to the 500L reactors 1 and 2, and respectively washed thereto down with methanol (9 kg each, 0.4w/w each). In so doing, as a result of analyzing by HPLC the solution prior to the two fraction division (225.65 kg), the target di-*tert*-butyl {3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate content was 19.37%, and the weight of di-*tert*-butyl {3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate contained was 43.71 kg (106.6 mol, yield: 95.7%).

For the 500L reactor 1 the processing was as described below. After stirring was started, at an internal temperature of from 35 to 45°C, water (35 kg, 1.5w/w) was added dropwise thereto over 30 minutes or longer, and di-*tert*-butyl {3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate (0.010 kg) was added thereto at an internal temperature of from 35 to 40°C. After stirring at an internal temperature of from 35 to 40°C for 30 minutes or longer, precipitation of solid was checked, and the solution was stirred further at the same temperature range for one hour or longer. Then, after water (three times 35 kg, 1.5w/w each) was added dropwise thereto over 30 minutes or longer at an internal temperature of from 35 to 45°C, the reactor was cooled to an internal temperature of from 5 to 15°C over 3 hours or longer, and the solution was stirred at the same temperature range for 12

hours or longer. Solid-liquid separation was carried out with a centrifuge dividing into two times, and the cake was washed with hydrous methanol (mixed solution of methanol (each time 7 kg, 0.3w/w) and water (each time 27 kg, 1.14w/w)). After washing was finished, centrifugal separation was carried out for 30 minutes or longer to obtain a wet solid of the title compound (25.80 kg). This wet solid was loaded into a mixing type vacuum dryer and vacuum dried at an external temperature from 45 to 55°C for 24 hours or longer to obtain the title compound (21.09 kg) as a pale yellow solid.

The operation for the 500L reactor 2 was carried out concurrently with the above, the same operations were performed to obtain the title compound (21.22 kg) as a pale yellow solid.

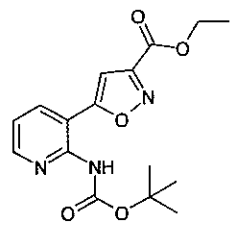
Accordingly, the title compound (42.31 kg, yield: 92.7%) was obtained.

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.33(18H,s), 4.63(2H,s), 6.66(1H,s), 7.45(1H,dd,J=4.8,8.0Hz), 8.30(1H,dd,J=2.0,8.0Hz), 8.62(1H,dd,J=2.0,4.8Hz).

HPLC conditions: column: YMC-Pack Pro C18 (5 μm, 150 × 4.6mmI.D., YMC), mobile phase: acetonitrile / water / ammonium acetate=300 / 700 / 1 to 900 / 100 / 1 (v/v/w).

GC Conditions: column: DB-624 (30m, 0.53mmI.D., Film 3 μm, Agilent).

[Reference Example 22] Ethyl 5-{2-[(2,2-dimethylpropoxycarbonyl)amino]pyridin-3-yl}isoxazol-3-carboxylate

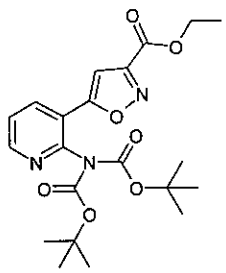


To a mixture of 4-methylen-2-oxo-4*H*-pyrido[2,3-d][1,3]oxazin-1-carboxylic acid *tert*-butyl ester (2.71 g, 10.37 mmol), which was synthesized according to the

methods described in the pamphlet of the International Publication WO 08/136279, Specifications, Preparation Example 3-3-1, triethylamine (4.2 mL, 30 mmol) and tetrahydrofuran (30 mL) was added ethyl 2-chloro-2-(hydroxyimino)acetate (4.5 g, 30 mmol) at 0°C over two hours and then stirred at room temperature for 14 hours. To the reaction mixture was added water, extracted with ethyl acetate and the organic layer was washed with water and saturated sodium chloride water. After drying over magnesium sulfate and filtering, the solvent was evaporated under a reduced pressure. The residue was suspended and washed with a 1:1 mixture of n-hexane and ethyl acetate to obtain the title compound (1.56 g).

^{1}H-NMR Spectrum (CDCl3) δ (ppm):1.44(3H,t,J=6.8Hz), 1.46(9H,s), 4.47(4H,q,J=7.2Hz), 6.95(1H,s), 7.22(1H,dd,J=4.8,8.0Hz), 7.42(1H,bs), 8.05(1H,dd,J=2.0,8.0Hz), 8.52(1H,dd,J=2.0,4.8Hz).

[Reference Example 23] Ethyl 5-{2-[bis (2,2-dimethylpropoxy-carbonyl)amino]pyridin-3-yl}isoxazol-3-carboxylate

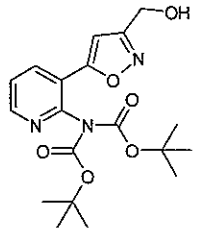


To a mixture of ethyl 5-{2-[(2,2-dimethylpropoxycarbonyl)amino]pyridin-3-yl}isoxazol-3-carboxylate (1.46 g, 4.38 mmol), di-*tert*-butyl dicarbonate (1.46 g, 6.69 mmol) and tetrahydrofuran (25 mL) was added 4-dimethylaminopyridine (30 mg, 0.25 mmol) at room temperature, which was stirred at room temperature for 14 hours. To the reaction mixture was added water, extracted with ethyl acetate, and the organic layer was washed with water and saturated sodium chloride water. After drying over magnesium sulfate and filtering, the solvent was evaporated under a reduced

pressure and the residue was purified by silica gel column chromatography (hexane : ethyl acetate = 3 : 1 then 1 : 1) to obtain the title compound (1.96 g).

^{1}H-NMR Spectrum (CDCl3) δ (ppm):1.36(18H,s), 1.46(3H,t,J=6.8Hz), 4.47(4H,q,J=6.8Hz), 6.93(1H,s), 7.46(1H,dd,J=4.8,7.6Hz), 8.29(1H,d,J=7.6Hz), 8.64(1H,d,J=4.8Hz).

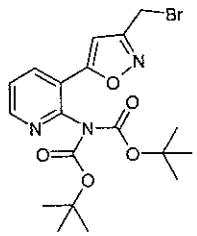
[Reference Example 24] Di-*tert*-butyl {3-[3-(hydroxymethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate



To a mixture of ethyl 5-{2-[bis(2,2-dimethylpropoxycarbonyl)amino]pyridin-3-yl}isoxazol-3-carboxylate (1.73 g, 4 mmol), ethanol (5 mL) and tetrahydrofuran (5 mL) was added sodium borohydride (0.15 g, 4 mmol) at 0°C, which was stirred at room temperature for one hour. Sodium borohydride (0.15 g, 4 mmol) was further added thereto and the solution was stirred at room temperature for three hours. To the reaction mixture was added water, extracted with ethyl acetate and the organic layer was washed with water and saturated sodium chloride water. After drying over anhydrous magnesium sulfate and filtering, the solvent was evaporated under a reduced pressure. The residue was added with a mixture of n-hexane-ethyl acetate (1 : 1), suspended and stirred, and then filtered to obtain the title compound (1.02 g).

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.33(18H,s), 4.81(2H,s), 6.60(1H,s), 7.43(1H,dd,J=4.8,8.0Hz), 8.27(1H,dd,J=2.0,8.0Hz), 8.60(1H,dd,J=2.0,4.8Hz).

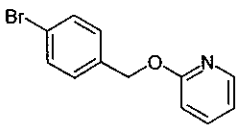
[Reference Example 25] Di-*tert*-butyl {3-[3-(bromomethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate



To a mixture of di-*tert*-butyl {3-[3-(hydroxymethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate (0.78 g, 2 mmol), triethylamine (1.95 mL, 14 mmol) and 1,2-dimethoxyethane (10 mL) was added phosphorus tribromide (0.37 mL, 4 mmol) dropwise at 0°C, which was stirred at room temperature for two hours, and then stirred at 50°C for 30 minutes. The reaction mixture was cooled to 0°C, then water was added thereto and extracted with ethyl acetate, and the organic layer was washed with saturated sodium chloride water. After drying over anhydrous magnesium sulfate and filtering, the solvent was evaporated under a reduced pressure, the residue was purified by silica gel column chromatography to obtain the title compound (0.14 g).

^{1}H-NMR Spectrum (CDCl3) δ (ppm):1.33(18H,s), 4.45(2H,s), 6.63(1H,s), 7.43(1H,dd,J=4.8,8.0Hz), 8.28(1H,dd,J=2.0,8.0Hz), 8.61(1H,dd,J=2.0,4.8Hz).

[Reference Example 26] Synthesis of 2-[(4-bromobenzyl)oxy]pyridine



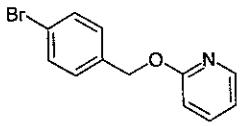
To a solution of 4-bromobenzyl alcohol (18 g, 94.3 mmol) in dimethylsulfoxide (85 mL) was added potassium *tert*-butoxide (11.5 g, 99 mmol) little by little under a nitrogen atmosphere at room temperature, which was stirred for 10 minutes. To this solution was added 2-fluoropyridine (12.3 g, 123 mmol) dropwise over 30 minutes under a water bath cooling. After stirring at room temperature for two hours, ethyl acetate and 5% sodium chloride water were added, and extracted. After the organic layer was washed sequentially with water and 5% sodium chloride water, the solvent

was evaporated under a reduced pressure to obtain the title compound (24.3 g) as a yellow oily product.

