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(54) Title: IMMUNOSUPPRESSIVE COMPOSITION

(57) Abstract: An immunosuppressive composition containing an IL-2 inhibitor for combination use with an adenosine deaminase inhibitor is provided. An adenosine deaminase inhibitor complements the defect of an IL-2 inhibitor. The combination use of the two compounds enables treatment and prevention of various diseases and conditions in need of immunosuppression.
DESCRIPTION

IMMUNOSUPPRESSIVE COMPOSITION

5 **Technical Field**

This invention relates to an immunosuppressive composition containing an interleukin 2 inhibitor and an adenosine deaminase inhibitor.

10 **Background Art**

Interleukin 2 (IL-2) is known to mediate immune system. Thus, IL-2 inhibitor is believed to have an immunosuppressive activity. "The effect of IL-2 inhibitor" may be the treatment and prevention of rejection by transplantation, graft-versus-host diseases by medulla ossium transplantation, autoimmune diseases, and so on.

Tricyclic macrolide compounds represented by FK506 inhibits synthesis of lymphokine, i.e., IL-2, dependent on the immunophilin-calcineurin complex. FK-506 binds to its specific immunophilin receptor (FKBP12) to form a complex with calcineurin that inhibits the ability of this enzyme to dephosphorylate the cytoplasmic subunit of the nuclear factor of activated T cells (NF-AT), thereby blocking its translocation to the nucleus and the transcription of IL-2 gene.

A tricyclic macrolide compound, FK506, has been widely used in clinical applications as an immunosuppressive agent. Its immunosuppressive effect is known to be based on the mechanism that inhibits intracellular signal transduction systems functioning upon activation of lymphocytes (Principle of Drug Development in Transplantation and Autoimmunity, Lieberman, R. and Mukherjee, A. eds., pp.107-116 (Chapter 8.1), R.G. Landes Company).

This drug inhibits allocytolytic T lymphocyte induction, but does not affect the ability of cytotoxic T lymphocytes to lyse targets (Yoshimura, N. et al., Transplantation 47, 351-356 (1989)). In other words, once the T lymphocytes are activated,
FK506 is not able to inhibit their functions.

Adenosine (Ado) is an endogenous purine nucleotide released by cells as part of the normal metabolic machinery. It shows the biological activities by binding to its receptors anchored in the cell membrane. For example, extracellular Ado reportedly shows immunosuppressive effects by sustained increase in intracellular cAMP through Ado receptors (Koshiba, M. et al., J. Biol. Chem. 272, 25881-25889 (1997) and Huang, S. et al., Blood 90, P.1600-1610 (1997)).

Adenosine deaminase (ADA) catalyzes an essentially irreversible deamination of extracellular Ado on the T-cell surface (Morimoto, C. et al., Immunological Review 161, 55-70 (1998)). Thus, ADA inhibitors would increase the level of the biological activities of Ado.


Disclosure of the Invention

An objective of this invention is to provide a pharmaceutical composition containing an IL-2 inhibitor with improved immunosuppressive effects.

FK506 has a potent immunosuppressive activity, but does not inhibit the action of activated cytotoxic T lymphocytes. The fact that Ado shows immunosuppressive effects even against cytotoxic T lymphocytes attracted the present inventors' attention. The inventors studied the effect of FK506 in combination with an ADA inhibitor on rat cardiac allograft and found that the combined use of the two drugs significantly prolonged rat cardiac allograft survival.

Specifically, this invention relates to a product
comprising an IL-2 inhibitor and an adenosine deaminase inhibitor for simultaneous, separate or sequential combination use in suppressing an immune reaction.

This invention also provides use of an IL-2 inhibitor in combination with an adenosine deaminase inhibitor for manufacturing a medicament for simultaneous, separate or sequential use of suppressing the immune reaction, and a method for suppressing the immune reaction by administering an effective amount of an IL-2 inhibitor in combination with an effective amount of an adenosine deaminase inhibitor to a human or an animal.

Furthermore, this invention relates to a pharmaceutical composition comprising an IL-2 inhibitor and an adenosine deaminase inhibitor.

The IL-2 inhibitor used in the present invention should not be limited and be considered to mean any compound possessing IL-2 inhibitory activity. The particular example is a compound possessing an inhibitory activity on the production of IL-2. Another example is a compound that inhibits the transmission of IL-2 signal.

The present invention is useful to suppress immune reaction to prolong the survival period of the graft, to reduce the administration amount of IL-2 inhibitor, and/or to reduce undesirable side effects caused by an IL-2 inhibitor.

Preferable "IL-2 inhibitors" are, for example, the tricyclic macrolides shown in EP-0184162, WO89/05303, WO93/05058, WO96/31514, and so on, the disclosure of which is incorporated herein by reference. It is well known that those tricyclic macrolides have strong IL-2 inhibitory activity.

As a particular example of the tricyclic macrolides compounds, the compound of the following formula (I) can be exemplified.
wherein each of adjacent pairs of $R^1$ and $R^2$, $R^3$ and $R^4$, and $R^5$ and $R^6$ independently

(a) is two adjacent hydrogen atoms, but $R^2$ may also be an alkyl group or
(b) may form another bond formed between the carbon atoms to which they are attached;

$R^7$ is a hydrogen atom, a hydroxy group, a protected hydroxy group, or an alkoxy group, or an oxo group together with $R^1$;

$R^8$ and $R^9$ are independently a hydrogen atom or a hydroxy group;

$R^{10}$ is a hydrogen atom, an alkyl group, an alkyl group substituted by one or more hydroxy groups, an alkenyl group, an alkenyl group substituted by one or more hydroxy groups, or an alkyl group substituted by an oxo group;

$X$ is an oxo group, a hydrogen atom and a hydroxy group, a hydrogen atom and a hydrogen atom, or a group represented by the formula $-\text{CH}_2\text{O}-$;

$Y$ is an oxo group, a hydrogen atom and a hydroxy group, a hydrogen atom and a hydrogen atom, or a group represented by the formula $\text{N-NR}^{11}\text{R}^{12}$ or $\text{N-OR}^{13}$;

$R^{11}$ and $R^{12}$ are independently a hydrogen atom, an alkyl group, an aryl group or a tosyl group;

$R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22}$ and $R^{23}$ are independently
a hydrogen atom or an alkyl group;

R²⁴ is an optionally substituted ring system which may contain one or more heteroatoms;

n is an integer of 1 or 2; and

in addition to the above definitions, Y, R¹⁰ and R²³, together with the carbon atoms to which they are attached, may represent a saturated or unsaturated 5- or 6-membered nitrogen, sulfur and/or oxygen-containing heterocyclic ring optionally substituted by one or more groups selected from the group consisting of alkyl, hydroxy, alkoxy, benzyl, a group of the formula -CH₂Se(C₅H₅), and an alkyl group substituted by one or more hydroxy groups.

Preferable R²⁴ may be cyclo(C₅₋₇)alkyl group, and (a) to (c) below.

(a) a 3,4-di-oxo-cyclohexyl group;

(b) a 3-R²⁰-4-R²¹-cyclohexyl group, in which

R²⁰ is hydroxy, alkoxy, oxo, or -OCH₂OCH₂CH₂OCH₃, and

R²¹ is hydroxy, -OCN, alkoxy, heteroaryloxy which may be substituted by suitable substituents, -OCH₂OCH₂CH₂OCH₃, protected hydroxy, chloro, bromo, iodo, aminooxaloyloxy, azido, p-tolloyxthiocarbonyloxy, or R²⁵R²⁶CHCOO-, in which R²⁵ is optionally protected hydroxy or protected amino, and R²⁶ is hydrogen or methyl, or

R²⁰ and R²¹ together form an oxygen atom in an epoxide ring;

or

(c) cyclopentyl group substituted by methoxymethyl, optionally protected hydroxymethyl, acyloxymethyl (in which the acyl moiety optionally contains either a dimethylamino group which may be quaternized, or a carboxy group which may be esterified), one or more amino and/or hydroxy groups which may be protected, or aminooxaloyxymethyl. A preferred example is a 2-formyl-cyclopentyl group.

The definitions used in the above general formula (I) and the specific and preferred examples thereof are now explained and set forth in detail.

The term "lower" means, unless otherwise indicated, a group
having 1 to 6 carbon atoms.

Preferable examples of the "alkyl groups" and an alkyl moiety of the "alkoxy group" include a straight or branched chain aliphatic hydrocarbon residue, for example, a lower alkyl group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, neopentyl and hexyl.

Preferable examples of the "alkenyl groups" include a straight or branched chain aliphatic hydrocarbon residue having one double-bond, for example, a lower alkenyl group such as vinyl, propenyl (e.g., allyl), butenyl, methylpropenyl, pentenyl and hexenyl.

Preferable examples of the "aryl groups" include phenyl, tolyl, xylyl, cumenyl, mesityl and naphthyl.

Preferable protective groups in the "protected hydroxy groups" and the "protected amino" are 1-(lower alkylthio)-(lower)alkyl group such as a lower alkylthiomethyl group (e.g., methylthiomethyl, ethylthiomethyl, propylthiomethyl, isopropylthiomethyl, butylthiomethyl, isobutylthiomethyl, hexylthiomethyl, etc.), more preferably C₁-C₄ alkylthiomethyl group, most preferably methylthiomethyl group; trisubstituted silyl group such as a tri(lower)alkylsilyl (e.g., trimethylsilyl, triethylsilyl, tributylsilyl, tert-butyldimethylsilyl, tritert-butyldisilyl, etc.) or lower alkyl-diarylsilyl (e.g., methylidiphenylsilyl, ethylidiphenylsilyl, propylidiphenylsilyl, tert-butyldiphenylsilyl, etc.), more preferably tri(C₁-C₄)alkylsilyl group and C₁-C₄ alkylidiphenylsilyl group, most preferably tert-butyldimethylsilyl group and tert-butyldiphenylsilyl group; and an acyl group such as an aliphatic, aromatic acyl group or an aliphatic acyl group substituted by an aromatic group, which are derived from a carboxylic acid, sulfonic acid or carbamic acid.

Examples of the aliphatic acyl groups include a lower alkanoyl group optionally having one or more suitable substituents such as carboxy, e.g., formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, carboxyacetyl, carboxypropionyl, carboxybutyryl,
carboxyhexanoyl, etc.; a cyclo(lower)alkoxy(lower)alkanoyl group optionally having one or more suitable substituents such as lower alkyl, e.g., cyclopropoxyacetyl, cyclobutyloxypropionyl, cycloheptoxybutyryl, menthloxyacetetyl, menthloxypropionyl, menthloxybutyryl, menthloxypropethyl, menthloxyhexanoyl, etc.; a camphorsulfonyl group; or a lower alkylcarbamoyle group having one or more suitable substituents such as carboxy or protected carboxy, for example, carboxy(lower)alkylcarbamoyle group (e.g., carboxymethylcarbamoyle, carboxyethylcarbamoyle, carboxypentylcarbamoyle, carboxybutylcarbamoyle, carboxypentylcarbamoyle, carboxyhexylcarbamoyle, etc.), tri-(lower)alkylsilyl(lower)alkoxy-carbonyl(lower)alkylcarbamoyle group (e.g., trimethylsilylmethoxycarbonyltrimethylsilylbutylcarbamoyle, trimethylsilylhexyloxycarbonylpropylcarbamoyle, triethysilylhexyloxycarbonylpropylcarbamoyle, tert-butyltrimethylsilylhexyloxycarbonylpropylcarbamoyle, trimethylsilylpropoxyalkylcarbamoyle, etc.) and so on.

Examples of the aromatic acyl groups include an aroyl group optionally having one or more suitable substituents such as nitro, e.g., benzoyl, toluyol, xyloyl, naphthoyl, nitrobenzoyl, dinitrobenzoyl, nitronaphthoyl, etc.; and an arenesulfonyl group optionally having one or more suitable substituents such as halogen, e.g., benzenesulfonyl, toluenesulfonyl, xylenesulfonyl, naphthalenesulfonyl, fluorobenzenesulfonyl, chlorobenzenesulfonyl, bromobenzenesulfonyl, iodosobenzenesulfonyl, etc.

Examples of the aliphatic acyl groups substituted by an aromatic group include ar(lower)alkanoyl group optionally having one or more suitable substituents such as lower alkoxy or trihalo(lower)alkyl, e.g., phenylacetyl, phenylpropionyl, phenylbutyryl, 2-trifluoromethyl-2-methoxy-2-phenylacetyl, 2-ethyl-2-trifluoromethyl-2-phenylacetyl, 2-trifluoromethyl-2-propoxy-2-phenylacetyl, etc.

More preferable acyl groups among the aforesaid acyl groups are C₁-C₄ alkanoyl group optionally having carboxy, cyclo(C₅-C₆)alkoxy(C₅-C₆)alkanoyl group having two (C₁-C₄) alkyls at the
cycloalkyl moiety, camphorsulfonyl group, carboxy(C₁-C₄)alkylcarbamoyl group, tri(C₁-C₄)alkylsilyl(C₁-C₄)-alkoxycarbonyl(C₁-C₄)alkylcarbamoyl group, benzoyl group optionally having one or two nitro groups, benzenesulfonyl group having halogen, or phenyl(C₁-C₄)alkanoyl group having C₁-C₄alkoxy and trihalo(C₁-C₄)alkyl group. Among these, the most preferable ones are acetyl, carboxypropionyl, menthylxoyacetyl, camphorsulfonyl, benzoyl, nitrobenzoyl, dinitrobenzoyl, iodobenzenesulfonyl and 2-trifluoromethyl-2-methoxy-2-phenylacetyl.

Preferable examples of the "5- or 6-membered nitrogen, sulfur and/or oxygen-containing heterocyclic ring" include a pyrrolyl group and a tetrahydrofuryl group.


Particular examples of the useful compounds are a compound designated as FK506, a compound designated as ascomycin, and the like products produced by microorganisms of the genus Streptomyces, such as Streptomyces tsukubaensis No. 9993 [deposited with National Institute of Bioscience and Human Technology Agency of Industrial Science and Technology (formerly Fermentation Research Institute Agency of Industrial Science and Technology), at 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki, Japan, since October 5, 1984, under the accession number FERM BP-927 in accordance with Budapest Treaty] or Streptomyces hygroscopicus subsp. yakushimaensis No. 7238 [deposited with National Institute of Bioscience and Human Technology Agency of
Industrial Science and Technology (formerly Fermentation Research Institute Agency of Industrial Science and Technology), at 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki, Japan, since January 12, 1985, under the accession number FERM BP-928 in accordance with Budapest Treaty] (EP-A-0184162).

FK506 (general name: tacrolimus, chemical name: 17-allyl-1,14-dihydroxy-12-[2-((4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxo-4-azatricyclo[22.3.1.0²,⁶]octacos-18-ene-2,3,10,16-tetraone), a preferable example of the tricyclocyclic macrolide compounds of the present invention, is represented by the following formula:

![Chemical structure of FK506]

The preferred examples of the tricyclic macrolide compounds (I) are the ones, wherein each of adjacent pairs of R¹ and R⁴ or R³ and R⁶ independently form another bond formed between the carbon atoms to which they are attached;

- each of R⁸ and R¹³ is independently a hydrogen atom;
- R⁹ is a hydroxy group;
- R¹⁰ is a methyl group, an ethyl group, a propyl group or an allyl group;
- X is a combination of a hydrogen atom and a hydrogen atom, or an oxo group;
- Y is an oxo group;
each of $R^{14}$, $R^{15}$, $R^{16}$, $R^{17}$, $R^{18}$, $R^{19}$, and $R^{22}$ is a methyl group; $R^{24}$ is a 3-$R^{20}$-4-$R^{21}$-cyclohexyl group, in which $R^{20}$ is hydroxy, alkoxy, oxo, or $-OCH_{2}OCH_{2}OCH_{3}$, and $R^{21}$ is hydroxy, $-OCN$, alkoxy, heteroaryloxy which may be substituted by suitable substituent(s), $-OCH_{2}OCH_{3}OCH_{3}$, protected hydroxy, chloro, bromo, iodo, aminooxaloxoxy, azido, p-tolyloxythiocarbonyloxy, or $R^{25}R^{26}CHCOO-$, in which $R^{25}$ is optionally protected hydroxy or protected amino, and $R^{26}$ is hydrogen or methyl, or $R^{20}$ and $R^{21}$ together form an oxygen atom in an epoxide ring; and

$n$ is an integer of 1 or 2.

The most preferable tricyclic macrolide compounds (I) are, in addition to FK506, ascomycin derivatives such as halogenated-ascomycin (e.g., 33-epi-chloro-33-desoxyascomycin), which is disclosed in EP 427,680, example 66a.

As the other preferable example of the tricyclic macrolide compounds, rapamycin [THE MERCK INDEX (12th edition), No. 8288] and its derivatives can be exemplified. A preferred example of the derivatives is an O-substituted derivative in which hydroxy in position 40 of formula A illustrated at page 1 of WO 95/16691, incorporated herein by reference, is replaced by $-OR_{1}$ in which $R_{1}$ is hydroxyalkyl, hydroalkoxyalkyl, acylaminoalkyl and aminoalkyl; for example 40-$O$-(2-hydroxy)ethyl-rapamycin, 40-$O$-(3-hydroxy)propyl-rapamycin, 40-$O$-[2-(2-hydroxy)ethoxy]-ethyl-rapamycin and 40-$O$-(2-acetaminoethyl)-rapamycin. These O-substituted derivatives may be produced by reacting rapamycin (or dihydro or deo xo-rapamycin) with an organic radical attached to a leaving group (for example RX where R is the organic radical which is desired as the O-substituent, such as an alkyl, allyl, or benzyl moiety, and X is a leaving group such as CCl$_{3}$C(NH)O or CF$_{3}$SO$_{2}$) under suitable reaction conditions. The conditions may be acidic or neutral conditions, for example, in the presence of an acid like trifluoromethanesulfonic acid, camphorsulfonic acid, p-toluensulfonic acid or their respective pyridinium or substituted pyridinium salts when X is CCl$_{3}$C(NH)O or in the presence of a base like pyridine, a substituted pyridine, diisopropylethylamine or pentamethylypiperidine when X is CF$_{3}$SO$_{2}$. 

The most preferable one is 40-O-(2-hydroxy)ethyl-rapamycin, which is disclosed in WO94/09010, the disclosure of which is incorporated herein by reference.

The tricyclic macrolide compounds (I), and rapamycin and its derivatives, may be in a form of their salt, which include conventional non-toxic and pharmaceutically acceptable salts such as the salt with inorganic or organic bases, specifically, an alkali metal salt such as sodium salt and potassium salt, an alkali earth metal salt such as calcium salt and magnesium salt, an ammonium salt and an amine salt such as triethylamine salt and N-benzyl-N-methylamine salt.

With respect to the IL-2 inhibitor of the present invention, particularly the tricyclic macrolide compounds, it is to be understood that there may be conformers and one or more stereoisomers such as optical and geometrical isomers due to asymmetric carbon atom(s) or double bond(s), and such conformers and isomers are also included within the scope of the present invention. Furthermore, the tricyclic macrolide compounds can be in the form of a solvate, which is included within the scope of the present invention. The solvate preferably include a hydrate and an ethanolate.

Further examples of the IL-2 inhibitor is cyclosporin and its derivatives such as cyclosporin A, B, C, D, E, F, G, etc, which are shown in THE MERCK INDEX (12th edition), No. 2821, U.S. Patents 4,117,118, 4,215,199, 4,288,431, and 4,388,307, Helv. Chim. Acta. 60, 1568(1977) and 65, 1655(1982), Transplant. Proc. 17, 1362(1985), and so on. Among these, the most preferable one is cyclosporin A. The disclosures of the above references are incorporated herein by reference.

The tricyclic macrolide compounds (I) and their pharmaceutically acceptable salts, and cyclosporin or its derivatives may be classified as "IL-2 production inhibitors", which show immunosuppressive activity by inhibiting the production of IL-2. Rapamycin or its derivatives may be classified as "IL-2 signal transmission inhibitors", which show immunosuppressive activity by inhibiting the transmission of
IL-2 signal.


A compound of the following formula (II), one of the representative imidazole compounds, can also be used as the ADA inhibitor of the present invention.

![Chemical Structure (II)](image)

wherein $R^{34}$ is hydrogen, hydroxy, protected hydroxy, or aryl optionally substituted with suitable substituent(s);

- $R^{35}$ is hydrogen or lower alkyl;
- $R^{33}$ is hydroxy or protected hydroxy;
- $R^{34}$ is cyano, (hydroxy)iminoamino(lower)alkyl, carboxy, protected carboxy, heterocyclic group optionally substituted with amino, or carbamoyl optionally substituted with suitable substituent(s); and

- $A$ is $-Q-$ or $-O-Q-$, wherein Q is single bond or lower alkylene,

provided that when $R^{32}$ is lower alkyl, then $R^{31}$ is hydroxy, protected hydroxy, or aryl optionally substituted with suitable substituent(s),
its prodrug, or their salt.

In preferable compounds of formula (II),

$R_1^{11}$ is hydrogen, hydroxy, protected hydroxy, or aryl optionally substituted with suitable substituent(s) selected from the group consisting of halo(lower)alkyl, halogen, hydroxy, protected carboxy, carbamoyl, lower alkylenedioxy, lower alkylthio, lower alkylsulfinyl, lower alkylsulfonyl, lower alkylo optionally substituted with hydroxy or protected carboxy, and lower alkoxy optionally substituted with aryl, halogen, amino or lower alkylamino;

$R_2^{12}$ is hydrogen or lower alkyl;

$R_3^{13}$ is hydroxy or protected hydroxy;

$R_4^{14}$ is cyano, (hydroxy)iminoamino(lower)alkyl, carboxy, protected carboxy, heterocyclic group optionally substituted with amino, or carbamoyl optionally substituted with suitable substituent(s) selected from the group consisting of amino, hydroxy, lower alkyl, lower alkylsulfonyl and aminoimino(lower)alkyl optionally substituted with hydroxy; and

- $A$ is $-Q-$ or $-O-Q-$, wherein Q is single bond or lower alkylene.

More preferably,

$R_1^{11}$ is aryl optionally substituted with suitable substituent(s) selected from the group consisting of halo(lower)alkyl, halogen, hydroxy, protected carboxy, carbamoyl, lower alkylenedioxy, lower alkylthio, lower alkylsulfinyl, lower alkylsulfonyl, lower alkylo optionally substituted with hydroxy or protected carboxy, and lower alkoxy optionally substituted with aryl, halogen, amino or lower alkylamino; and

$R_2^{12}$ is lower alkyl.

Still more preferably,

$R_3^{13}$ is hydroxy;

$R_4^{14}$ is carbamoyl; and

$A$ is $-Q-$ or $-O-Q-$, wherein Q is ethylene.

The compound (II), its prodrug, or their salt can be
prepared by the following processes. In the following formulae, compounds may be prodrugs or their salts.

**Process 1**

\[ \text{R}^{31}, \text{R}^{32}, \text{R}^{33}, \text{R}^{34}, \text{and } \text{A} \text{ are each as defined above, and } \text{X}^{1} \text{ is hydroxy or a leaving group, provided that } \text{R}^{33} \text{ is not hydroxy.} \]

In this process the compound (II) can be produced by reacting the compound (IV), where \( \text{X}^{1} \) is hydroxy, with alkanesulfonyl chloride (i.e., methanesulfonyl chloride, etc.) or arylsulfonyl chloride (i.e., toluenesulfonyl chloride, etc.) in the presence of a base such as triethylamine or pyridine in a solvent, which does not adversely affect the reaction, such as dichloromethane, chloroform, tetrahydrofuran, or diethyl ether from 0°C to room temperature for about 1 hour and reacting the resulting sulfonate with the compound (III) in the presence of a base such as sodium hydride, potassium tert-butoxide, or potassium carbonate in a solvent such as dimethylformamide (DMF) from room temperature to 100°C. Alternatively, the compound (III) can be reacted with the compound (IV) in the presence of a base such as sodium methoxide, potassium tert-butoxide, or sodium hydride to give the compound (II).

The compound (II) wherein \( \text{R}^{33} \) is hydroxy can be obtained by the following process:
Process 2

In the reaction formula $R^{31}$ and $R^{34}$ are as defined above and $R'$ is a hydroxy protective group.

In process 2, the compound (II-1) can be produced by reducing the compound (V) using a reducing agent such as sodium borohydride in a solvent such as methanol, ethanol, tetrahydrofuran, or water at 0°C to reflux temperature for 30 minutes to 72 hours.

When the compound (II) contains a protected hydroxy group, the protected hydroxy group can be converted to a hydroxy group by a known method, for example, by reacting the compound with a deprotecting agent such as palladium hydroxide on carbon/cyclohexane, iodotrimethylsilane or tetrabutylammonium fluoride in a solvent, which does not adversely affect the reaction, such as ethanol, chloroform or tetrahydrofuran. The reaction temperature is not critical and the reaction is usually carried out under cooling or warming.

The compound (II) where $R^{34}$ is (hydroxy)iminoamino(lower)alkyl, heterocyclic group or substituted carbamoyl can be prepared from the compound (II) where $R^{34}$ is cyano or protected carboxy by reacting the latter with the compound corresponding to $R^{34}$ of the former with or without a condensing agent such as sodium methoxide at room temperature to 120°C for 2 to 72 hours.

The starting compound (V) can be prepared by the following reaction.
In the reaction formula $R^{31}$, $R^{34}$, $R'$, $X^1$, and $A$ are as defined above.

This reaction can be performed in the same manner as in Process 1.

Process 3

The compound (II) wherein $R^{33}$ is not hydroxy can be obtained by reacting a compound of formula (VI)

$$R^{31}-OH \quad \text{(VI)}$$

wherein $R^{31}$ is as defined above, with a compound of formula (VII)

$$R^{34} \quad \text{(VII)}$$

wherein $R^{32}$, $R^{31}$, $R^{34}$, $X^1$, and $Q$ are as defined above, provided that $R^{33}$ is not hydroxy.

The reaction may be carried out in a manner such as the Mitsunobu reaction or the modification thereof. This reaction can be preferably carried out in the presence of di(lower)alkyl azodicarboxylate (e.g., diethyl azodicarboxylate, etc.) and trialkyl or triarylphosphines (e.g., triphenylphosphine, etc.) in a solvent, which does not adversely affect the reaction, such as tetrahydrofuran, diethyl ether, or the like. The reaction temperature is not critical and the reaction is usually carried
out under cooling to warming.