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):5.33(2H,s), 6.87-6.70(1H,m), 6.98-7.02(1H,m), 7.38-7.44(2H,m), 7.55-7.60(2H,m), 7.71-7.76(1H,m), 8.15-8.18(1H,m).

Reference Example 27 is the other synthesis method to Reference Example 26.

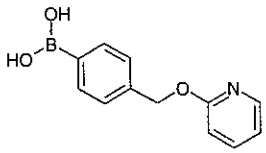
[Reference Example 27] Synthesis of 2-[(4-bromobenzyl)oxy]pyridine



Under a nitrogen atmosphere, to a solution of 4-bromobenzyl alcohol (600 g, 3.21 mol) and 2-fluoropyridine (343 g, 3.53 mol) in tetrahydrofuran (1069 mL) was added a solution of potassium *tert*-butoxide (396 g, 3.53 mol) in tetrahydrofuran (3208 mL) (63min, 9.2 to 20.5°C) dropwise under a 7°C cooling. After stirring at 22°C for three hours, an aqueous solution of 5% sodium bicarbonate (prepared from sodium bicarbonate: 160 g and water: 3208 mL) was added thereto dropwise (20min, 21.0 to 23.9°C). Then, heptane (3220 mL) was added thereto and extracted, the organic layer was washed with water (800 mL). The organic layer was concentrated under a reduced pressure (to approximately 3200 mL), ethanol (1604 mL) was added thereto and concentrated under a reduced pressure (to approximately 3200 mL). Then, to the layer was added heptane (3200 mL), concentrated under a reduced pressure, heptane (3200 mL) was further added thereto and concentrated under a reduced pressure to obtain a solution of the title compound in heptane (2603 g, containing 789 g of the target product) as a brown oily product (yield: 93.2%).

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):5.33(2H,s), 6.87-6.70(1H,m), 6.98-7.02(1H,m), 7.38-7.44(2H,m), 7.55-7.60(2H,m), 7.71-7.76(1H,m), 8.15-8.18(1H,m).

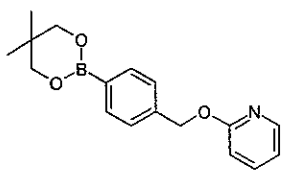
[Reference Example 28] Synthesis of {4-[(pyridin-2-yloxy)methyl]phenyl}boronic acid



Under a nitrogen atmosphere, a solution of 2-[(4-bromobenzyl)oxy]pyridine (50 g, 190 mmol) in tetrahydrofuran (200 mL) was cooled to -78°C and a 2.6M *n*-butyllithium hexane solution (88 mL, 228 mmol) was added thereto dropwise. After stirring for 45 minutes, trimethoxy borane (29.6 g, 285 mmol) was added thereto dropwise at the same temperature. After 30 minutes, a saturated aqueous solution of ammonium chloride and water were added thereto for quenching, and the solution was extracted with ethyl acetate. After the organic layer was washed with a mixture of saturated aqueous solution of ammonium chloride and saturated sodium chloride water and dried over anhydrous sodium sulfate, the solvent was evaporated under a reduced pressure. To the residue was added acetonitrile (200 mL), followed by suspending and stirring at 70°C for 30 minutes, then, cooled, and stirred overnight at 4°C. The precipitated solid was filtered to obtain the title compound (11.2 g) as a white solid.

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):4.62(2H,s), 5.42(2H,s), 6.83(1H,d,J=8.4Hz), 6.87-6.92(1H,m), 7.50(2H,d,J=8.0Hz), 7.57-7.62(1H,m), 7.75(2H,d,J=8.0Hz), 8.16-8.19(1H,m).

[Reference Example 29] Synthesis of 2-{[4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzyl]oxy}pyridine



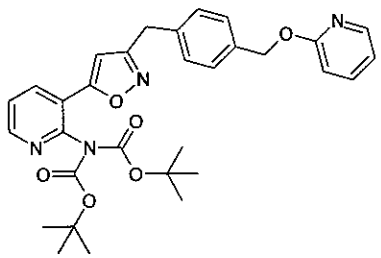
To a solution of 2-[(4-bromobenzyl)oxy]pyridine (789 g, 2.99 mol) in heptane

(2603 g) were added heptane (939 mL) and tetrahydrofuran (1199 mL), which was cooled slowly with dry ice / ethanol bath under a nitrogen atmosphere, while stirring. After 45 minutes, cooling was stopped and 2-[(4-bromobenzyl)oxy]pyridine (0.9 g) was added thereto at an internal temperature of -12°C. Cooling was resumed, with cooling at -20°C / h. Approximately 3 hours later, 1.66M *n*-butyllithium hexane solution (1980 mL, 3.29 mol) was added thereto dropwise (80min,-67.0 to 61.4°C). After stirring for 0.5 hours, at the same temperature, triisopropoxy borane (674 g, 3.56 mol) was added thereto dropwise (134min, -68.2 to 60.3°C). At the same temperature, after stirring for 0.5 hours, switching to an ice water bath cooling, the solution was stirred overnight (external temperature: 0°C). On the next day, water (5600 mL) was added thereto dropwise, the solution was transferred to a separator vessel and was extracted into the aqueous layer (pH: 11.2). Ethyl acetate (4800 mL) was added thereto and concentrated hydrochloric acid (approximately 280 mL) was added thereto dropwise (at an internal temperature of 20°C or lower) while stirring, to adjust the solution to pH: 7.1. The organic layer was separated and washed with 5% sodium chloride water (approximately 900 g) and then concentrated under a reduced pressure. To the residue was added isopropyl alcohol (3300 mL), concentrated under a reduced pressure, isopropyl alcohol (3300 mL) was further added thereto and concentrated under a reduced pressure to obtain a solution of {4-[(pyridin-2-yloxy)methyl]phenyl}boronic acid (646 g) in isopropyl alcohol (2671 g) (yield: 94.4%). The obtained solution was heated to 60°C, which was added to a container containing 2,2-dimethyl-1,3-propanediol (354 g, 3.41 mol) while eliminating insoluble matter by suction-filtration, and then which was washed thereto down with isopropyl alcohol (685 mL). After confirming dissolution, the solution was stirred at a bath temperature of 20°C and crystal precipitation was observed at an internal

temperature of 28.8°C. After 1.5 hours, the bath temperature was set to -20°C, and the solution was stirred overnight. The precipitated crystal was filtered, and the crystal was washed with a small amount of isopropyl alcohol cooled to 0°C. The crystal was dried under a reduced pressure to obtain the title compound (779 g) as a white crystal (yield: 92.2%).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm):0.94(6H,s), 3.74(4H,s), 5.35(2H,s), 6.87(1H,d,J=8.4Hz), 6.96-7.00(1H,m), 7.39(2H,d,J=8.0Hz), 7.67-7.74(3H,m), 8.14-8.17(1H,m).

[Reference Example 30] Synthesis of di-*tert*-butyl [3-(3-{4[(pyridin-2-yloxy)methyl]benzyl}isoxazol-5-yl)pyridin-2-yl]imidodicarbonate

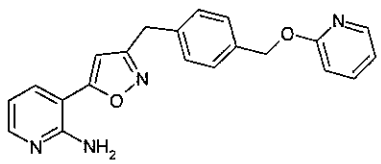


Under nitrogen atmosphere, to a mixture of di-*tert*-butyl {3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate (164 mg, 0.40 mmol), {4-[(pyridin-2-yloxy)methyl]phenyl}boronic acid (138 mg, 0.60 mmol), cesium carbonate (391 mg, 1.20 mmol), copper(I) iodide (3.9 mg, 5 mol%) and 1,2-dimethoxyethane (2.0 mL) was added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium (II) · dichloromethane complex (16.4 mg, 5 mol%) ,which was stirred at 80°C for 1.5 hours. {4-[(Pyridin-2-yloxy)methyl]phenyl}boronic acid (46 mg, 0.20 mmol) was added thereto and the solution was further stirred for 4.5 hours. After cooling, ethyl acetate and 5% sodium chloride solution were added thereto, insoluble matter was filtered out, then, the filtrate was transferred to a separatory funnel and separated. After the organic layer was washed with 5% sodium chloride water and dried over anhydrous

magnesium sulfate, the solvent was evaporated under a reduced pressure. The residue was purified by silica gel column chromatography to obtain the title compound (173 mg) as a pale yellow oily product.

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):1.23(9H,s), 4.05(2H,s), 5.34(2H,s), 6.32(1H,s), 6.76-6.79(1H,m), 6.86-6.90(1H,m), 7.28(2H,d, J=8.0Hz), 7.38-7.43(3H,m), 7.55-7.60(1H,m), 8.15-8.18(1H,m), 8.27(1H,dd,J=2.0,8.0Hz), 8.57(1H,dd,J=2.0,7.6Hz).