This reaction may also be carried out in a similar manner to Process 1.

In the following, suitable examples of the definitions of the formula (II) to (VII) to be included within the scope of the invention are explained in detail.

The term "lower" means a group having 1 to 6 carbon atom(s), unless otherwise provided.

Suitable "lower alkyl" and lower alkyl moiety of "halo(lower) alkyl," "lower alkoxy," "lower alkylamino," "lower alkylthio," "lower alkylsulfinyl," and "lower alkylsulfonyl" include a straight or branched one such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl, or the like, with methyl being preferred.

Suitable "lower alkylamino" includes "mono(lower)alkylamino," "di(lower)alkylamino," such as methylamino, dimethylamino, ethylamino, diethylamino, or the like.

Suitable "lower alkylene" may be straight or branched one having 1 to 8 carbon atom(s), such as methylene, ethylene, trimethylene, tetramethylene, pentamethylene, hexamethylene, or the like.

Suitable "lower alkylenedioxy" includes methylenedioxy, ethylenedioxy, methylethylenedioxy, or the like.

Suitable "protected hydroxy" includes lower alkoxy optionally substituted with aryl; acyloxy; or tri(lower)alkylsilyloxy (i.e., trimethylsilyloxy, tert-butyldimethylsilyloxy, etc.); or the like.

Suitable hydroxy protective groups in the protected hydroxy group include lower alkyl optionally substituted with aryl; acyl; tri(lower)alkylsilyl (i.e., trimethylsilyl, tert-butyldimethylsilyl, etc.); or the like.

Suitable "halogen" and halogen moiety of "halo(lower)alkyl" includes fluorine, chlorine, bromine, or iodine.

Suitable "aryl" and aryl moiety of "aroyl" include phenyl,
naphthyl, tolyl, xylyl, or the like, with phenyl and naphthyl being preferred.

Suitable "protected carboxy" includes lower alkoxy carbonyl (e.g., methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, 2,2,2-trichloroethoxycarbonyl, etc.), aryloxy-carbonyl (e.g., phenoxy carbonyl, 4-nitrophenoxy carbonyl, etc.), ar(lower)alkoxy carbonyl (e.g., benzyloxy carbonyl, 4-nitrobenzyloxycarbonyl, etc.), or the like.

Suitable carboxy protective groups in the protected carboxy group include lower alkyl (e.g., methyl, ethyl, or tert-butyl), halo(lower)alkyl (e.g., 2-iodomethyl or 2,2,2-trichloroethyl), ar(lower)alkyl (e.g., benzyl, trityl, 4-methoxy benzyl, 4-nitro benzyl, phenethyl, bis(methoxyphenyl)methyl, 3,4-dimethoxy benzyl or 4-hydroxy-3,5-di-tert-butyl benzyl), aryl (e.g., phenyl, naphthyl, tolyl, or xylyl), and the like. More suitable examples are lower alkyl such as methyl, ethyl, or tert-butyl, and ar(lower)alkyl such as benzyl.

Suitable "acyl" and acyl moiety of "acyloxy" include lower alkanoyl, aroyl, or the like.

Suitable "lower alkanoyl" includes formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, or the like.

Suitable "aroyl" may be benzoyl, naphthoyl, toluoyl, xyloyl, or the like.

In the definition, unless stated otherwise, "lower alkanoyl" and "aroyl" may be substituted with one or more substituent(s) selected from halogen, cyano, nitro, lower alkyl, and a combination thereof.

Suitable "acyloxy" includes acetyloxy, trifluoroacetyloxy, or the like.

Suitable "leaving group" may be halogen, acyloxy (e.g., acetyloxy, trifluoroacetyloxy, etc.), lower alkylsulfonyloxy (e.g., methanesulfonyloxy, etc.), triarylphosphinoxy (e.g., -O-P^+(C_6H_5)_3, etc.), or the like.
Suitable "substituent(s)" of "carbamoyl" include amino, hydroxy, lower alkyl, lower alkylsulfonyl, and aminomino(lower)alkyl optionally substituted with hydroxy, or the like.

Suitable "substituent(s)" of "aryl" include halo(lower)alkyl, halogen, hydroxy, protected carboxy, carbamoyl, lower alkylenedioxy, lower alkylthio, lower alkylsulfinyl, lower alkylsulfonfonyl, lower alkyl optionally substituted with hydroxy or protected carboxy, lower alkoxy optionally substituted with aryl, halogen, amino, or alkylamino, or the like.

Suitable "heterocyclic group" contains at least one hetero atom selected from nitrogen, sulfur, and oxygen atom and may be saturated or unsaturated, monocyclic or polycyclic heterocyclic group. Preferable examples of the heterocyclic group include N-containing heterocyclic group described below.

1. unsaturated 3 to 7-membered, preferably 5- or 6-membered heteromonocyclic group containing 1 to 4 nitrogen atoms, for example, pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, pyrimidinyl. pyrazinyl, pyridazinyl, triazolyl (e.g., 4H-1,2,4-triazolyl, 1H-1,2,3-triazolyl, 2H-1,2,3-triazolyl, etc.), tetrazolyl (e.g., 1H-tetrazolyl, 2H-tetrazolyl, etc.), etc.;

2. saturated 3 to 7-membered, preferably 5- or 6-membered heteromonocyclic group containing 1 to 4 nitrogen atoms (e.g., pyrrolidinyl, imidazolidinyl, piperidyl, piperazinyl, etc.);

3. unsaturated 3 to 7-membered, preferably 5- or 6-membered heteromonocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms, for example, oxazolyl, isoxazolyl, oxadiazolyl (e.g., 1,2,4-oxadiazolyl, 1,2,4-oxadiazolinyl, 1,3,4-oxadiazolyl, 1,2,5-oxadiazolyl, etc.), etc.;

4. saturated 3 to 7-membered, preferably 5- or 6-membered heteromonocyclic group containing 1 to 2 oxygen atoms and 1 to 3 nitrogen atoms (e.g., morpholinyl, etc.);

5. unsaturated 3 to 7-membered, preferably 5- or 6-membered heteromonocyclic group containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms, for example, thiazolyl, thiadiazolyl (e.g.,
1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, etc.), etc.;

(6) saturated 3 to 7-membered preferably 5- or 6-membered heteromonocyclic group containing 1 to 2 sulfur atoms and 1 to 3 nitrogen atoms (e.g., thiomorpholinyl, thiazolidinyl, etc.) and the like.

Among the above, more preferable heterocyclic group included in \( R^n \) is above-mentioned (1), in which the most preferable one is triazolyl or tetrazolyl.

Suitable salts of the compounds (II) of the present invention are pharmaceutically acceptable conventional nontoxic salts and can be an organic acid addition salt (e.g. formate, acetate, trifluoroacetate, maleate, tartarate, oxalate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.), an inorganic acid addition salt (e.g. hydrochloride, hydrobromide, sulfate, phosphate, etc.), a salt with an amino acid (e.g. aspartic acid salt, glutamic acid salt, etc.), or the like.

The "prodrug" means the derivatives of compounds of the present invention having a chemically or metabolically degradable group, which becomes pharmaceutically active after biotransformation.

The compounds (II) may contain one or more asymmetric centers and thus they can exist as enantiomers or diastereoisomers. Furthermore, certain compound (II) which contain alkenyl groups may exist as cis- or trans-isomers. In each instance, the invention includes both mixtures and separate individual isomers.

The compounds (II) may also exist in tautomeric forms and the invention includes both mixtures and separate individual tautomers.

The compounds (II) and its salt can be in a form of a solvate, which is included within the scope of the present invention. The solvate preferably include a hydrate and an ethanolate.

The compounds (II) of the present invention can be purified by any conventional purification methods employed for purifying organic compounds, such as recrystallization, column
chromatography, thin-layer chromatography, high-performance liquid chromatography and the like. The compounds can be identified by conventional methods such as NMR spectroscopy, mass spectroscopy, IR spectroscopy, elemental analysis, and measurement of melting point.

The IL-2 inhibitor and the ADA inhibitor contained in the product of this invention and the pharmaceutical composition of this invention is used in the form of a conventional pharmaceutical preparation in admixture with a conventional pharmaceutically acceptable carrier such as an organic or inorganic solid or liquid excipient which is suitable for oral, parenteral or external administration. The pharmaceutical composition may be compounded in a solid form such as granule, capsule, tablet, dragee, suppository, or ointment, or in a liquid form such as solution, suspension, or emulsion for injection, intravenous drip, ingestion, eye drop, etc. If needed, the above preparation may further include an auxiliary substance such as a stabilizer, a wetting agent, an emulsifier, buffer, or any other commonly used additives. It may further comprise additional ingredients, such as, mycophenolate mofetil (CellCept), steroids, Azathiopurine, and so on.

For practical use, the pharmaceutical composition may be put in a commercial package. Such a commercial package usually carries a written matter which states that the pharmaceutical composition can or should be used for suppressing the immune reaction.

The method for immunosuppression of the present invention can be effected by administering to a human or a mammal in need of immunosuppression the above-described IL-2 inhibitor and the ADA inhibitor simultaneously, separately, or sequentially through the same or different administration route.

In this method, the above preparation can be administered to the subject 1 to 4 times per day. Alternatively, these compounds are formulated together into a pharmaceutical preparation as described above, and the preparation is administered to the subject.
The IL-2 inhibitor is administered in an amount effective for immunosuppression, for example, in a unit dose of 0.05 mg/kg to 500 mg/kg, preferably 0.01 mg/kg to 100 mg/kg. The ADA inhibitor is administered in an amount effective to inhibit ADA and increase the efficacy of the IL-2 inhibitor, for example, in a unit dose of from 0.001 mg/kg to 500 mg/kg, preferably 0.01 mg/kg to 10 mg/kg. However, the above dosage may be increased or decreased according to age, body weight, or conditions of the patient or the administration route.

The subject which may be treated by the present invention is preferably a human. Animals to be treated include livestock mammals such as cows, horses, etc., domestic animals such as dogs, cats, rats, etc.

The pharmaceutical composition of the present invention is useful for increasing the effect of the treatment and/or prevention of the following diseases and conditions because of the pharmacologic activities possessed by the IL-2 inhibitors:

- rejection reactions by transplantation of organs or tissues such as the heart, kidney, liver, bone marrow, skin, cornea, lung, pancreas, small intestine, limb, muscle, nerve, intervertebral disc, trachea, myoblast, cartilage, etc.; graft-versus-host reactions following bone marrow transplantation;
- autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type I diabetes, etc.; and infections caused by pathogenic microorganisms (e.g. Aspergillus fumigatus, Fusarium oxysporum, Trichophyton asteroides, etc.);
- Inflammatory or hyperproliferative skin diseases or cutaneous manifestations of immunologically-mediated diseases (e.g. psoriasis, atopic dermatitis, contact dermatitis, eczematoid dermatitis, seborrheic dermatitis, lichen planus, pemphigus, bullous pemphigoid, epidermolysis bullosa, urticaria, angioedema, vasculitides, erythema, dermal eosinophilia, lupus erythematosus, acne, and alopecia areata); autoimmune diseases of the eye (e.g. keratoconjunctivitis, vernal conjunctivitis, uveitis associated with Behcet's disease, keratitis, herpetic
keratitis, conical keratitis, corneal epithelial dystrophy, keratoleukoma, ocular premphigus, Moore's ulcer, scleritis, Graves' ophthalmopathy, Vogt-Koyanagi-Harada syndrome, keratoconjunctivitis sicca (dry eye), phlyctenule, iridocyclitis, sarcoidosis, endocrine ophthalmopathy, etc.);

reversible obstructive airways diseases [asthma (e.g. bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma, and dust asthma), particularly chronic or invertebrate asthma (e.g. late asthma and airway hyper-responsiveness) bronchitis, etc.];

mucosal or vascular inflammations (e.g. gastric ulcer, ischemic or thrombotic vascular injury, ischemic bowel diseases, enteritis, necrotizing enterocolitis, intestinal damages associated with thermal burns, leukotriene B4-mediated diseases);

intestinal inflammations/allergies (e.g. coeliac diseases, proctitis, eosinophilic gastroenteritis, mastocytosis, Crohn's disease and ulcerative colitis);

food-related allergic diseases with symptomatic manifestation remote from the gastrointestinal tract (e.g. migrain, rhinitis and eczema);

renal diseases (e.g. intestitial nephritis, Goodpasture's syndrome, hemolytic uremic syndrome, and diabetic nephropathy);

nervous diseases (e.g. multiple myositis, Guillain-Barre syndrome, Meniere's disease, multiple neuritis, solitary neuritis, cerebral infarction, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and radiculopathy);

cerebral ischemic disease (e.g., head injury, hemorrhage in brain (e.g., subarachnoid hemorrhage, intracerebral hemorrhage), cerebral thrombosis, cerebral embolism, cardiac arrest, stroke, transient ischemic attack (TIA), hypertensive encephalopathy, cerebral infarction);

endocrine diseases (e.g. hyperthyroidism, and Basedow's disease);