[Reference Example 31] 3-(3-(4-Pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine



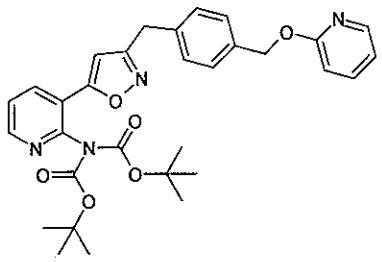
Di-*tert*-butyl [3-(3-{4[(pyridin-2-yloxy)methyl]benzyl}isoxazol-5-yl)pyridin-2-yl]imidodicarbonate (28.8 mg, 51.6 μmol) was dissolved in acetonitrile (0.6 mL), concentrated hydrochloric acid (60 μL, 690 μmol) was added thereto dropwise under an ice water cooling, which was stirred at the same temperature for one hour. Concentrated hydrochloric acid (140 μL, 1.61 mmol) was further added thereto dropwise and the solution was stirred at the same temperature for one hour and at 20°C for 3.5 hours. Under an ice water cooling, to the reaction solution were added 0.5N aqueous sodium hydroxide and ethyl acetate and extracted. After the organic layer was washed with 5% sodium chloride water and dried over anhydrous magnesium sulfate, the solvent was evaporated under a reduced pressure to obtain the title compound (18.3 mg) as a pale yellow oily product.

^{1}H-NMR Spectrum ($CDCl_3$) δ (ppm):4.07(2H,s), 5.37(2H,s), 5.42(2H,brs), 6.25(1H,s), 6.71(1H,dd,J=5.2,7.6Hz), 6.80(1H,d,J=8.4Hz), 6.87-6.91(1H,m),

7.30(2H,d,J=7.6Hz), 7.44(2H,d,J=7.6Hz), 7.56-7.61(1H,m), 7.70(1H,dd,J=2.0,7.6Hz), 8.14(1H,dd,J=2.0,4.8Hz), 8.16-8.19(1H,m).

Reference Examples 32 to 33 are other synthesis methods to Reference Examples 30 to 31.

[Reference Example 32] Synthesis of di-*tert*-butyl [3-(3-{4[(pyridin-2-yloxy)methyl]benzyl}isoxazol-5-yl)pyridin-2-yl]imidodicarbonate



The operation of the first batch was carried out as described below. Under a nitrogen gas stream, after di-*tert*-butyl {3-[3-(chloromethyl)isoxazol-5-yl]pyridin-2-yl}imidodicarbonate (20.80 kg, 50.75 mol), 2-{[4-(5,5-dimethyl-1,3,2-dioxaborinan-2-yl)benzyl]oxy}pyridine (19.61 kg, 66.00 mol, 1.30M/M), (oxydi-2,1-phenylene)bis(diphenylphosphine) (1.367 kg, 2.54 mol, 0.05M/M), potassium carbonate (9.11 kg, 65.91 mol, 1.30M/M) were added to a 500L reactor 2 which was nitrogen-substituted beforehand, the interior of the reactor was nitrogen-substituted again, N,N-dimethyl formamide (147 kg, 7.08w/w) was added thereto and stirring was started. Then, after maintaining at an internal temperature of from 15 to 25°C and a level of the reduced pressure of -0.090MPa or greater for three to five minutes maintenance, the reduced pressure was released with nitrogen. This operation was repeated five times to degas the solution. After degassing was finished, solution of palladium acetate in N,N-dimethyl formamide (mixed solution of palladium acetate (0.570 kg, 2.54 mol, 0.05M/M) and degassed N,N-dimethyl formamide (9.8 kg, 0.5w/w, a portion of which was set aside for thorough wash)) was added thereto, and

washed thereto down with the set aside degassed N,N-dimethyl formamide. Next, after stirring for 10 minutes, immediately degassed water (10.4 kg, 0.5w/w) was added thereto dropwise at an internal temperature of from 20 to 30°C, and the operation of reducing the pressure to -0.087MPa level of the reduced pressure and releasing the reduced pressure with nitrogen was repeated three times. Thereafter, hot water at approximately 60°C was circulated quickly to adjust the internal temperature to from 55 to 65°C and the solution was stirred for three hours from the start of the heating. After the end of the reaction was confirmed by HPLC analysis, toluene (90 kg, 4.34w/w) was added thereto at an internal temperature of from 0 to 25°C and water (156 kg, 7.5w/w) was added thereto dropwise at the same temperature range. Then, after stirring at an internal temperature of from 15 to 30°C for 30 minutes, the solution was left standing still for 30 minutes or longer standing, and the lower layer was eliminated. To the upper layer inside of the reactor was added water (104 kg, 5.0w/w), which was stirred at an internal temperature of from 15 to 30°C for five minutes, then left standing still overnight, and only the lower layer containing no insoluble matter was eliminated. The upper layer and the lower layer containing insoluble matter were filtered with Celite 503 RV (2.8 kg, 0.135w/w) under pressure by a filtration device, and the reactor and the filtration device were pour-washed with toluene (18.0 kg, 0.867w/w, a portion was set aside for flushing and thorough washing). The obtained filtrate and washes were returned to the 500L reactor 2 and washed thereto down with the previously set aside toluene. Subsequently, after the internal temperature was adjusted to 15 to 30°C, the solution was left standing still for 30 minutes or longer, and the lower layer was eliminated. After the stirring speed was adjusted to approximately maximum and n-heptane (152 kg, 7.32w/w) was added thereto dropwise over one hour or longer at an internal

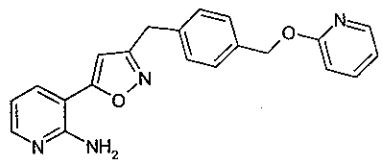
temperature of from 15 to 30°C, the solution was stirred at the same temperature range for two hours or longer. Then, after thiocyanuric acid (0.90 kg, 5.08 mol, 0.1M/M) was added thereinto with divided fractions over 30 minutes or longer at an internal temperature of from 15 to 30°C, the solution was stirred at the same temperature range for one hour or longer. Once more, thiocyanuric acid (0.90 kg, 5.08 mol, 0.1M/M) was added thereinto with divided fractions over 30 minutes or longer at an internal temperature of from 15 to 30°C and the solution was stirred overnight at the same temperature range. After overnight stirring, the solution in the reactor was filtered with activated carbon by a filtration device which was prepared beforehand, and the reactor and the filtration device were washed thoroughly with a mixed solution of n-heptane-toluene (mixed solution of n-heptane (130 kg) and toluene (83 kg),a portion of which was set aside for wetting the activated carbon (Seisei Shirasagi)). After adding thiocyanuric acid (1.80 kg, 10.16 mol, 0.2M/M) thereinto once more, filtration with activated carbon was carried out using the same amount of Celite 503 RV, activated carbon (Seisei Shirasagi) and mixed solution of n-heptane-toluene. The obtained filtrate and washes were added to the 500L reactor 1, which was concentrated under a reduced pressure and hot water circulation at 40 to 70°C until the content solution was approximately 100L visually. And, the residue was left standing still under a nitrogen atmosphere and at an internal temperature of 30°C or lower until activated carbon filtration of the second batch was finished.

As a second batch, the same operation as described above was carried out. The filtrate and washes of the second batch were added to the 500L reactor 1 to be pooled with the residue of the first batch, and concentration under a reduced pressure was started. Under hot water circulation at 60 to 70°C, when the distillate outflow had weakened, toluene (144 kg) was added thereto, then, again, under hot

water circulation at 60 to 70°C, concentration under a reduced pressure was carried out until distillate outflow weakened. At this point, the residue was analyzed and the toluene / target product ratio (0.167 w / w) was calculated from the di-*tert*-butyl [3-(3-{4[(pyridin-2-yloxy)methyl]benzyl}isoxazol-5-yl)pyridin-2-yl]imido dicarbonate content and the toluene content in the concentration residue. Toluene (29.66 kg, corresponding to a toluene / target product ratio of 0.700 w / w) was added thereto, the solution was stirred at an internal temperature of from 15 to 30°C for 30 minutes or longer to obtain a toluene solution of title compound (containing 42.37 kg of the target product; yield: 74.7%).

HPLC conditions: column: CAPCELL PAK C18 MGII (5 μm, 150 × 4.6mmI.D., SHISEIDO), mobile phase: acetonitrile / water / trifluoroacetic acid = 180 / 820 / 1 to 900 / 100 / 1 (v / v / v).

[Reference Example 33] 3-(3-(4-Pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine



To a toluene solution of di-*tert*-butyl [3-(3-{4[(pyridin-2-yloxy)methyl]benzyl}isoxazol-5-yl)pyridin-2-yl]imido dicarbonate (containing 42.37 kg (75.85 mol)) was added formic acid (181 kg, 4.27w/w) dropwise at an internal temperature of from -5 to 20°C, which was stirred at an internal temperature of from 22 to 32°C for 19 to 20 hours. After the end of the reaction was confirmed by HPLC analysis, the internal temperature was cooled to -5 to 10°C and the content solution was divided into two fractions and added to the 500L reactors 1 and 2, respectively.

For the 500L reactor 1, post-processing was carried out as described below.

Under stirring, water (74 kg, 1.75w/w) was added thereto dropwise at an internal temperature of from -5 to 20°C, further, *tert*-butylmethylether (31.4 kg, 0.74w/w) and n-heptane (29.0 kg, 0.684w/w) were added thereto at an internal temperature of from 0 to 25°C. The solution was stirred at an internal temperature of from 15 to 25°C for five minutes, left standing still for 30 minutes or longer to separate the lower layer. The lower layer was returned to the reactor, *tert*-butylmethylether (31.4 kg, 0.74w/w) and n-heptane (29.0 kg, 0.684w/w) were added thereto again at an internal temperature of from 0 to 25°C, the solution was stirred at an internal temperature of from 15 to 25°C for five minutes, then, left standing still for 30 minutes or longer, and the lower layer was separated again. The lower layer was returned to the reactor and, first, an aqueous solution of 48% sodium hydroxide (116 kg; 1392.0 mol, 18.35M/M as sodium hydroxide) was added dropwise at an internal temperature of from 0 to 25°C. Next, at the same temperature range, ethyl acetate (96 kg, 2.26w/w) was added thereto and an aqueous solution of 48% sodium hydroxide (20.5 kg; 246.0 mol, 3.24M/M as sodium hydroxide) was added thereto dropwise. In addition, herein, at same temperature range, an aqueous solution of approximately 8% sodium hydroxide (mixed solution of an aqueous solution of 48% sodium hydroxide (12.7 kg; 152.4 mol, 2.00M/M as sodium hydroxide) and water (64 kg, 1.5w/w)) was added thereto dropwise until a pH of the lower layer was pH 8.00 to 9.00, (actual value: pH 8.58) (0.75 kg used). Thereafter, the solution was stirred for one hour or longer at an internal temperature of from 20 to 30°C and left standing still overnight, then, the pH of the lower layer was checked again (actual value: pH 8.29) and the lower layer was eliminated. To the upper layer remaining in the reactor was added an aqueous solution of approximately 5% sodium bicarbonate (mixed solution of sodium bicarbonate (5.3 kg, 63.09 mol) and water (101 kg, 2.375w/w)), which was stirred at

an internal temperature of from 20 to 30°C for one hour or longer and then left standing still for 30 minutes or longer. After the lower layer (pH8.60) was eliminated, to the upper layer was added water (106 kg, 2.5w/w), which was stirred at an internal temperature of from 20 to 30°C for one hour or longer, then, left standing still for 30 minutes or longer, and the lower layer (pH7.17) was eliminated again.

For the 500L reactor 2, the same post-processing was carried out concurrently with the 500L reactor 1.

The content solution of the 500L reactor 1 was transferred to the 500L reactor 2 and concentrated under a reduced pressure under hot water circulation at 55 to 65°C until the content solution was approximately 100L. Next, to the concentration residue were added ethanol (42 kg, 1.0w/w) and ethyl acetate (96 kg, 2.26w/w), which was stirred for five minutes, then, concentrated under a reduced pressure under hot water circulation at 55 to 65°C until a level of the reduced pressure of -0.092MPa or greater and almost no distillate outflow was observed. At this point, as precipitation of crystal was observed, ethyl acetate was added little by little until the crystal dissolved completely (13.85 kg used). After ethanol (18.3 kg) and ethyl acetate (6.7 kg) were further added, the internal temperature was adjusted to 50 to 55°C, and after confirming visually that the crystal was dissolved, n-heptane (33.5 kg, 0.79w/w) was added thereto over 30 minutes or longer at an internal temperature of from 45 to 55°C. Then, after 3-(3-(4-pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine (0.011 kg), which can be synthesized according to the methods described in the pamphlet of the International Publication WO 08/136279, Specifications, Example 18, was added thereto at an internal temperature of from 45 to 50°C and precipitation of crystal was observed, the solution was stirred at the same temperature range for one hour or longer. After n-heptane (66.9 kg, 1.58w/w)

was added dropwise over one hour or longer at an internal temperature of from 45 to 55°C, the internal temperature was cooled to 0 to 10°C over four hours or longer, and the solution was stirred at the same temperature range for five hours or longer. After the content solution was sampled and it was confirmed that the rate of crystallization of the target product was 94%, the suspension was filtered under pressure, the crystal was pour-washed in the order with mixed solution of ethanol-ethyl acetate-n-heptane (mixed solution of ethanol (3.60 kg, 0.085w/w), ethyl acetate (4.15 kg, 0.098w/w) and n-heptane (18.81 kg, 0.444w/w)), and mixed solution of ethanol-n-heptane (mixed solution of ethanol (7.25 kg, 0.171w/w) and n-heptane (18.81 kg, 0.444w/w)) to obtain a wet crude crystal of the title compound (36.52 kg) as a slightly yellow crystal.

The obtained wet crude crystal of 3-(3-(4-pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine (36.52 kg) and ethanol (57.9 kg, 2.37w/w) were added sequentially to a 500L dissolution can, which was nitrogen-substituted beforehand, and heated to an internal temperature of from 70 to 75°C to dissolve the crystal. While maintaining the temperature, this dissolution solution was transferred through a SUS filter to a 500L crystallization can, and the 500L dissolution can and SUS filter were washed thoughtly with ethanol (19.3 kg, 0.8w/w) which was kept heated at an external temperature of approximately 65°C. Next, the filtrate was adjusted to an internal temperature of from 55 to 60°C, and it was confirmed that the solution inside the can was homogenous. Thereafter, when the internal temperature was cooled slowly to 48 to 51°C, crystal precipitated. After heating again to an internal temperature of from 55 to 60°C to dissolve the crystal, immediately the internal temperature was cooled to 48 to 51°C and 3-(3-(4-pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine (0.011 kg), which can be synthesized

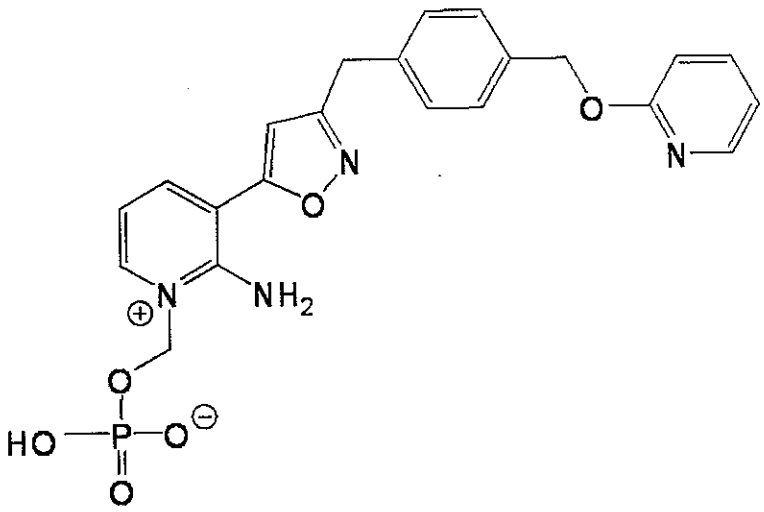
according to the methods described in the pamphlet of the International Publication WO 08/136279, Specifications, Example 18, was added immediately. Then, after precipitation of crystal was confirmed visually at an internal temperature of from 45 to 50°C, the suspension was stirred at an internal temperature of from 43 to 47°C for one hour to one hour and 30 minutes, and the internal temperature was cooled to 0 to 10°C over four hours or longer. At this point, after a precipitated crystal was sampled and the crystal form thereof was confirmed to be identical to the reference sample, the suspension was stirred overnight at the same temperature range. On the next day, after the crystal form was confirmed to be identical to the reference sample, the crystal was solid-liquid separated with a centrifuge divided in two times, and respectively pour-washed with approximately half of the amount of 19.3 kg of ethanol to obtain a wet crystal of the target product (24.23 kg). This wet crystal was placed into a mixed type vacuum dryer and dried under a reduced pressure at an external temperature of from 20 to 30°C for 6 hours or longer and an external temperature of from 35 to 45°C for 12 hours or longer to obtain the title compound (23.52 kg, 65.63 mol, yield: 86.8%) as a pale yellow crystal.

^{1}H-NMR Spectrum ($CDCl_3$)δ(ppm):4.07(2H,s), 5.37(2H,s), 5.42(2H,brs), 6.25(1H,s), 6.71(1H,dd,J=5.2,7.6Hz), 6.80(1H,d,J=8.4Hz), 6.87-6.91(1H,m), 7.30(2H,d,J=7.6Hz), 7.44(2H,d,J=7.6Hz), 7.56-7.61(1H,m), 7.70(1H,dd,J=2.0,7.6Hz), 8.14(1H,dd,J=2.0,4.8Hz), 8.16-8.19(1H,m).

HPLC conditions: column: CAPCELL PAK C18 MGII (5 μm, 150 × 4.6mmI.D., SHISEIDO), mobile phase: acetonitrile / water / trifluoroacetic acid = 180 / 820 / 1 to 900 / 100 / 1 (v / v / v).