hematic diseases (e.g. pure red cell aplasia, aplastic anemia, hypoplastic anemia, idiopathic thrombocytopenic purpura,
autoimmune hemolytic anemia, agranulocytosis, pernicious anemia, megaloblastic anemia, and anerythroplasia); bone diseases (e.g. osteoporosis); respiratory diseases (e.g. sarcoidosis, pulmonary fibrosis, and idiopathic interstitial pneumonia); skin diseases (e.g. dermatomyositis, leukoderma vulgaris, ichthyosis vulgaris, photosensitivity, and cutaneous T-cell lymphoma); circulatory diseases (e.g. arteriosclerosis, atherosclerosis, aortitis syndrome, polyarteritis nodosa, and myocardosis); collagen diseases (e.g. scleroderma, Wegener's granuloma, and Sjogren's syndrome); adiposis; eosinophilic fasciitis; periodontal diseases (e.g. damage to gingiva, periodontium, alveolar bone or substantia ossea dentis); nephrotic syndrome (e.g. glomerulonephritis); male pattern alopecia, alopecia senile; muscular dystrophy; pyoderma and Sezary syndrome; chromosome abnormality-associated diseases (e.g. Down's syndrome); Addison's disease; active oxygen-mediated diseases [e.g. organ injury (e.g. ischemic circulation disorders of organs (e.g. heart, liver, kidney, digestive tract, etc.) associated with preservation, transplantation, or ischemic diseases (e.g. thrombosis, cardial infarction, etc.)): intestinal diseases (e.g. endotoxin shock, pseudomembranous colitis, and drug- or radiation-induced colitis): renal diseases (e.g. ischemic acute renal insufficiency, chronic renal failure): pulmonary diseases (e.g. toxicosis caused by pulmonary oxygen or drugs (e.g. paracort, bleomycin, etc.), lung cancer, and pulmonary emphysema): ocular diseases (e.g. cataracta, iron-storage disease (siderosis bulbi), retinitis, pigmentosa, senile plaques, vitreous scarring,
corneal alkali burn): dermatitis (e.g. erythema multiforme, linear immunoglobulin A bullous dermatitis, cement dermatitis); and other diseases (e.g. gingivitis, periodontitis, sepsis, pancreatitis, and diseases caused by environmental pollution (e.g. air pollution), aging, carcinogen, metastasis of carcinoma, and hypobaropathy)];
diseases caused by histamine release or leukotriene C4 release;
restenosis of coronary artery following angioplasty and prevention of postsurgical adhesions;
autoimmune diseases and inflammatory conditions (e.g., primary mucosal edema, autoimmune atrophic gastritis, premature menopause, male sterility, juvenile diabetes mellitus, pemphigus vulgaris, pemphigoid, sympathetic ophthalmitis, lens-induced uveitis, idiopathic leukopenia, active chronic hepatitis, idiopathic cirrhosis, discoid lupus erythematosus, autoimmune orchitis, arthritis (e.g. arthritis deformans), or polychondritis);
Human Immunodeficiency Virus (HIV) infection, AIDS;
allergic conjunctivitis;
hypertrophic cicatrix and keloid due to trauma, burn, or surgery.
In addition, the IL-2 inhibitor have liver regenerating activity and/or activities of stimulating hypertrophy and hyperplasia of hepatocytes. Therefore, the product of the present invention is useful for increasing the effect of the therapy and/or prophylaxis of liver diseases [e.g. immunogenic diseases (e.g. chronic autoimmune liver diseases such as autoimmune hepatic diseases, primary biliary cirrhosis or sclerosing cholangitis), partial liver resection, acute liver necrosis (e.g. necrosis caused by toxins, viral hepatitis, shock, or anoxia), hepatitis B, non-A non-B hepatitis, hepatocirrhosis, and hepatic failure (e.g. fulminant hepatitis, late-onset hepatitis and "acute-on-chronic" liver failure (acute liver failure on chronic liver diseases))].

Furthermore, the present product is useful for increasing
the effect of the prevention and/or treatment of various
diseases because of the useful pharmacological activity of the
IL-2 inhibitors, such as augmenting activity of chemotherapeutic
effect, activity of cytomegalovirus infection, anti-
inflammatory activity, inhibiting activity against peptidyl-
prolyl isomerase or rotamase, antimalarial activity, antitumor
activity, and so on.

In addition to the above effect of the IL-2 inhibitors,
the product of the present invention is useful for treating or
preventing the diseases described below based on the
pharmacological activity of the ADA inhibitor.

Autoimmune diseases and inflammatory conditions, e.g.,
various pains collagen diseases, autoimmune diseases, various
immunity diseases, and the like in human beings or animals, and
more particularly for the treating and/or preventing
inflammation and pain in joint and muscle (e.g. rheumatoid
arthritis, rheumatoid spondylitis, osteoarthritis, gouty
arthritis, etc.), inflammatory skin condition (e.g. sunburn,
eczema, etc.), inflammatory eye condition (e.g. conjunctivitis,
etc.), lung disorder in which inflammation is involved (e.g.
asthma, bronchitis, pigeon fancier's disease, farmer's lung,
etc.), condition of the gastrointestinal tract associated with
inflammation (e.g. aphthous ulcer, Crohn's disease, atrophic
gastritis, ulcerative colitis, coeliac disease, regional ileitis,
irritable bowel syndrome, etc.), gingivitis, (inflammation, pain
and tumescence after operation or injury), pyrexia, pain and other
conditions associated with inflammation, systemic lupus
erythematosus, scleroderma, polymyositis, polychondritis,
periarteritis nodosa, ankylosing spondylitis, inflammatory
chronic renal condition (e.g. nephrotic syndrome,
glomerulonephritis, membranous nephritis, etc.), acute
nephritis, rheumatic fever, Sjogren's syndrome, Behcet disease,
thyroiditis, type I diabetes, dermatomyositis, chronic active
hepatitis, acute hepatitis, myasthenia gravis, idiopathic sprue,
Grave's disease, multiple sclerosis, primary biliary cirrhosis,
Reiter's syndrome, autoimmune hematological disorders (e.g.
hemolytic anemia, pure red cell anemia, idiopathic thrombocytopenia; aplastic anemia, etc.), myasthenia gravis, uveitis, contact dermatitis, psoriasis, Kawasaki disease, sarcoidosis, Wegner's granulomatosis, Hodgkin's disease, or the like;

Organ or tissue allo- or xeno-transplant rejection, e.g., kidney, liver, heart, lung, combined heart-lung, bone marrow, islet cells, pancreatic, skin, chromaffin or dopamine producing cells, small bowel, or corneal transplantation. Treating and/or preventing graft-versus-host disease, such as occurs following bone marrow transplantation;

Various leukemias, including virus induced, or various induced lymphomas; and

Diseases that arise from, or are aggravated by, insufficient blood flow through a particular organ or portion thereof, e.g., heart attacks or strokes, the microvascular disease of diabetes mellitus, atherosclerosis, or events resulting in a less prolonged loss of blood flow (e.g., angina pectoris, transient ischemic attacks, bowel ischemia, kidney ischemia, intermittent claudication of skeletal muscle, migraine headaches, Raynaud's phenomenon), or the like.

Any patents, patent applications, and publications cited herein are incorporated by reference.

25 **Brief Description of the Drawings**

Figure 1 compares rat cardiac allograft survival between a group to which FK506 alone was administered and a group to which FK506 and an ADA inhibitor were administered.

30 **Best Mode for Carrying out the Invention**

The following Examples are given for the purpose of illustrating the present invention in detail, but are not to be construed to limit the scope of the present invention.

35 **Example 1**

Protective effect of the ADA inhibitor combined with tricyclic
macrolide compound on rat cardiac allograft

**Test Compound**
1-[(2S,3R)-2-hydroxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxamide (hereinafter, described as "Compound A." See Reference Example 21 below.)

**Method**

Experiments were performed on male Lewis and ACI rats weighing 175-200 g. The ADA inhibitor used was Compound A. FK506 was used as a tricyclic macrolide compound. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and subjected to allogeneic (Lewis donor to ACI recipient) heterotopic intra-abdominal cardiac transplantation. Experimental groups were divided into single-drug group and combined-drug groups. Single-drug dose of FK506 was 0.32 mg/kg p.o. Combined-dose groups were FK506 (0.32 mg/kg p.o.) + ADA inhibitor (10 mg/kg s.c.). The grafted hearts were monitored by daily palpation and complete rejection was defined as the cessation of palpable contractile activity. The ADA inhibitor was dissolved in polyethylene glycol #400, and administered by daily subcutaneous injection in a volume of 2 ml/kg of body weight for 14 days.

**Results**

The ADA inhibitor was examined in combination with a potent immunosuppressive agent (FK506) to determine whether they could improve rat cardiac allograft survival. Graft survival is shown in Table 1 and Figure 1 in terms of number of surviving grafts and median survival time (MST).

Table 1: Protective effect of ADA inhibitor combined with FK506 on rat cardiac allograft

<table>
<thead>
<tr>
<th>Test compound(s)</th>
<th>MST (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FK506 (0.32 mg/kg)</td>
<td>11</td>
</tr>
<tr>
<td>Compound A (10 mg/kg) + FK506 (0.32 mg/kg)</td>
<td>20**</td>
</tr>
</tbody>
</table>
Significant vs. FK506 (0.32mg/kg) at **: p<0.001 by Mann-Whitney test.

Example 2

Protective effect of ADA inhibitor combined with cyclosporin A (CsA) on rat cardiac allograft

Test Compound

1-[(2S,3R)-2-hydroxy-5-(2,3-dichlorophenyl)-3-pentyl]-imidazole-4-carboxamide hydrochloride (hereinafter, described as "Compound B." See Reference Example 49 below.)

Method

Experiments were performed according to Example 1 except that Compound B and CsA were used instead of Compound A and FK506, respectively. Single-drug dose of CsA was 3.2mg/kg p.o. Combined-dose groups were CsA (3.2mg/kg p.o.) + Compound B (100mg/kg p.o.). Cyclosporin was dissolved in olive oil. Compound B was dissolved in 0.5% methylcellulose solution.

Result

Compound B was examined in combination with a potent immunosuppressive agent (CsA) to determine whether they could improve rat cardiac allograft survival. Graft survival is shown in Table 2.

Table 2: Protective effect of ADA inhibitor combined with cyclosporinA(CsA) on rat cardiac allograft.

<table>
<thead>
<tr>
<th>Test compound(s)</th>
<th>MST (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CsA (3.2 mg/kg)</td>
<td>14</td>
</tr>
<tr>
<td>Compound B (100mg/kg) + CsA (3.2 mg/kg)</td>
<td>17*</td>
</tr>
</tbody>
</table>

Significant vs. CsA (3.2mg/kg) at *: p<0.05 by Mann-Whitney test.
Reference Example

1. Production of ADA inhibitor

Preparation 1

A mixture of methyl 4-imidazolecarboxylate (5.0 g) and ammonium chloride (539 mg) in aqueous 28% NH₃ solution (75 ml) was heated at 100°C in a steel sealed tube for 5.5 hours. After cooling, the reaction mixture was concentrated in vacuo. The residue was stirred in a mixed solvent of acetone, ethanol and water (5:5:1, total 25 ml). The resulting precipitates were collected by filtration and washed with the same mixed solvent, and dried in vacuo to give 4-imidazolecarboxamide (4.63 g) as a white solid.

mp: 211-214°C
IR (KBr): 3500-2600, 1652 cm⁻¹
NMR (DMSO-d₆, δ): 7.06 (1H, br s), 7.34 (1H, br s), 7.58 (1H, s), 7.69 (1H, s)
MASS: 112 (M+H)⁺

Preparation 2

Triethylamine (583 mg) was added dropwise to a stirred mixture of ethyl (R)-2-hydroxy-4-phenylbutyrate (1.0 g) and methanesulfonyl chloride (660 mg) in dichloromethane (10 ml) at ice-bath temperature. After 40 minutes, the reaction mixture was partitioned between dichloromethane and water. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo to give ethyl (R)-2-methanesulfonyloxy-4-phenylbutyrate (1.37 g) as an oil. This material was used immediately without further purification. NaH (60% in mineral oil, 192 mg) was added to a solution of 4-imidazolecarboxamide (534 mg) in DMF (8 ml) at room temperature. The reaction mixture was stirred for 30 minutes. The methanesulfonate prepared above was added and the resulting mixture was stirred for 3 hours at 60°C.

The reaction mixture was cooled to 10°C in an ice bath, and the insoluble material was filtered and washed thoroughly with
methylene chloride. The filtrate and the washing were combined
and then washed with brine. The organic layer was dried over
sodium sulfate and concentrated in vacuo. The residue was
purified by silica gel (45 g) chromatography eluting with
chloroform/methanol (30:1) to give ethyl 2-(4-carbamoyl-1-
imidazolyl)-4-phenylbutyrate (556 mg).

IR (neat): 3500-2800, 1741, 1666 cm⁻¹

NMR (CDCl₃, δ): 1.26 (3H, t, J=7.1Hz), 2.3-2.68 (4H, 
m), 4.20 (3H, q, J=7.1Hz), 4.60 (1H, dd, J=9.8, 9.8Hz), 
5.44 (1H, br s), 6.96 (1H, br s), 7.08-7.35 (5H, m), 
7.46 (1H, s), 7.72 (1H, s)

MASS: 302 (M+H)⁺

**Preparation 3**

2-Hydroxyoctanoic acid (1.0 g) was stirred in 10% hydrogen
chloride methanol solution (20 ml) at room temperature. After
1.5 hours, the reaction mixture was evaporated under reduced
pressure. The residue was partitioned between ethyl acetate and
water. The organic layer was washed with aqueous NaHCO₃ solution
and dried over sodium sulfate. Evaporation of the solvent under
reduced pressure gave methyl 2-hydroxyoctanoate (0.684 g) as a
colorless oil.