Example 1: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-

pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium



To a mixture of phosphoric acid di-*tert*-butyl ester chloromethyl ester (1.5 g, 5.6 mmol), sodium iodide (420 mg, 2.8 mmol), and tetrahydrofuran (10 mL) was added 3-(3-(4-(pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine (1.0 g, 2.8 mmol) described in Reference Example 1, which was stirred overnight at room temperature. The reaction mixture was cooled to 0°C, and trifluoroacetic acid (17 mL) was added thereto dropwise at the same temperature. The reaction mixture was allowed to room temperature and stirred for 30 minutes. The reaction solution was concentrated under a reduced pressure, and the residue was performed gel filtration (CHP20P, Mitsubishi Kasei), eluted with water, and then methanol). The eluent was concentrated under a reduced pressure, the residue was then purified in an ODS column (water : acetonitrile : trifluoroacetic acid = 1000 : 10 : 1, then 1000 : 200 : 1.2, then 1000 : 250 : 1.25, and then 1500 : 500 : 2). The eluent was concentrated under a reduced pressure, small amounts of methanol and ether were added to the residue, and the precipitated solids were collected by filtration. These were dried with forced air for 2.5 hours at 60°C to obtain the title compound (380 mg, 29%).

^{1}H-NMR spectrum (CD_3OD : D_2O = 1 : 1) δ (ppm): 4.08 (2H, s), 5.33 (2H, s), 5.75 (2H, d, J = 10 Hz), 6.85 (1H, d, J = 8.4 Hz), 6.96-7.40 (3H, m), 7.35 (2H, d, J =

8.0 Hz), 7.41 (2H, d, J = 8.0 Hz), 7.69-7.74 (1H, m), 8.15-8.18 (1H, m), 8.26 (1H, dd, J = 1.2, 7.2 Hz), 8.35 (1H, dd, J = 1.2, 6.8 Hz).

Example 2 : 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium anhydrous form (type 1)

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (30mg) was suspended in dimethylsulfoxide at 80°C, then, while stirring, water and acetic acid were added to turn the solution transparent. Then, the solution was returned to room temperature and the precipitated solid was recovered by filtration to obtain the title compound (15mg).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 4.07 (2H,s), 5.33 (2H,s), 5.72 (2H,d,J=9.2Hz), 6.84-6.87 (1H,m), 6.97-7.20 (2H,m), 6.98 (1H,s), 7.35 (2H,d,J=8.4Hz), 7.42 (2H,d,J=8.4Hz), 7.69-7.74 (1H,m), 8.15-8.18 (1H,m), 8.25 (1H,dd,J=1.6,7.2Hz), 8.33-8.37 (1H,m).

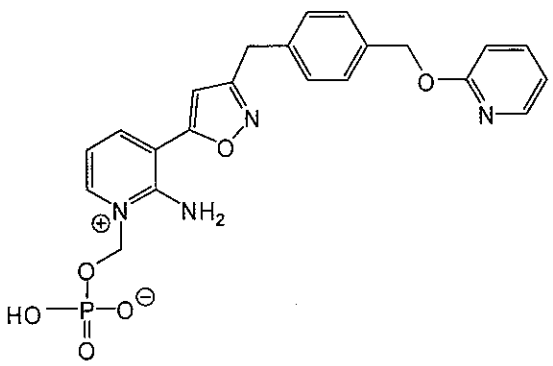
Example 3 : 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium anhydrous form (type 2)

To a mixture of phosphoric acid di-*tert*-butyl ester chloromethyl ester (541mg, 2.1mmol) and tetrahydrofuran (10mL), 3-(3-(4-(Pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine described in Reference Example 1 (300mg, 0.84mmol) and sodium iodide (251mg, 1.7mmol) were added at 4°C, which was stirred at 4°C for 66 hours. To the reaction mixture, under ice cooling, an aqueous solution of 2N hydrochloric acid (10mL) was added, which was stirred at room temperature for 3.5 hours. To the reaction mixture was added ethyl acetate (10mL) and water (10mL), and was then extracted. The aqueous layer was separated, ethyl acetate (10mL) was added to the aqueous layer, and then, 1N aqueous sodium

hydroxide was added until a pH of the aqueous layer reached 2, which was extracted. The aqueous layer was separated, ethyl acetate (10mL) was added to the aqueous layer, and then, 1N aqueous sodium hydroxide was added until a pH of the aqueous layer reached 2.5, which was extracted. The aqueous layer was separated, ethyl acetate (10mL) was added to the aqueous layer, and then, 1N aqueous sodium hydroxide was added until a pH of the aqueous layer reached 3, which was extracted. The aqueous layer was separated, 1N aqueous sodium hydroxide was added until a pH of the aqueous layer reached 4, and the aqueous layer was stirred overnight at room temperature. The precipitated crystal was filtered and washed sequentially with water at pH=4 (2mL) and ethyl acetate (2mL) to obtain the title compound (68mg, 17%).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 4.08 (2H,s), 5.33 (2H,s), 5.73 (2H,d,J=9.2Hz), 6.84-6.87 (1H,m), 6.97-7.04 (3H,m), 7.35 (2H,d,J=8.1Hz), 7.42 (2H,d,J=8.1Hz), 7.70-7.74 (1H,m), 8.16-8.18 (1H,m), 8.25 (1H,dd,J=1.5,7.5Hz), 8.34-8.36 (1H,m).

Example 4 : 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium anhydrous form (type 3)



To a mixture of chloromethyl di-tert-butylphosphate (6.73 g, 26 mmol) and tetrahydrofuran (22 mL), sodium iodide (6.03 g, 40 mmol) and cupper powder (127 mg, 2 mmol) were added, which was stirred at room temperature. After 5 hours, 3-

(3-(4-(pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine (7.17 g, 20 mmol) described in Example 1, toluene (20 mL), and 5N aqueous sodium hydroxide (22 mL) were added thereto, followed by stirring at room temperature. After 23 hours, a mixture of chloromethyl di-tert-butylphosphate (3.63 g, 14 mmol), sodium iodide (3.25 g, 21.5 mmol), cupper powder (68 mg, 1.08 mmol), and tetrahydrofuran (12 mL), which was prepared as described above, was added thereto, which was stirred at room temperature. After 20 hours, the reaction mixture was filtered to remove the insoluble matter, and the organic layer was separated. To the resulting organic layer, 5N hydrochloric acid (22 mL) was added dropwise with an ice-water cooling bath, followed by stirring at room temperature. After 2.5 hours, ethyl acetate (35 mL) was added to the reaction mixture, and 5N aqueous sodium hydroxide was added dropwise with an ice-water cooling bath so as to adjust a pH to be from 10.5 to 11.0. The aqueous layer was separated, followed by washing with ethyl acetate (35 mL), and the same procedure was repeated twice. To the resulting aqueous layer, acetonitrile (70 mL) was added, followed by stirring at room temperature for one hour, so as to remove the insoluble matters by filtration. The aqueous layer was separated, acetonitrile (70 mL) was added thereto, followed by stirring for 30 minutes with an ice-water cooling bath. Then, the solution was stored in a refrigerator over night. Next day, the insoluble matters was removed by filtration. To the resulting aqueous layer, 5N hydrochloric acid was added dropwise, so as to adjust a pH to be 3.5. A small amount of solid of 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium was added into the solution, followed by stirring at room temperature over night. After 18 hours, the precipitated solid was filtrated, followed by washing with water (8 mL), water-acetonitrile (1:1, 8 mL), and acetnitrile (8 mL) in this order, and dried under a reduced

pressure to obtain the crude solid (3.39 g) as a pale-brown solid.

To the resulting crude solid (3.39 g), sodium sulfite (3.39 g) and water (67.7 mL) were added followed by stirring at room temperature for 0.5 hours. After it was confirmed that the insoluble matters was not observed, acetonitrile (13.5 mL) was added thereto. Addition of 5N hydrochloric acid dropwise resulted in gradual formation of the crystal. After adjusting a pH to be 3.5, the stirring was carried out at room temperature overnight. After 17 hours, the precipitated crystal was filtrated, then washed with water-acetonitrile (1:1; 14 mL), acetonitrile (21 mL) in this order, and dried under a reduced pressure to obtain the title compound (2.98 g) as a pale-yellow crystal.

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 4.08 (2H,s), 5.33 (2H,s), 5.73 (2H,d,J=9.2Hz), 6.84-6.87 (1H,m), 6.97-7.04 (3H,m), 7.35 (2H,d,J=8.1Hz), 7.42 (2H,d,J=8.1Hz), 7.70-7.74 (1H,m), 8.16-8.18 (1H,m), 8.25 (1H,dd,J=1.5,7.5Hz), 8.34-8.36 (1H,m).

Example 5 : 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium hydrate

To a mixture of phosphoric acid di-*tert*-butyl ester chloromethyl ester (43mg, 0.17mmol) and acetone (1mL), 3-(3-(4-(Pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine described in Reference Example 1 (30mg, 0.084mmol) and sodium iodide (25mg, 0.17mmol) were added at room temperature, which was stirred at 4°C for 17 hours, then, stirred at room temperature for 3 days. To the reaction mixture, tetrahydrofuran (1mL) and an aqueous solution of 2N hydrochloric acid (1mL) were added at room temperature, which was stirred at room temperature for 1.5 hours. To the reaction mixture, ethyl acetate was added under ice cooling, and then, 5N aqueous sodium hydroxide was added at the same temperature until a pH of the

aqueous layer reached 7.5. The aqueous layer was separated and an aqueous solution of 1N hydrochloric acid was added at room temperature until a pH of the aqueous layer reached 5. The aqueous layer was added with ethyl acetate at room temperature and was stirred overnight at the same temperature. The precipitated crystal was filtered and sequentially washed with water at pH=4 (2mL) and isopropanol (2mL) to obtain the title compound (9.7mg, 23%).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 4.07 (2H,s), 5.32 (2H,s), 5.69 (2H,d,J=9.3Hz), 6.84-6.89 (2H,m), 6.97-7.00 (2H,m), 7.34 (2H,d,J=8.1Hz), 7.41 (2H,d,J=8.2Hz), 7.69-7.74 (1H,m), 8.16-8.19 (2H,m), 8.27 (1H,d,J=6.6Hz).

Example 6 : 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium acetic acid solvate

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (30mg) was suspended in ethanol at 80°C, then, while stirring, acetic acid was added to obtain a transparent solution. Then, the solution was returned to room temperature and the precipitated solid was recovered by filtration to obtain the title compound (22mg).

^{1} H-NMR Spectrum (DMSO-d_6) δ (ppm): 1.91 (3H,s), 4.08 (2H,s), 5.33 (2H,s), 5.73 (2H,d,J=9.6Hz), 6.84-6.87 (1H,m), 6.97-7.30 (2H,m), 6.98 (1H,s), 7.35 (2H,d,J=8.0Hz), 7.42 (2H,d,J=8.0Hz), 7.69-7.74 (1H,m), 8.15-8.18 (1H,m), 8.25 (1H,dd,J=1.6,7.6Hz), 8.36 (1H,dd,J=1.6,6.4Hz).

Example 7: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium amorphous

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (50mg) was dissolved in 0.023% aqueous ammonia (25mL), then lyophilized to obtain the title compound.

Example 8: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium monohydrochloride

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (100mg) was suspended in water (1mL) and methanol (1mL), then, while stirring, 2N hydrochloric acid (0.5ml) was added to obtain a transparent solution. Then, acetone (13mL) was added thereto, and the solid precipitated by subjecting to ultrasound treatment was recovered by filtration to obtain a white solid (97mg). The resulting solid (97mg) was dissolved in water (1mL), acetone (19mL) was added, and the solid precipitated by subjecting to ultrasound treatment was recovered by filtration to obtain the title compound (92mg).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 4.10 (2H,s), 5.33 (2H,s), 5.96 (2H,d,J=12.8Hz), 6.84-6.87 (1H,m), 6.97-7.01 (1H,m), 6.99 (1H,s), 7.08 (1H,t,J=7.2Hz), 7.35 (2H,d,J=8.0Hz), 7.42 (2H,d,J=8.0Hz), 7.70-7.75 (1H,m), 8.15-8.18 (1H,m), 8.31 (2H,d,J=7.2Hz).

Example 9: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium monohydrochloride

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (50mg) was suspended in water (0.5mL) and methanol (0.5mL), then, while stirring, 2N hydrochloric acid (214µL) was added to obtain a transparent solution. Then, acetone was added until the solution turned slightly turbid, which was stirred for 30 minutes. The precipitated solid was recovered by filtration to obtain the title crystal (33mg).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 4.10 (2H,s), 5.33 (2H,s), 5.98 (2H,d,J=12.8Hz), 6.84-6.87 (1H,m), 6.97-7.01 (1H,m), 6.99 (1H,s), 7.07-7.11 (1H,m),

7.35 (2H,d,J=7.6Hz), 7.42 (2H,d,J=7.6Hz), 7.70-7.75 (1H,m), 8.15-8.18 (1H,m), 8.28-8.33 (2H,m).

Example 10: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium hemisulfate

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (50mg) was dissolved in water (0.5mL) methanol (0.5mL) and 2N sulfuric acid (52.5mg). Acetone (approximately0.5mL) was added to cloud the solution, which was stirred briefly at room temperature. The cloudy solution was subjected to the ultrasound treatment, then, the precipitated solid was recovered by filtration to obtain the title crystal (38mg).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 4.10 (2H,s), 5.33 (2H,s), 5.94 (2H,d,J=12.4Hz), 6.84-6.87 (1H,m), 6.97-7.01 (2H,m), 7.06-7.10 (1H,m), 7.35 (2H,d,J=8.4Hz) 7.42 (2H,d,J=8.4Hz), 7.70-7.75 (1H,m), 8.15-8.18 (1H,m), 8.28-8.32 (2H,m).

Example 11: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium mononitrate

An aqueous solution of 9% nitric acid was added to 2-amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridine (50mg) at room temperature until a transparent solution was obtained. After concentration, water was added to the residue and insoluble matter was removed by filtration. The filtrate was concentrated, and small amounts of methanol and acetone were added to the residue. After concentration, addition of a small amount of ethanol to the residue, followed by subjecting to the ultrasound treatment, the mixture was refluxed for two minutes. After returning to room temperature, the precipitated solid was recovered by filtration to obtain the title crystal (35mg).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 4.10 (2H,s), 5.33 (2H,s), 5.98 (2H,d,J=13.6Hz), 6.86 (1H,d,J=8.4Hz), 6.97-7.01 (2H,m), 7.09 (1H,dd,J=7.6,7.6Hz), 7.35 (2H,d,J=8.0Hz), 7.42 (2H,d,J=8.0Hz), 7.70-7.75 (1H,m), 8.15-8.18 (1H,m), 8.26-8.34 (2H,m).

Example 12: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium monohydrobromide

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (50mg) was dissolved in water (0.5mL), methanol (0.5mL) and 2N hydrobromic acid (0.3mL). A small amount of acetone was added to cloud the solution, which was left to stand still for a while. The precipitated solid was recovered by filtration to obtain the title crystal (44mg).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 4.10 (2H,s), 5.33 (2H,s), 5.98 (2H,d,J=13.2Hz), 6.84-6.87 (1H,m), 6.98-7.01 (2H,m), 7.09 (1H,dd,J=7.6,7.6Hz), 7.35 (2H,d,J=8.0Hz), 7.42 (2H,d,J=8.0Hz), 7.70-7.75 (1H,m), 8.15-8.18 (1H,m), 8.27-8.33 (2H,m).

Example 13: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium sodium salt

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (50mg) was suspended in methanol (3mL) and 1N aqueous sodium hydroxide (0.107mL) was added thereto. This solution was stirred for two hours at room temperature, then, concentrated under a reduced pressure. An appropriate amount of acetone was added to this residue to let a yellow crystal precipitate. The precipitated yellow crystal was recovered by filtration and dried under a reduced pressure at room temperature to obtain the title compound (26mg).

^{1}H-NMR Spectrum (D_2O) δ (ppm): 3.96 (2H,s), 5.12 (2H,s), 5.62 (2H,d,J=2.3Hz), 6.60 (1H,s), 6.70 (1H,d,J=2.1Hz), 6.84-6.88 (2H,m), 7.21 (2H,d,J=1.8Hz), 7.29 (2H,d,J=1.8Hz), 7.27-7.59 (1H,m), 7.90-7.98 (3H,m).

Example 14: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium hemicalcium salt

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (30mg) was suspended in water (0.5mL), 1N aqueous sodium hydroxide (0.064mL) was added at room temperature, followed by adding calcium acetate monohydrate (5.6mg) in water (0.32mL). The reaction mixture was stirred overnight at room temperature. The precipitated crystal was centrifuged to separate it from the supernatant. The crystal was washed twice with water (2mL) and then washed once with acetone (2mL) to obtain the title compound (12mg).

^{1}H-NMR Spectrum (CD_3OD/D_2O=1/1) δ (ppm): 4.13 (2H,s), 5.29 (2H,s), 5.79 (2H,d,J=8.8Hz), 6.85 (1H,s), 6.88-6.90 (1H,m), 7.02-7.06 (2H,m), 7.37 (2H,d,J=8.2Hz), 7.44 (2H,d,J=8.4Hz), 7.74-7.78 (1H,m), 8.09-8.11 (1H,m), 8.17-8.19 (1H,m), 8.23 (1H,dd,J=1.6,7.6Hz).

Example 15: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium lithium salt

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (50mg) was suspended in methanol (3mL) and an aqueous solution of 4M lithium hydroxide (0.027mL) was added thereto. This mixture was stirred for 20 hours at room temperature and then concentrated under a reduced pressure. An appropriate amount of acetone was added to this residue to let a yellow crystal precipitate. The precipitated yellow

crystal was recovered by filtration and dried under a reduced pressure at room temperature to obtain the title compound (35mg).

^{1}H-NMR Spectrum (D_2O) δ (ppm): 3.96 (2H,s), 5.13 (2H,s), 5.59 (2H,d,J=2.2Hz), 6.59 (1H,s), 6.71 (1H,d,J=2.1Hz), 6.79 (1H,t,J=1.8Hz), 6.85-6.88 (1H,m), 7.21 (2H,d,J=2.1Hz), 7.29 (2H,d,J=2.1Hz), 7.56-7.60 (1H,m), 7.88-7.94 (3H,m).