IR (neat): 3463, 2952, 2927, 2859, 1735 cm⁻¹

NMR (CDCl₃, δ): 0.88 (3H, t, J=6.5Hz), 1.25-1.90 (10H, 
m), 2.70 (1H, br s), 3.79 (3H, s), 4.19 (1H, br)

MASS: 175 (M+H)⁺

**Preparation 4**

The following compounds were obtained according to a similar
manner to that of Preparation 2.

(1) Methyl α-(4-carbamoyl-1-imidazolyl)

IR (KBr): 3500-2800, 1752, 1675 cm⁻¹

NMR (CDCl₃, δ): 3.84 (3H, s), 5.48 (1H, br s), 5.93 (1H,
s), 7.06 (1H, br s), 7.24-7.46 (5H, m), 7.60 (1H, s), 7.67 (1H, s)

MASS: 260 (M+H)^+

5 (2) Methyl 2-(4-carbamoyl-1-imidazolyl)
mp: 63.5-65.5°C
IR (KBr): 3400-2800, 1753, 1671 cm⁻¹

NMR (CDCl₃, δ): 0.87 (3H, t, J=6.5Hz), 1.05-1.45 (6H, m), 1.90-2.20 (4H, m), 3.77 (3H, s), 4.71 (1H, dd, J=9.6, 5.6Hz), 5.52 (1H, s), 7.10 (1H, s), 7.59 (1H, s), 7.72 (1H, s)

MASS: 268 (M+H)^+

Preparation 5

NaH (60% in mineral oil, 60 mg) was added to a stirred solution of 4-imidazolecarboxamide (obtained in Preparation 1) (167 mg) in DMF (3.5 ml), and the reaction mixture was stirred for 1.5 hours at 55°C. Ethyl 2-bromovalerate (0.153 ml) was added to this mixture, and the reaction mixture was stirred for 3 hours at 55-60°C. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by silica gel (12 g) chromatography eluting with chloroform/methanol (25:1) to give ethyl 2-(4-carbamoyl-1-imidazolyl)valerate (150 mg).

mp: 95°C

IR (KBr): 3343, 3197, 2964, 1751, 1681 cm⁻¹

NMR (DMSO-d₆, δ): 0.86 (3H, t, J=7.2Hz), 1.13 (2H, m), 1.20 (3H, t, J=7.1Hz), 2.05 (2H, q, J=7.2Hz), 4.14 (2H, q, J=7.1Hz), 5.16 (1H, t, J=7.2Hz), 7.10 (1H, s), 7.30 (1H, s), 7.73 (1H, s), 7.78 (1H, s)

MASS: 240 (M+H)^+
Preparation 6

1-(2-Oxotetrahydrofuran-3-yl)imidazole-4-carboxamide was obtained from 4-imidazolecarboxamide obtained in Preparation 1 and α-bromo-γ-butyrolactone according to a similar manner to that of Preparation 5.

IR (KBr): 3700-3100, 1779, 1745, 1600 cm⁻¹
MASS: 196 (M+H)⁺

Preparation 7

Trifluoromethanesulfonic acid (1.13 g) was added to a stirred mixture of ethyl (S)-(−)-lactate (5.90 g) and benzyl 2,2,2-trichloroacetimidate (15.15 g) in cyclohexane (70 ml) and methylene chloride (35 ml) at room temperature under nitrogen atmosphere. After being stirred for 18 hours, the reaction mixture was filtered. The filtrate was diluted with cyclohexane, and then washed successively with saturated NaHCO₃ solution (100 ml) and H₂O (100 ml). The organic layer was dried over sodium sulfate and concentrated in vacuo. The residue was purified by silica gel (260 g) chromatography eluted with hexane/ethyl acetate (30:1) to give ethyl (S)-2-(benzyloxy)propionate (6.48 g).

IR (neat): 3100-2800, 1743, 1139 cm⁻¹

NMR (CDCl₃, δ): 1.26 (3H, t, J=7.0Hz), 1.44 (3H, d, J=6.8Hz), 4.05 (1H, q, J=6.8Hz), 4.22 (2H, q, J=7.0Hz), 4.40-4.75 (2H, m), 7.10-7.39 (5H, m)

MASS: 231 (M+Na)⁺

[α]D²⁸.⁵ = -76.0° (C=0.50, EtOH)

Preparation 8

A solution of 1.0M DIBAL (diisobutylaluminum hydride) in hexane (10 ml) was added dropwise to a stirred solution of ethyl (S)-2-(benzyloxy)propionate (obtained in Preparation 7) (2.08 g) in methylene chloride (20 ml) at -78°C (dry-ice/acetonitrile) for 5 minutes under nitrogen atmosphere. After 20 minutes, methanol
(1.6 ml) was added dropwise to the mixture at -78°C, and the resulting mixture was stirred at room temperature for 30 minutes. The mixture was filtered through a pad of Celite, and the solid on the filter was washed with methylene chloride. The combined filtrates were concentrated in vacuo. The obtained residue was purified by silica gel (35 g) chromatography eluted with hexane/ethyl acetate (30:1) to give (S)-2-(benzyloxy)propionaldehyde (810 mg).

IR (neat): 3100-2800, 1735, 1095 cm⁻¹

NMR (CDCl₃, δ): 1.33 (3H, d, J=6.9 Hz), 3.90 (1H, m), 4.60 (2H, m), 7.10-7.40 (5H, m), 9.67 (1H, s)

MASS: 163 (M-H)⁺

[α]²⁶.⁸ = -34.7° (C=0.50, EtOH)

Preparation 9

Trimethylsulfoxonium iodide (1.22 g) was added to a stirred suspension of sodium hydride (60% in mineral oil, 234 mg) in dimethylsulfoxide (12 ml) and dimethoxyethane (10 ml) at -3°C to -4°C under nitrogen atmosphere. After 10 minutes, a solution of (S)-2-(benzyloxy)propionaldehyde (obtained in Preparation 8)(800 mg) in dimethoxyethane (2 ml) was added dropwise to the mixture for a period of 5 minutes at the same temperature, and the resulting mixture was stirred for 30 minutes at room temperature. The mixture was poured into a cold saturated ammonium chloride solution (50 ml) and extracted with ethyl acetate (100 ml). The organic layer was washed with brine (50 ml), dried over magnesium sulfate and concentrated in vacuo. The residue was purified by silica gel (20 g) chromatography eluted with hexane/ethyl acetate (30:1) to give (3S)-3-benzyloxy-1,2-epoxybutane (507 mg).

IR (neat): 2981, 2927, 2865, 1241, 1103 cm⁻¹
NMR (CDCl₃, δ): 1.29 (3H, m), 2.40-3.55 (4H, m), 4.50-4.85 (2H, m), 7.10-7.40 (5H, m)

MASS: 201 (M+Na)⁺

5 Preparation 10

A solution of 2.0M benzylmagnesium chloride in tetrahydrofuran (2.38 ml) was added dropwise to a stirred mixture of lithium chloride (20.2 mg) and copper(II) chloride (32 mg) in tetrahydrofuran (10 ml) at -78°C (dry-ice/acetone) for a period of 10 minutes under nitrogen atmosphere. A solution of (3S)-3-benzyloxy-1,2-epoxybutane (obtained in Preparation 9) (425 mg) in tetrahydrofuran (10 ml) was added dropwise to this mixture at -78°C over 10 minutes. The resulting mixture was stirred at -78°C for 2.5 hours and then allowed to warm to room temperature, and stirred overnight. The reaction mixture was treated with saturated ammonium chloride solution (20 ml) at an ice-bath temperature, and then diluted with ethyl acetate (100 ml). The organic layer was washed with H₂O (50 ml) and brine (50 ml), dried over magnesium sulfate and concentrated in vacuo. The residue was purified by silica gel (20 g) chromatography eluted with hexane/ethyl acetate (10:1) to give (2S)-2-benzyloxy-5-phenylpentan-3-ol (620 mg).

IR (neat): 3444, 2931, 2865 cm⁻¹

NMR (CDCl₃, δ): 1.14-2.00 (3H, m), 1.60-1.85 (1H, m), 2.55-3.00 (3H, m), 3.30-3.85 (3H, m), 4.35-4.75 (2H, m), 7.05-7.40 (10H, m)

MASS: 293 (M+Na)⁺

Preparation 11

The following compounds were obtained according to a similar manner to that of Preparation 10.

(1) (2S)-2-benzyloxy-6-phenylhexan-3-ol
IR (neat): 3436, 2933, 2861 cm\(^{-1}\)

NMR (CDCl\(_3\), \(\delta\)): 1.05-1.20 (3H, m), 1.30-2.00 (4H, m), 2.00-2.80 (3H, m), 3.25-3.85 (2H, m), 4.35-4.75, (2H, m), 7.05-7.45 (10H, m)

MASS: 285 (M+Na)\(^+\)

(2) (2S)-2-benzylloxy-5-(1-naphthyl)

IR (neat): 3700-3100, 3100-2800, 1087, 1076 cm\(^{-1}\)

NMR (CDCl\(_3\), \(\delta\)): 1.10-1.20 (3H, m), 1.75-2.00 (2H, m), 2.15-2.75 (1H, m), 2.95-3.95 (4H, m), 4.40-4.75 (2H, m), 7.20-7.60 (9H, m), 7.65-7.20 (3H, m)

MASS: 307 (M+Na)\(^+\)

(3) (2S,3S)-2-(benzylloxy)-5-(2-methylphenyl)pentan-3-ol

NMR (CDCl\(_3\), \(\delta\)): 1.19 (3H, d, J=6 Hz), 1.6-1.8 (2H, m), 2.32 (3H, s), 2.64 (1H, d, J=3 Hz), 2.6-3.0 (2H, m), 3.3-3.6 (2H, m), 4.43 (1H, d, J=11 Hz), 4.67 (1H, d, J=11 Hz), 7.1-7.3 (9H, m)

MASS: 307 (M+Na)\(^+\)

(4) (2S,3S)-2-(benzylloxy)-5-(2-chlorophenyl)pentan-3-ol

NMR (CDCl\(_3\), \(\delta\)): 1.17 (3H, d, J=5 Hz), 1.6-1.9 (2H, m), 2.64 (1H, d, J=3 Hz), 2.7-3.1 (2H, m), 3.4-3.5 (2H, m), 4.44 (1H, d, J=12 Hz), 4.67 (1H, d, J=12 Hz), 7.1-7.4 (9H, m)

MASS: 327 (M+Na)\(^+\)

(5) (2S,3S)-2-(benzylloxy)-5-(2-methoxyphenyl)pentan-3-ol

NMR (CDCl\(_3\), \(\delta\)): 1.18 (3H, d, J=6 Hz), 1.6-1.9 (2H, m), 2.6-3.0 (3H, m), 3.4-3.5 (2H, m), 3.82 (3H, s), 4.44 (1H, d, J=12 Hz), 4.66 (1H, d, J=12 Hz), 6.8-7.0 (2H, m), 7.1-7.4 (7H, m)

MASS: 323 (M+Na)\(^+\)

(6) (2S,3S)-2-(benzylloxy)-5-(2-hexyloxyphenyl)pentan-3-ol

NMR (CDCl\(_3\), \(\delta\)): 0.90 (3H, t, J=6 Hz), 1.18 (3H, d, J=6 Hz), 1.2-1.6 (6H, m), 1.6-1.9 (4H, m), 2.66 (1H, d, J=3 Hz), 2.7-2.9 (2H, m), 3.4-3.5 (2H, m), 3.96 (2H, t, J=6 Hz), 4.66 (1H, d, J=12 Hz), 5.8-7.0 (2H, m), 7.1-7.4 (7H, m)
4.44 (1H, d, J=11Hz), 4.66 (1H, d, J=11Hz), 6.8-7.0 (2H, m), 7.1-7.3 (7H, m)

**MASS**: 393 (M+Na)^+

5) **(7) (2S,3S)-2-(benzyloxy)-5-(2,3-dichlorophenyl)pentan-3-ol**

NMR (CDCl₃, δ): 1.19 (3H, d, J=5Hz), 1.6-1.9 (2H, m), 2.65 (1H, d, J=3Hz), 2.7-3.1 (2H, m), 3.3-3.5 (2H, m), 4.43 (1H, d, J=11Hz), 4.67 (1H, d, J=11Hz), 7.0-7.5 (8H, m)

**MASS**: 361 (M+Na)^+

10) **(8) (2S,3S)-2-(benzyloxy)-5-(2-phenethyloxyphenyl)pentan-3-ol**

NMR (CDCl₃, δ): 1.14 (3H, d, J=6Hz), 1.6-1.8 (2H, m), 2.5-3.0 (3H, m), 3.10 (2H, t, J=7Hz), 3.3-3.5 (2H, m), 4.18 (2H, t, J=7Hz), 4.43 (1H, d, J=11Hz), 4.65 (1H, d, J=11Hz), 6.7-7.4 (14H, m)

15) **MASS**: 413 (M+Na)^+

10) **(9) (2S,3S)-2-(benzyloxy)-5-(2,3-dimethylphenyl)pentan-3-ol**

NMR (CDCl₃, δ): 1.19 (3H, d, J=6Hz), 1.6-1.8 (2H, m), 2.22 (3H, s), 2.28 (3H, s), 2.6-3.0 (3H, m), 3.3-3.6 (2H, m), 4.43 (1H, d, J=11Hz), 4.67 (1H, d, J=11Hz), 7.02 (3H, s), 7.2-7.4 (5H, m)

**MASS**: 321 (M+Na)^+

25) **(10) (2S,3S)-2-(benzyloxy)-5-[2,3-(methyleneoxy)phenyl]-pentan-3-ol**

NMR (CDCl₃, δ): 1.19 (3H, d, J=6Hz), 1.6-1.9 (2H, m), 2.6-2.9 (3H, m), 3.3-3.5 (2H, m), 4.43 (1H, d, J=12Hz), 4.67 (1H, d, J=12Hz), 5.92 (2H, s), 6.6-6.8 (3H, m), 7.33 (5H, s)

**MS**: 337 (M+Na)^+

**Preparation 12**

To a stirred solution of Pd(OAc)$_2$ (340 mg), nBu$_3$P (613 mg), and Et$_3$N (1.99 g) in DMF (30 ml) was added methyl 2-hydroxy-3-butenoate (1.76 g) followed by 1-iodonaphthalene (5.0 g), and
the reaction mixture was stirred at 100°C for 2.5 hours. The reaction mixture was poured into water (300 ml) and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, and evaporated in vacuo. The residue was purified by silica gel (130 g) column chromatography eluting with hexane/ethyl acetate (50:1) to give methyl 4-(1-naphthyl)-2-oxobutyrate (254 mg) as a red oil.

IR (neat): 3050, 2954, 1739, 1725 cm⁻¹
NMR (CDCl₃, δ): 3.25-3.55 (4H, m), 3.86 (3H, s), 7.25-8.10 (7H, m)

Preparation 13

NaBH₄ (22 mg) was added portionwise to an ice cooled solution of methyl 4-(1-naphthyl)-2-oxobutyrate (obtained in Preparation 12) (252.5 mg) in THF (5 ml)-H₂O (1 ml). After the addition was completed, the reaction mixture was stirred at ice-bath temperature for 30 minutes. Water (4 ml) was added, and the resulting mixture was stirred for several minutes and then extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate, and evaporated in vacuo. The residue was purified by silica gel (5 g) column chromatography eluting with hexane/ethyl acetate (10:1) to give methyl 2-hydroxy-4-(1-naphthyl)butyrate (84.4 mg) as a colorless oil.

IR (neat): 3700-3100, 3052, 2954, 1739, 1236, 1103 cm⁻¹
NMR (CDCl₃, δ): 2.03-2.31 (2H, m), 2.91 (1H, d, J=5.2Hz), 3.23 (2H, t, J=7.9Hz), 3.75 (3H, s), 4.29 (1H, m), 7.30-8.10 (7H, m)

MASS: 245 (M+H)⁺

Preparation 14

NaBH₄ (1.82 g) was added portionwise to an ice cooled solution of ethyl (R)-2-hydroxy-4-phenylbutyrate (2.0 g) in methanol (40 ml). After the addition was completed, the reaction mixture was stirred at room temperature for 45 minutes. Water (20 ml) was added, and the resulting mixture was stirred for several minutes and then evaporated under reduced pressure. The
residue was extracted with ethyl acetate. The extract was washed with brine and dried over magnesium sulfate. Evaporation of the solvent under reduced pressure gave (R)-4-phenylbutane-1,2-diol (1.63 g) as a colorless oil. This material was used for the next reaction without further purification.

Imidazole (1.96 g) was added to an ice cooled solution of the diol in DMF (20 ml) followed by tert-butyldimethylsilyl chloride (1.52 g). After 1 hour, the ice-bath was removed and then the mixture was stirred overnight at room temperature.

The reaction mixture was poured into water (200 ml) and extracted with ethyl acetate. The organic layer was washed with brine, dried over magnesium sulfate and evaporated in vacuo. The residue was purified by silica gel (50 g) column chromatography eluting with hexane/ethyl acetate (50:1) to give (R)-1-(tert-butyldimethylsilyloxy)-4-phenylbutan-2-ol (2.10 g) as a colorless oil.

IR (neat): 3800-3100, 2950, 2931, 2859, 1253, 1116, 1081 cm⁻¹

NMR (CDCl₃, δ): 0.52 (6H, s), 0.90 (9H, s), 1.60-1.85 (2H, m), 2.45 (1H, d, J=3.6Hz), 2.60-2.95 (2H, m), 3.35-3.75 (3H, m), 7.15-7.35 (5H, m)

MASS: 281 (M+H)⁺

**Preparation 15**

The following compounds were prepared by a similar procedure to that of Preparation 12.

(1) Methyl 4-(3-methylphenyl)-2-oxobutyrate

IR (neat): 2954, 2923, 1731, 1238, 1074 cm⁻¹

NMR (CDCl₃, δ): 2.33 (3H, s), 2.92 (2H, t, J=7.5Hz), 3.18 (2H, t, J=7.5Hz), 3.86 (3H, s), 6.90-7.25 (4H, m)

(2) Methyl 4-[3-(trifluoromethyl)phenyl]

IR (neat): 2958, 1739, 1728, 1241 cm⁻¹

NMR (CDCl₃, δ): 3.03 (2H, t, J=7.4Hz), 3.22 (2H, t, J=7.4Hz), 3.87 (3H, s), 7.35-7.55 (4H, m)
(3) Methyl 4-[3-(tert-butyldimethylsilyloxy)phenyl]-2-oxo-butyrate

IR (neat): 2954, 2935, 2857, 1731, 1594, 1244 cm⁻¹
NMR (CDCl₃, δ): 0.19 (6H, s), 0.98 (9H, s), 2.90 (2H, t, J=7.6Hz), 3.16 (2H, t, J=7.6Hz), 3.86 (3H, s), 6.65-6.85 (3H, m), 7.15 (1H, m)
MASS: 323 (M+H)⁺

Preparation 16
The following compounds were prepared by a similar procedure to that of Preparation 13.

(1) Methyl 2-hydroxy-4-(3-methylphenyl)butyrate

IR (neat): 3700-3100, 3016, 2954, 2859, 1733, 1234, 1099 cm⁻¹
NMR (CDCl₃, δ): 1.80-2.20 (2H, m), 2.33 (3H, s), 2.65-2.85 (3H, m), 3.76 (3H, s), 4.20 (1H, m), 6.95-7.25 (4H, m)
MASS: 209 (M+H)⁺

(2) Methyl 2-hydroxy-4-[3-(trifluoromethyl)phenyl]butyrate

IR (neat): 3700-3200, 3016, 2956, 1739, 1328, 1122, 703 cm⁻¹
NMR (CDCl₃, δ): 1.85-2.25 (2H, m), 2.70-2.95 (3H, m), 3.76 (3H, s), 4.18 (1H, m), 7.35-7.55 (4H, m)

(3) Methyl 2-hydroxy-4-[3-(tert-butyldimethylsilyloxy)-phenyl]butyrate

IR (neat): 3700-3100, 2954, 2857, 1739, 1595, 1479, 1444, 1273 cm⁻¹
NMR (CDCl₃, δ): 0.19 (6H, s), 0.98 (9H, s), 1.80-2.20 (2H, m), 2.65-2.80 (3H, m), 3.77 (3H, s), 4.18 (1H, m), 6.65-6.90 (3H, m), 7.13 (1H, m)
MASS: 325 (M+H)⁺
Preparation 17

The following compounds were prepared by a similar procedure to that of Preparation 2.

(1) Methyl 2-(4-carbamoyl-1-imidazolyl)-4-(1-naphthyl)-butyrate

IR (KBr): 3343, 3185, 1745, 1662 cm⁻¹

NMR (CDCl₃, δ): 2.40-3.25 (4H, m), 3.73 (3H, m), 4.71 (1H, m), 5.42 (1H, brs), 6.98 (1H, brs), 7.19 (1H, d, J=6.9Hz), 7.35-7.60 (4H, m), 7.74-7.95 (4H, m)

MASS: 338 (M+H)⁺

(2) Methyl 2-(4-carbamoyl-1-imidazolyl)-4-(3-methylphenyl)-butyrate

IR (neat): 3800-2800, 1745, 1658 cm⁻¹

NMR (CDCl₃, δ): 2.25-2.75 (7H, m), 3.75 (3H, s), 4.64 (1H, m), 5.43 (1H, br s), 6.85-7.25 (5H, m), 7.45 (1H, s), 7.71 (1H, s)

MASS: 302 (M+H)⁺

(3) Methyl 2-(4-carbamoyl-1-imidazolyl)-4-[3-(trifluoromethyl)phenyl]butyrate

IR (neat): 3700-2800, 1743, 1236 cm⁻¹

NMR (CDCl₃, δ): 2.25-2.80 (4H, m), 3.77 (3H, m), 4.65 (1H, m), 5.43 (1H, br s), 6.96 (1H, br s), 7.20-7.55 (5H, m), 7.73 (1H, s)

MASS: 356 (M+H)⁺

(4) Methyl 2-(4-carbamoyl-1-imidazolyl)-4-(3-hydroxyphenyl)-butyrate

IR (neat): 3700-2800, 1745, 1664, 1590, 1267, 1234 cm⁻¹

NMR (CDCl₃, δ): 2.20-2.80 (4H, m), 3.76 (3H, s), 4.65 (1H, m), 5.64 (1H, br s), 6.50-6.85 (3H, m), 6.90-7.30 (2H, m), 7.55 (1H, s), 7.73 (1H, s)

MASS: 304 (M+H)⁺
Preparation 18

To a stirred solution of Pd (OAc)$_2$ (40 mg, 0.18 mmol), nBu$_3$P (71 mg, 0.35 mmol), and Et$_3$N (232 mg, 2.29 mmol) in DMF (5 ml) was added 3-butene-1,2-diol (155 mg, 1.76 mmol) followed by 4-iodotoluene (500 mg, 2.29 mmol), and the reaction mixture was stirred at 100°C for 1.5 h. The reaction mixture was poured into water (50 ml) and extracted with ethyl acetate. The organic layer was washed with brine, dried (magnesium sulfate) and evaporated in vacuo. The residue was purified by silica gel (10 g) column chromatography eluting with toluene/ethyl acetate (50:1) to give 1-hydroxy-4-(p-tolyl)butan-2-one (230 mg, 73.4%) as a pale yellow solid.

To an ice cooled solution of 1-hydroxy-4-(p-tolyl)butan-2-one in DMF (5 ml) was added imidazole (264 mg, 3.88 mmol) followed by tert-butylidimethylsilyl chloride (234 mg, 1.55 mmol). After 30 minutes the ice-bath was removed and then the mixture was stirred overnight at room temperature. The reaction mixture was poured into water (50 ml) and extracted with ethyl acetate. The organic layer was washed with brine, dried (magnesium sulfate), and concentrated in vacuo. The residue was purified by silica gel (8 g) column chromatography eluting with hexane/ethyl acetate (50:1) to give 1-(tert-butylidimethylsilyloxy)-4-(4-methylphenyl)butan-2-one (350 mg, 67.9%) as a colorless oil.

IR (neat): 2933, 2857, 1726, 1255, 1105, 842 cm$^{-1}$
NMR (CDCl$_3$, $\delta$): 0.07 (6H, s), 0.91 (9H, s), 2.31 (3H, s), 2.75-2.95 (4H, m), 4.14 (2H, s), 7.08 (4H, s)

Preparation 19

1-(Tert-butylidimethylsilyloxy)-4-[3-(ethoxycarbonyl)-phenyl]butan-2-one (1.62 g, 42.7%) was prepared as a colorless oil by a similar procedure to that of Preparation 18 from ethyl 3-iodobenzoate and 3-butene-1,2-diol.

IR (neat): 2929, 2858, 1720, 1238, 1103 cm$^{-1}$
NMR (CDCl$_3$, $\delta$): 0.07 (6H, s), 0.91 (9H, s), 1.40 (3H, t, J = 7.1 Hz), 2.75-3.05 (4H, m), 4.15 (2H, s), 4.37 (2H, s)
q, J=7.1Hz), 7.30-7.45 (2H, m), 7.85-7.95 (2H, m)
MASS: 351 (M+H)'

Preparation 20

To a stirred solution of Pd (OAc)$_2$ (75 mg, 0.34 mmol), nBu$_3$P (136 mg, 0.67 mmol), and Et$_3$N (442 mg, 4.37 mmol) in DMF (10 ml) was added 3-butene-1,2-diol (296 mg, 3.36 mmol) followed by methyl 3-bromophenylacetate (1.0 g, 4.37 mmol), and the reaction mixture was stirred at 100°C for 5 h. The reaction mixture was poured into water (100 ml) and extracted with ethyl acetate. The organic layer was washed with brine, dried (magnesium sulfate) and evaporated in vacuo. The residue was purified by silica gel (25 g) column chromatography eluting with toluene/ethyl acetate (20:1) to give methyl 3-(4-hydroxy-3-oxobutyl)phenylacetate (193 mg, 24.4%) as an oil.