Example 16: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium potassium salt

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (65mg) was suspended in methanol (3mL) and 1M potassium hydroxide (0.107mL) was added thereto. This mixture was stirred for 16 hours at room temperature and then concentrated a under reduced pressure. An appropriate amount of acetone was added to this residue to let a yellow crystal precipitate. The precipitated yellow crystal was recovered by filtration and dried under a reduced pressure at room temperature to obtain the title compound (23mg).

^{1}H-NMR Spectrum (D_2O) δ (ppm): 3.96 (2H,s), 5.12 (2H,s), 5.62 (2H,d,J=2.3Hz), 6.60 (1H,s), 6.70 (1H,d,J=2.1Hz), 6.85-6.88 (2H,m), 7.21 (2H,d,J=2.1Hz), 7.29 (2H,d,J=2.1Hz), 7.55-7.60 (1H,m), 7.90-7.92 (2H,m), 7.93-7.97 (1H,m).

Example 17: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium phosphate

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (30mg) was suspended in methanol (0.50mL) and water (0.50mL), and phosphoric acid (85%, 0.11mL) was

added thereto at room temperature for dissolution. Acetone (3.0mL) was added dropwise to the reaction mixture, which turned into a colorless suspension. The solvent was eliminated by decantation to obtain the title compound (27mg).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 4.16 (2H,s), 5.38 (2H,s), 5.92 (2H,d,J=12.0Hz), 6.87 (1H,s), 7.11 (1H,dd,J=7.0,8.0Hz), 7.15 (1H,d,J=8.0Hz), 7.21 (1H,ddd,J=1.5,6.0,7.0Hz), 7.40 (2H,d,J=8.0Hz), 7.48 (2H,d,J=8.0Hz), 8.01 (1H,ddd,J=1.5,7.0,9.0Hz), 8.17-8.20 (2H,m), 8.29 (1H,dd,J=1.5,8.0Hz).

Example 18: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium N-methyl-D-glucamine salt

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (30mg) was suspended in water (1.0mL), and N-methyl-D-glucamine (99%, 15.1mg) and water (0.12ml) were added thereto for dissolution. The reaction mixture was concentrated under a reduced pressure, and the residue was dissolved by adding methanol (1.0mL). The solution was concentrated under a reduced pressure, then, acetone (4.0mL) was added thereto, the solvent was eliminated by decantation to obtain the title compound (69%, 27mg).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 2.76 (3H,s), 3.15 (1H,dd,J=9.5,13.0Hz), 3.20 (1H,dd,J=3.5,13.0Hz), 3.62-3.67 (3H,m), 3.71-3.76 (2H,m), 3.79-3.82 (3H,m), 4.05-4.10 (2H,m), 4.12 (2H,s), 5.28 (2H,s), 5.79 (2H,d,J=9.0Hz), 6.83 (1H,s), 6.88 (1H,d,J=8.5Hz), 7.01-7.05 (2H,m), 7.36 (2H,d,J=8.0Hz), 7.43 (2H,d,J=8.0Hz), 7.72-7.78 (1H,m), 8.09 (1H,dd,J=1.5,5.0Hz), 8.17 (1H,dd,J=1.5,6.5Hz), 8.20 (1H,dd,J=1.5,7.5Hz).

Example 19: 2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-

pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium 1 lysine salt

2-Amino-1-((phosphonoxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium (50mg), L- lysine (15.6mg) was dissolved in ethanol (2mL) and water (1mL) and then, the solvent was concentrated under a reduced pressure. Ethanol (1mL) was added to the residue to suspend and recover by filtration the solid. The resulting solid was dissolved in methanol and then concentrated. A small amount of methanol was added to the residue, which was heated to 60 to 70°C. Once the solid was solved, crystal began to precipitate, which was recovered by filtration to obtain the title crystal (25mg).

^{1}H-NMR Spectrum (DMSO-d_6) δ (ppm): 1.25-1.42 (4H,m), 1.53-1.62 (2H,m), 1.72-1.80 (2H,m), 2.86 (2H,t,J=8.0Hz), 3.59 (1H,t,J=5.6Hz), 3.95 (2H,s), 5.10 (2H,s), 5.62 (2H,d,J=9.2Hz), 6.58 (1H,s), 6.67 (1H,d,J=8.4Hz), 6.82-6.88 (2H,m), 7.19 (2H,d,J=7.6Hz), 7.27 (2H,d,J=7.6Hz), 7.53-7.58 (1H,m), 7.86-7.91 (2H,m), 7.97 (1H,d,J=7.2Hz).

(Powder X-ray diffraction measurement)

For each crystal and each amorphous obtained in the Examples, approximately 5mg of sample was ground with a mortar, then, placed on an aluminum measurement pan and measured under the following conditions:

Apparatus used: X-ray DSC System: TTR-III (Manufactured by Rigaku Corporation)

X-ray used: CuK alpha beam

Goniometer: TTR-III horizontal goniometer

Counter: scintillation counter

Sample plate: aluminum

Target: Cu

Tube voltage: 50kV

Tube current: 300mA

Scanning speed: 2.000°/min

Scanning axis: 2θ/θ

Scanning range: 2θ=2.000~35.000°

Divergence slit: 0.5mm

Vertical divergence limiting slit: 2mm

Scattering slit: open

Sensor slit: open

Sampling width: 0.02°

Integration time: 1

The X-ray diffraction patterns of the powders for each crystal and each amorphous obtained in each of Example are shown respectively in Fig. 1 to Fig. 14, and representative peaks and relative intensities for the diffraction angle (2θ) of each crystal described above are shown in Table 1.

Table 1

Example crystal	Free anhydrous form type 1		Free anhydrous form type 2		Free anhydrous form type 3		Free anhydrous form	
	Peak diffraction angle (2θ)	Relative intensity	Peak diffraction angle (2θ)	Relative intensity	Peak diffraction angle (2θ)	Relative intensity	Peak diffraction angle (2θ)	Relative intensity
	4.08	100	3.74	100	3.860	100	4.46	100
	8.24	31	9.98	34	17.800	57	7.18	54
	9.20	14	17.38	71	18.240	23	7.86	52
	16.54	95	18.22	52			18.52	99
	17.06	100						

Example crystal	Free acetic acid-added compound		Free amorphous	
	Peak diffraction angle (2θ)	Relative intensity	Peak diffraction angle (2θ)	Relative intensity
	2.72	100		
	16.60	13		
	18.10	25		

Example crystal	Monohydrochloride		Hemisulfate		Nitrate		Hydrobromide	
	Peak diffraction angle (2θ)	Relative intensity	Peak diffraction angle (2θ)	Relative intensity	Peak diffraction angle (2θ)	Relative intensity	Peak diffraction angle (2θ)	Relative intensity
	6.26	67	7.84	52	11.88	83	12.40	29
	12.54	56	19.42	100	12.22	69	17.82	16
	20.38	100	20.24	59	16.7	75	20.20	100
			21.14	62	19.72	100		
			21.52	62	21.5	75		

Example crystal	Sodium salt		1/2 Ca		Lithium		Potassium	
	Peak diffraction angle (2θ)	Relative intensity	Peak diffraction angle (2θ)	Relative intensity	Peak diffraction angle (2θ)	Relative intensity	Peak diffraction angle (2θ)	Relative intensity
	2.32	100	3.78	56	2.10	100	2.10	100
	4.60	6	14.36	32	4.36	15	4.30	12
			15.78	34	10.88	7	10.98	6
			18.22	100				
			18.44	60				

The compounds according to the present invention represented by formula (I), (IA), or (IB) were used in a Candida systemic infection experiment with mice, which showed that there was pronounced improvement in the average number of survival days, and that the properties were excellent, especially in terms of solubility in water and stability in aqueous solutions, as well as safety and in-vivo kinetics, demonstrating that these compounds are extremely useful for the prevention or treatment of fungal infections.

1. Comparative Test Example of Solubility in Water

3-(3-(4-(Pyridin-2-yloxymethyl)-benzyl)-isoxazol-5-yl)-pyridin-2-ylamine described in Reference Example 1, which is the parent compound, and the compound of Example 1 were compared for solubility in a Britton-Robinson buffer (ionic strength: 0.3) at 25°C. Table 2 shows the results.

Table 2

	Solubility (mg/mL)			
	pH 5	pH 7	pH 8	pH 9
Parent compound	< 0.1	< 0.1	< 0.1	< 0.1
Compound of Example 1	1.9	10.5	16.4	> 21.1

As can be clear from the results in Table 2, the compound of Example 1 has markedly higher solubility in water than does the parent compound over various pH regions.

Pharmacological Test Examples

2. Experimental systemic candidal infection model in mice

(1) Preparation of fungal inoculant

A standing culture of C. *albicans* E81022 strain was carried out for 24 hours at

35°C in sabouraud dextrose agar medium (Difco), and the recovered fungal cells were suspended in sterile physiological saline (Otsuka Pharmaceutical). By counting the fungal number on cytometry plate, the suspension was diluted to 2×10^7 cells / mL with sterilized physiological saline to serve fungal inoculum.

(2) Infection

The fungal inoculum was used in an amount of 0.2 mL to inoculate the mice in the tail vein (4×10^6 cells/mouse).