IR (neat): 3700-3100, 2950, 1732, 1261, 1159, 1069 cm$^{-1}$
NMR (CDCl$_3$, δ): 2.73 (2H, t, J=7.5Hz), 2.96 (2H, t, J=7.5Hz), 3.06 (1H, t, J=4.8Hz), 3.60 (2H, s), 3.70 (3H, s), 4.19 (2H, d, J=4.8Hz), 7.05-7.35 (4H, m)
MASS: 237 (M+H)'

Preparation 21

The following compounds were prepared by a similar procedure to that of Preparation 13.

(1) 1-(tert-Butyldimethylsilyloxy)-4-(4-methylphenyl)butan-2-ol

IR (neat): 3442, 2931, 2859, 1463, 1254, 1116 cm$^{-1}$
NMR (CDCl$_3$, δ): 0.06 (6H, s), 0.90 (9H, s), 1.60-1.85 (2H, m), 2.32 (3H, s), 2.44 (1H, d, J=3.5Hz), 2.55-2.90 (2H, m), 3.30-3.80 (3H, m), 7.10 (4H, s)
MASS: 295 (M+H)'

(2) 1-(tert-Butyldimethylsilyloxy)-4-[3-(ethoxycarbonyl)-phenyl]butan-2-ol

IR (neat): 3700-3100, 2933, 2860, 1718, 1279, 1110 cm$^{-1}$
NMR (CDCl₃, δ): 0.07 (6H, s), 0.90 (9H, s), 1.40 (3H, t, J=7.1Hz), 1.65-1.85 (2H, m), 2.46 (1H, d, J=3.4Hz), 2.65-3.00 (2H, m), 3.35-3.75 (3H, m), 4.37 (2H, q, J=7.1Hz), 7.30-7.45 (2H, m), 7.80-7.95 (2H, m)

MASS: 353 (M+H)⁺

(3) Methyl 3-[4-(tert-butyldimethylsilyloxy)-3-hydroxybutyl]-phenylacetate

IR (neat): 3800-3100, 2931, 2858, 1741, 1250, 1119 cm⁻¹
NMR (CDCl₃, δ): 0.07 (6H, s), 0.90 (9H, s), 1.60-1.80 (2H, m), 2.45 (1H, d, J=3.6Hz), 2.55-2.95 (2H, m), 3.35-3.75 (8H, m), 7.05-7.35 (4H, m)

MASS: 353 (M+H)⁺

**Preparation 22**

To an ice cooled solution of methyl 3-(4-hydroxy-3-oxo-butyl)phenylacetate (472 mg, 2.00mmol) in DMF (10 ml) was added imidazole (264 mg, 3.88 mmol) followed by tert-butyldimethylsilyl chloride (408 mg, 5.99 mmol). After 30 minutes the ice-bath was removed and then the mixture was stirred overnight at room temperature. The reaction mixture was poured into water (100ml) and extracted with ethyl acetate. The organic layer was washed with brine, dried (magnesium sulfate), and concentrated in vacuo. The residue was purified by silica gel (20 g) column chromatography eluting with hexane/ethyl acetate (10:1) to give methyl 3-[4-(tert-butyldimethylsilyloxy)-3-oxobutyl]phenylacetate (664 mg, 94.9%) as a colorless oil.

IR (neat): 2952, 2933, 2856, 1738, 1250, 1153, 1101 cm⁻¹
NMR (CDCl₃, δ): 0.07 (6H, s), 0.91 (9H, s), 2.75-3.00 (4H, m), 3.60 (2H, s), 3.69 (3H, s), 4.15 (2H, s), 7.05-7.30 (4H, m)

MASS: 351 (M+H)⁺

**Preparation 23**

A solution of ethyl 2-(4-carbamoyl-1-imidazolyl)-4-phenyl-butyrate (obtained in Preparation 2) in DMF (5 ml) was
added to an ice-cooled solution of POCl₃ (0.71 ml) in DMF (6 ml) under nitrogen atmosphere. After 1.5 h, the solvent was poured into water (50 ml) and the solution was neutralized with saturated NaHCO₃aq. The resulting mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel (16 g) column chromatography eluting with chloroform/methanol (100:1) to give ethyl 2-(4-cyano-1-imidazolyl)-4-phenylbutyrate (435 mg, 101.2%).

IR (neat): 3132, 2978, 2933, 2235, 1741, 1236, 1157 cm⁻¹
NMR (CDCl₃, δ): 1.28 (3H, t, J=7.1 Hz), 2.20-2.80 (4H, m), 4.23 (2H, q, J=7.1 Hz), 4.63 (1H, m), 7.00-7.40 (5H, m), 7.53 (1H, s), 7.58 (1H, s)
MASS: 284 (M+H)⁺

Preparation 24

1-(tert-Butyldimethylsilyloxy)-3-phenoxypropan-2-ol was prepared from 3-phenoxy-1,2-propanediol and tert-butyldimethylsilyl chloride by a similar procedure to that of Preparation 22.

IR (neat): 3700-3100, 2931, 2860, 1244, 1092 cm⁻¹
NMR (CDCl₃, δ): 0.08 (6H, s), 0.90 (9H, s), 2.54 (1H, d, J=5.4 Hz), 3.75-4.15 (5H, m), 6.85-7.05 (3H, m), 7.25-7.35 (2H, m)
MASS: 283 (M+H)⁺

Preparation 25

1-Naphthylmethylmagnesium chloride was prepared from magnesium turnings (2.88 g) and 1-(chloromethyl)naphthalene (6.98 g) in ether (80 ml) by the method of J. Am. Chem. Soc. (1943) 65, 295. A solution of lithium chloride (167 mg) and copper (II) chloride (266 mg) in THF (10 ml) was added dropwise to the ethereal solution of the Grignard reagent followed by addition of a solution of (2RS,3S)-3-(benzyloxy)-1,2-epoxybutane (3.52 g) in ether (30 ml) below -70 °C. The mixture was stirred at -78°C for 1 h, and then allowed to warm to room temperature and stirred
overnight. After cooling, the mixture was quenched with saturated aqueous ammonium chloride solution (100 ml). The insoluble material was filtered through Celite and the filter cake was washed with ether. The filtrate and washings were combined, and the organic layer was washed with water and brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give an oil. Flash chromatography (hexane:ethyl acetate = 9:1 → 4:1) gave (2S,3S)-2-benzyloxy-5-(1-naphthyl)pentan-3-ol (2.66 g, 42.0%) as the first eluate and (2S,3R)-2-benzyloxy-5-(1-naphthyl)pentan-3-ol (1.36 g, 21.5%) as the second eluate.

(2S,3S)-2-benzyloxy-5-(1-naphthyl)pentan-3-ol
IR (neat): 3558, 3458, 2870, 1078 cm⁻¹
NMR (CDCl₃, d): 1.17 (3H, d, J=6.0 Hz), 1.89 (2H, m), 2.70 (1H, d, J=4.0 Hz), 3.15 (1H, m), 3.30-3.60 (3H, m), 4.43 (1H, d, J=11.4 Hz), 4.67 (1H, d, J=11.4 Hz), 7.20-8.15 (12H, m)
[α]_D^26 -27.8° (c 0.5, EtOH)

(2S,3R)-2-benzyloxy-5-(1-naphthyl)pentan-3-ol
IR (neat): 3556, 3458, 2871, 1088 cm⁻¹
NMR (CDCl₃, d): 1.16 (3H, d, J=6.3 Hz), 1.86 (2H, m), 2.19 (1H, d, J=4.0 Hz), 3.11 (1H, m), 3.32-3.55 (2H, m), 3.87 (1H, m), 4.47 (1H, d, J=11.8 Hz), 4.60 (1H, d, J=11.8 Hz), 7.19-8.06 (12H, m)
[α]_D^26 +33.5° (c 0.5, EtOH)

Preparation 26
A solution of (S)-2-(benzylkoxy)propanal (Bull. Chem. Soc. Jpn. (1989) 62, 3038, 16.25 g) in ether (200 ml) was added to a suspension of zinc bromide (26.75 g) in ether (50 ml) below 6 °C and then an ethereal solution of 2-(1-naphthyl)ethyl magnesium bromide, prepared from 2-(1-naphthyl)ethyl bromide (46.55 g) and magnesium turnings (9.63 g) in ether (300 ml), was added below 8°C. The mixture was stirred at 4°C for 1 h and then THF (200 ml) was added. The final mixture
was stirred overnight at room temperature. After cooling, the mixture was quenched with saturated aqueous ammonium chloride solution (200 ml) and insoluble material was filtered. The filtrate was extracted with ethyl acetate and the extract was washed with brine, dried and concentrated in vacuo. Flash chromatography (hexane:ethyl acetate = 9:1) gave (2S,3S)-2-benzylxo-5-(1-naphthyl)pentan-3-ol (9.78 g, 30.8%) as an oil.

**Preparation 27**

To an ice-cooled solution of (2S,3S)-2-benzylxo-5-(1-naphthyl)pentan-3-ol (obtained in Preparation 26) (7.43 g) in dichloromethane (100 ml) was added methanesulfonyl chloride (2.15 ml) followed by triethylamine (3.88 ml). The mixture was stirred at 4 °C for 40 min. After being diluted with dichloromethane, the mixture was washed with water and brine, dried and concentrated in vacuo to give (2S,3S)-2-benzylxo-5-(1-naphthyl)-3-pentyl methanesulfonate (9.92 g, 107.4%) as an oil. The product was used directly in the next step without further purification.

IR (neat): 1344, 1173 cm⁻¹
NMR (CDCl₃, δ): 1.23 (3H, d, J=6.4Hz), 2.04-2.25 (2H, m), 2.98 (3H, s), 3.06-3.33 (2H, m), 3.82 (1H, m), 4.44 (1H, d, J=11.5Hz), 4.64 (1H, d, J=11.5Hz), 4.80 (1H, m), 7.25-8.02 (12H, m)

**Preparation 28**

The following compound was prepared by a similar procedure to that of Preparation 25.

(1) (2S,3S)-2-(benzylxo)-5-[2-(trifluoromethyl)phenyl]-pentan-3-ol
NMR (CDCl₃, δ): 1.19 (3H, d, J=6Hz), 1.6-1.9 (2H, m), 2.67 (1H,d,J=3Hz), 2.7-3.2 (2H,m), 3.3-3.6 (2H,m), 4.44 (1H,d,J=11Hz),4.67 (1H,d,J=11Hz), 7.2-7.7 (9H, m)

MASS: 361 (M+Na)⁺
(2) (2S,3S)-2-(tert-butyldimethylsilyloxy)-5-phenyl-pentan-3-ol

IR (neat): 3573, 3473, 2935, 1078 cm⁻¹

NMR (CDCl₃, δ): 0.09 (6H, s), 0.90 (9H, s), 1.13 (3H, d, J=6.2Hz), 1.66-1.77 (2H, m), 2.42 (1H, d, J=5.3Hz), 2.60-2.95 (2H, m), 3.30 (1H, m), 3.65 (1H, m), 7.14-7.32 (5H, m)

MS (ESI, m/z): 317(M+Na)⁺

[α]D²⁷ = -31.6° (c 0.5, EtOH)

(3) (2S,3S)-2-(tert-dimethylsilyloxy)-5-[2-(benzyloxy)-phenyl]pentan-3-ol

NMR (CDCl₃, δ): 0.05 (3H, s), 0.06 (3H, s), 0.88 (9H, s), 1.08 (3H, d, J=6Hz), 1.6-1.9 (2H, m), 2.40 (1H, d, J=5Hz), 2.6-3.0 (2H, m), 3.2-3.4 (1H, m), 3.6-3.7 (1H, m), 5.09 (2H, s), 6.8-7.5 (9H, m)

MS: 423 (M+Na)⁺

(4) (2S,3S)-2-(benzyloxy)-5-(2-naphthyl)pentan-3-ol

IR (neat): 3442, 1078 cm⁻¹

NMR (CDCl₃, δ): 1.18 (3H, d, J=6Hz), 1.7-2.0 (2H, m), 2.64 (1H, d, J=3Hz), 2.7-3.1 (2H, m), 3.3-3.6 (2H, m), 4.43 (1H, d, J=11Hz), 4.67 (1H, d, J=11Hz), 7.2-7.6 (8H, m), 7.64 (1H, s), 7.6-7.9 (3H, m)

MS: 343 (M+Na)⁺

(5) (2S,3S)-2-(benzyloxy)-6-(1-naphthyl)hexan-3-ol

IR (neat): 3437, 1081 cm⁻¹

NMR (CDCl₃, δ): 1.18 (3H, d, J=6Hz), 1.5-1.7 (2H, m), 1.7-2.2 (2H, m), 2.59 (1H, d, J=4Hz), 3.0-3.2 (2H, m), 3.3-3.6 (2H, m), 4.41 (1H, d, J=11Hz), 4.66 (1H, d, J=11Hz), 7.2-7.6 (9H, m), 7.70 (1H, d, J=8Hz), 7.7-8.1 (2H, m)

MS: 357 (M+Na)⁺
Preparation 29

The following compounds were prepared according to the procedure of Preparation 27.