(3) Preparation of Agent Solution, and Treatment

The compound of Example 1 was dissolved in concentrations of 13 mg / mL and 3.25 mg / mL with a 50mM glycine buffer with a pH of 10 (Wako Pure Chemicals), these solutions were then diluted 10 times with 5% glucose (Otsuka Pharmaceutical). At 0.5 and 9 hours after infection, 0.2 mL of the agent solution was administered into a tail vein of the mice. The agent concentrations were 2.5 and 10 mg / kg (calculated as the active form). The number of animals in one group was 5 animals.

(4) Evaluation of Effect

The protective effect was determined by observing life / death until 14 days after infection and calculating the mean survival days. The protective effect on the systemic candidal infection was measured for the compound of Example 1 by the method described in section 2. As a result, as shown in Table 3, the mice administered with the compound of Example 1 survived for a longer time as compared to the untreated group, and the compound of Example 1 have found to demonstrate an *in vivo* protective effect.

Table 3 Average Number of Survival Days in Candida Infection Experiment

Administration Group	Mean Survival Days
Control	2.4
Compound of Example 1: 3.25 mg/kg (calculated as active form : 2.5 mg/kg)	7.4
Compound of Example 1, 13 mg/kg (calculated as active form : 10 mg/kg)	12.8

Note that, *in vitro*, the compound of Example 1 demonstrated an activity of only 1/100 or less with respect to the parent compound (Reference Example 1). From these results, the compound of Example 1 was shown to act as a prodrug *in vivo*.

3. Pharmacokinetic Evaluation in Mice

(1) Preparation of Administration Solution

The compound of Example 1 was dissolved in a concentration of 0.45 mg / mL with a 50mM glycine buffer with a pH of 9 (Wako Pure Chemicals), and the active form was dissolved in a concentration of 0.45 mg / mL in a 10mM hydrochloric acid solution (Wako Pure Chemicals) containing 5% glucose (Otsuka Pharmaceutical).

(2) Administration, and Sampling of Blood and Plasma

Using five-week-old female ICR mice (Charles River Japan), with the number of mouse in one group being 2 mice, the compound according to the present invention and an active form thereof were administered into the tail vein in a dose of 3 mg / kg. The tail vein was punctured 0.25, 0.5, 1.5, 3, 5, and 8 hours after administration, and blood was collected with a heparin-treated pipette. The blood samples were put into sampling tubes and stored under ice cooling. Thereafter, they

were centrifuged for 5 minutes at 10,500 x g and at 4°C. The plasma thus obtained was accurately divided into 5 μL amounts and stored at -20°C until they were analyzed.

(3) Plasma Concentration Measurement Method

The plasma concentrations of the compound of Example 1 and the active form thereof were measured using a liquid chromatography mass spectrometer (LC-MS), and quantified by internal standard method. Imipramine hydrochloride (Sigma) was dissolved in a mixed solution of acetonitrile and methanol (1:1) so that the concentration would be 0.5 μmol / L, to prepare an internal standard substance solution (IS solution). The plasma was melted, 50 μL of IS solution was then added thereto and mixed, and this was centrifuged (deproteinated) for 10 minutes at 1500 x g and at 4°C, the supernatant was then analyzed by LC-MS (Waters). In the chromatogram thus obtained, the surface area of the peaks for the compound of Example 1 and the active form thereof (the parent compound of the compound of Example 1), and of the peak corresponding to the internal standard substance, were analyzed by MassLynx (Waters), and the concentration of the compound included in the plasma was calculated by internal standard method.

The plasma concentration was measured for the compound of Example 1 and the active form by the method described in section 3. As a result, as shown in Figure15, the plasma concentration of the compound of Example 1 decreased quickly after administration, and the plasma concentration of the active form increased quickly from immediately after administration of the compound of Example 1, exhibiting a plasma concentration change that was similar to that at the time of active form administration. The above suggests that the compound of Example 1 is rapidly converted into the active form in the bodies of the mice.

4. Pharmacokinetic Evaluation in Monkeys

(1) Preparation of Administration Solution

The compound of Example 1 was dissolved in a concentration of 1.5 mg / mL with a 50mM glycine buffer with a pH of 9 (Wako Pure Chemicals), and the active form (the parent compound thereof) was dissolved in a concentration of 1 mg / mL in a 10mM hydrochloric acid solution (Wako Pure Chemicals) containing 5% glucose (Otsuka Pharmaceutical).

(2) Administration, and Sampling of Blood and Plasma

Using five-year-old male crab-eating macaques (GFD), with the number of monkeys in one group being 2 monkeys, the compound of Example 1 and the active form thereof were administered into a radial cutaneous vein in a dose of 0.4 mg/kg. Blood was collected from the radial cutaneous vein 0.25, 0.5, 1, 2, 4, and 6 hours after administration using a heparin-treated syringe. The blood samples were put into sampling tubes and stored under ice cooling, they were then centrifuged for 5 minutes at 10,500 x g and at 4°C. The plasma thus obtained was accurately divided into 5 µL amounts and stored at -20°C until they were analyzed.

(3) Plasma Concentration Measurement Method

The plasma concentrations of the compound of Example 1 and the active form thereof were measured using a liquid chromatography mass spectrometer (LC-MS), and quantified by internal standard method. Imipramine hydrochloride (Sigma) was dissolved in a mixed solution of acetonitrile and methanol (1:1) so that the concentration would be 0.5 µmol / L, to prepare an internal standard substance solution (IS solution). The plasma was melted, 200 µL of IS solution was then added thereto and mixed, and this was centrifuged (deproteinated) for 10 minutes at 1500 x g and at 4°C, the supernatant was then analyzed by LC-MS (Waters). In the

chromatogram thus obtained, the surface area of the peaks for the compound of Example 1 and the active form compound, and of the peak corresponding to the internal standard substance, were analyzed by MassLynx (Waters), and the concentration of the compound included in the plasma was calculated by internal standard method.

The plasma concentration of the active form after administration of the compound of Example 1 was measured by the method described in section 4. As a result, as shown in Figure 16, the plasma concentration of the active form immediately after administration of the compound of Example 1 rose quickly, exhibiting a plasma concentration change similar to that at the time of active form administration. The plasma concentration of the compound of Example 1 was under the detection limit. The above suggests that the compound of Example 1 is quickly converted into the active form inside the bodies of the monkeys.

The present application is based on an United States Provisional Patent Application No. 61/017,053 filed with the United States Patent and Trademark Office on December 27, 2007 and a Japanese Patent Application (Japanese Patent Application No. 2007-336977) filed with the Japan Patent Office on December 27, 2007, the contents of which are incorporated herein by reference.

Industrial Applicability

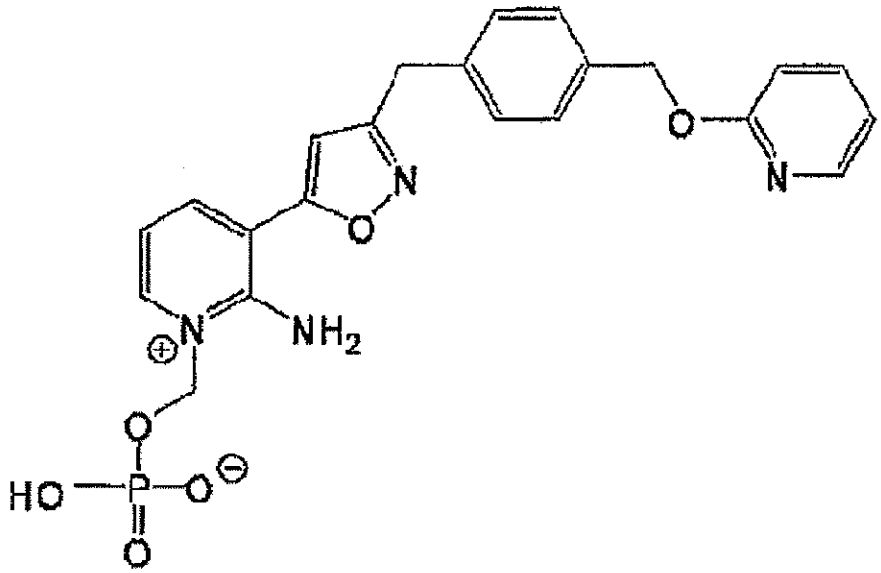
According to the present invention, the compound according to the present invention represented by Formula (I), (IA), or (IB): 1) acts against the onset, development and persistence of infections by inhibiting fungal GPI biosynthesis, thereby inhibiting expression of cell wall proteins and blocking cell wall assembly while preventing the fungus from attaching to cells so that the pathogen cannot

become pathogenic, and 2) is superior in terms of physical properties, and particularly its solubility in water, its stability in an aqueous solution, and its safety and in-vivo kinetics, making the above compounds extremely useful as a preventive or therapeutic agent for fungal infections.

5

CLAIMS

1. A compound of 2-amino-1-((phosphonooxy)methyl)-3-(3-((4-((2-pyridinyloxy)methyl)phenyl)methyl)-5-isoxazolyl)-pyridinium represented by:



or a salt thereof.

2. A pharmaceutical composition comprising the compound or the salt thereof according to claim 1.