(1) (2S,3R)-2-Benzylxyloxy-5-(1-naphthyl)-3-pentyl methanesulfonate

IR (neat): 1346, 1171 cm\(^{-1}\)

NMR (CDCl\(_3\), d): 1.23 (3H, d, J=6.4Hz), 1.80-2.25 (2H, m), 3.08 (3H, s), 3.10 (1H, m), 3.40 (1H, m), 3.64 (1H, m), 4.58 (2H, s), 5.04 (1H, m), 7.30-8.05 (12H, m)

(2) (2S,3S)-2-(tert-Butyldimethylsilyloxy)-5-phenyl-3-pentyl methanesulfonate

IR (neat): 2935, 1352, 1174 cm\(^{-1}\)

NMR (CDCl\(_3\), d): 0.03 (3H, s), 0.06 (3H, s), 0.86 (9H, s), 1.17 (3H, d, J=6.2Hz), 1.80-2.20 (2H, m), 2.60-2.90 (2H, m), 3.01 (3H, s), 4.10 (1H, m), 4.53 (1H, m), 7.10-7.40 (5H, m)

Reference Example 1

NaBH\(_4\) (491 mg) was added portionwise to an ice cooled solution of ethyl 2-(4-carbamoyl-1-imidazolyl)-4-phenylbutyrate (obtained in Preparation 2) (391 mg) in methanol (20 ml) under an nitrogen atmosphere. After the addition was completed, the reaction mixture was stirred at room temperature for 30 minutes. Water was added, and the resulting mixture was stirred for several minutes and then evaporated under reduced pressure. The residue was partitioned between chloroform and water. The organic layer was washed with brine and dried over sodium sulfate. Evaporation of the solvent under reduced pressure gave 1-(1-hydroxy-4-phenyl-2-butyl)imidazole-4-carboxamide (347 mg) as a white solid.

mp: 127.0-129.5°C

IR (KBr): 3500-2700, 1664 cm\(^{-1}\)

NMR (DMSO-d\(_6\), \(\delta\) ): 2.06 (2H, q, J=7.6Hz), 2.39 (2H, t,
Reference Example 2

The following compounds were obtained according to the similar manner to that of Reference Example 1.

(1) 1-(2-Hydroxy-1-phenylethyl)imidazole-4-carboxamide

mp: 147-149°C

IR (KBr): 3324, 3187, 1668 cm⁻¹

NMR (CDCl₃, δ): 4.26 (2H, d, J=5.4Hz), 5.35 (2H, br), 7.05 (1H, br), 7.10-7.50 (5H, m), 7.64 (1H, s), 7.75 (1H, s)

MASS: 232 (M+H)⁺

(2) 1-(1-Hydroxy-2-octyl)imidazole-4-carboxamide

mp: 97.5-100.5°C

IR (KBr): 3324, 3178, 2927, 2857, 1662 cm⁻¹

NMR (CDCl₃, δ): 0.83 (3H, t, J=6.5Hz), 0.90-1.35 (8H, m), 1.60-1.80 (2H, m), 3.60 (2H, t, J=5.6Hz), 4.09 (1H, qui, J=6.5Hz), 4.98 (1H, t, J=5.3Hz), 7.00 (1H, s), 7.22 (1H, s), 7.67 (2H, s)

MASS: 240 (M+H)⁺

(3) 1-(1-Hydroxy-2-pentyl)imidazole-4-carboxamide

mp: 160°C

IR (KBr): 3336, 3172, 1654 cm⁻¹

NMR (DMSO-d₆, δ): 0.84 (3H, t, J=7.2Hz), 1.10 (2H, m), 1.70 (2H, q, J=7.5Hz), 3.61 (2H, t, J=5.4Hz), 4.12 (1H, qui, J=6.5Hz), 4.99 (1H, t, J=5.4Hz), 7.01 (1H, s), 7.24 (1H, s), 7.69 (2H, s)

MASS: 198 (M+H)⁺
(4) 1-(1,4-Dihydroxy-2-butyl)imidazole-4-carboxamide

IR (KBr): 3700-3100, 1670 cm\(^{-1}\)

NMR (DMSO-\(d_6\), \(\delta\)): 1.86 (2H, m), 3.10-3.45 (2H, m), 3.62 (2H, t, \(J=5.5\)Hz), 4.29 (1H, m), 4.60 (1H, t, \(J=5.0\)Hz), 5.01 (1H, t, \(J=5.3\)Hz), 7.02 (1H, s), 7.25 (1H, s), 7.65 (1H, s), 7.68 (1H, s)

MASS: 200 (M+H)\(^+\)

(5) 1-[1-hydroxy-4-(1-naphthyl)-2-butyl]imidazole-4-carboxamide

mp: 138-140°C

IR (KBr): 3600-2800, 1660, 1598 cm\(^{-1}\) (M+H)\(^+\)

NMR (DMSO-\(d_6\), \(\delta\)): 2.17 (2H, t, \(J=7.7\)Hz), 2.70-3.10 (2H, m), 3.68 (2H, t, \(J=5.4\)Hz), 4.27 (1H, m), 5.04 (1H, t, \(J=5.3\)Hz), 7.06 (1H, brs), 7.20-7.60 (5H, m), 7.75-8.00 (5H, m)

MASS: 310 (M+H)\(^+\)

(6) 1-[1-Hydroxy-4-(3-methylphenyl)-2-butyl]imidazole-4-carboxamide

mp: 115.5-117.5°C

IR (KBr): 3325, 3195, 3110, 2935, 2854, 1662, 1604 cm\(^{-1}\)

NMR (DMSO-\(d_6\), \(\delta\)): 1.90-2.50 (7H, m), 3.62 (2H, m), 4.10 (1H, m), 5.01 (1H, br), 6.85-7.40 (6H, m), 7.70 (1H, s), 7.74 (1H, s)

MASS: 274 (M+H)\(^+\)

(7) 1-{1-Hydroxy-4-[3-(trifluoromethyl)phenyl]-2-butyl}-imidazole-4-carboxamide

mp: 103-106°C

IR (KBr): 3332, 3195, 3143, 1670, 1335 cm\(^{-1}\)

NMR (DMSO-\(d_6\), \(\delta\)): 2.11 (2H, q, \(J=8.0\)Hz), 2.35-2.75 (2H, m), 3.64 (2H, m), 4.13 (1H, m), 5.03 (1H, br s), 7.03 (1H, br s), 7.26 (1H, br s), 7.40-7.65 (4H, m), 7.71
(1H, s), 7.77 (1H, s)
MASS: 328 (M+H)⁺

(8) 1-[(1-Hydroxy-4-(3-hydroxyphenyl)-2-butyl]imidazole-4-carboxamide

IR (KBr): 3700-2800, 1658, 1600 cm⁻¹
NMR (DMSO-d₆, δ): 1.90-2.50 (4H, m), 3.62 (2H, m), 4.14 (1H, m), 5.09 (1H, t, J=5.3Hz), 6.45-6.65 (3H, m), 6.95-7.60 (4H, m), 7.74 (1H, s), 7.80 (1H, s), 9.37 (1H, s)
MASS: 276 (M+H)⁺

Reference Example 3
The following compounds were obtained according to a similar manner to that of Preparation 2.

(1) 1-[(2S)-2-(Benzyloxy)-5-phenyl-3-pentyl]imidazole-4-carboxamide

IR (neat): 3700-2800, 1673, 1658 cm⁻¹
NMR (CDCl₃, δ): 0.98-1.08 (3H, m), 2.10-2.75 (4H, m), 3.60-4.00 (2H, m), 4.05-4.70 (2H, m), 5.39 (1H, brs), 6.90-7.10 (3H, m), 7.15-7.45 (9H, m), 7.67 (1H, dd, J=6.1, 1.3Hz)
MASS: 364 (M+H)⁺, 386 (M+Na)⁺

(2) 1-[(2S)-2-(Benzyloxy)-6-phenyl-3-hexyl]imidazole-4-carboxamide

IR (neat): 3500-2800, 1666, 1589, 1236, 1095 cm⁻¹
NMR (CDCl₃, δ): 0.98-1.08 (3H, m), 1.30-2.20 (4H, m), 2.30-3.20 (2H, m), 3.50-4.10 (2H, m), 4.20-4.65 (2H, m), 5.37 (1H, br s), 6.95 (1H, brs), 7.00-7.80 (12H, m)
MASS: 378 (M+H)⁺

(3) 1-[(2S)-2-(Benzyloxy)-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxamide
IR (neat): 3700-2800, 1666, 1594, 1236, 1097 cm\(^{-1}\)

NMR (CDCl\(_3\), \(\delta\)): 1.04 (3H, d, J=6.2Hz), 2.10-2.60 (2H, m), 2.70-3.15 (2H, m), 3.50-4.10 (2H, m), 4.20-4.65 (2H, m), 5.41 (1H, brs), 7.01 (1H, brs), 7.10-7.60 (9H, m), 7.65-7.95 (5H, m)

**MASS: 414 (M+H)\(^+\)**

(4) 1-[1-(tert-Butyldimethylsilyloxy)-4-(4-methylphenyl)-2-buty]imidazole-4-carboxamide

NMR (CDCl\(_3\), \(\delta\)): -0.07 (3H, s), -0.05 (3H, s), 0.84 (9H, s), 1.95-2.25 (2H, m), 2.32 (3H, s), 2.35-2.80 (2H, m), 3.65-4.10 (3H, m), 5.40 (1H, br s), 6.90-7.15 (5H, m), 7.44 (1H, s), 7.64 (1H, s)

**MASS: 388 (M+H)\(^+\)**

(5) 1-{1-(tert-Butyldimethylsilyloxy)-4-[3-(ethoxycarbonyl)-phenyl]-2-buty]imidazole-4-carboxamide

IR (neat): 3700-3050, 2931, 2860, 1716, 1666, 1595, 1240, 1095 cm\(^{-1}\)

NMR (CDCl\(_3\), \(\delta\)): -0.06 (3H, s), -0.04 (3H, s), 0.83 (9H, s), 1.41 (3H, t, J=7.1Hz), 2.18 (2H, m), 2.40-2.80 (2H, m), 3.60-4.10 (3H, m), 3.65-4.10 (3H, m), 4.39 (2H, q, J=7.1Hz), 5.37 (1H, br s), 6.95 (1H, br s), 7.20-7.42 (2H, m), 7.45 (1H, s), 7.65 (1H, s), 7.75-7.95 (2H, m)

**MASS: 446 (M+H)\(^+\)**

(6) 1-{1-(tert-Butyldimethylsilyloxy)-4-{3-[(methoxy-carbonyl)methyl]phenyl]-2-buty]imidazole-4-carboxamide

IR (neat): 3800-3000, 2952, 2858, 1739, 1676, 1257, 1126 cm\(^{-1}\)

NMR (CDCl\(_3\), \(\delta\)): -0.07 (3H, s), -0.04 (3H, s), 0.83 (9H, s), 2.05-2.25 (2H, m), 2.30-2.75 (2H, m), 3.60 (2H, s), 3.70-3.85 (5H, m), 3.98 (1H, m), 5.39 (1H, br s), 6.90-7.35 (5H, m), 7.46 (1H, s), 7.65 (1H, s)
MASS: 446 (M+H)⁺

(7) 1-(1-Hydroxy-3-phenoxy-2-propyl)imidazole-4-carboxamide
mp: 147.5–149.5°C
IR (KBr): 3330, 3188, 1662, 1600, 1246 cm⁻¹
NMR (DMSO-d₆, δ): 3.80–4.30 (5H, m), 5.53 (1H, d, J=4.1Hz), 6.85–7.10 (4H, m), 7.20–7.40 (3H, m), 7.63 (2H, s)
MASS: 262 (M+H)⁺

(8) 1-[(2S,3R)-2-(benzyloxy)-5-(2-methylphenyl)-3-pentyl]-imidazole-4-carboxamide
NMR (CDCl₃, δ): 1.07 (3H, d, J=6Hz), 2.0–2.6 (4H, m), 2.18 (3H, s), 3.6–3.8 (1H, m), 3.9–4.1 (1H, m), 4.38 (1H, d, J=11Hz), 4.58 (1H, d, J=11Hz), 5.39 (1H, s), 6.9–7.4 (10H, m), 7.45 (1H, d, J=1Hz), 7.67 (1H, d, J=1Hz)
MASS: 378 (M+H)⁺

(9) 1-[(2S,3R)-2-(benzyloxy)-5-(2-chlorophenyl)-3-pentyl]-imidazole-4-carboxamide
NMR (CDCl₃, δ): 1.08 (3H, d, J=6Hz), 2.0–2.5 (2H, m), 2.5–2.7 (2H, m), 3.6–3.7 (1H, m), 3.9–4.1 (1H, m), 4.38 (1H, d, J=12Hz), 4.58 (1H, d, J=12Hz), 5.37 (1H, s), 6.9–7.4 (10H, m), 7.48 (1H, d, J=1Hz), 7.67 (1H, d, J=1Hz)
MASS: 420 (M+Na)⁺

(10) 1-[(2S,3R)-2-(benzyloxy)-5-(2-methoxyphenyl)-3-pentyl]-imidazole-4-carboxamide
NMR (CDCl₃, δ): 1.04 (3H, d, J=6Hz), 2.0–2.6 (4H, m), 3.6–3.7 (1H, m), 3.80 (3H, s), 3.9–4.1 (1H, m), 4.39 (1H, d, J=12Hz), 4.57 (1H, d, J=12Hz), 5.38 (1H, s), 6.8–7.4 (10H, m), 7.45 (1H, d, J=1Hz), 7.69 (1H, d, J=1Hz)
MASS: 394 (M+H)⁺
(11) 1-[(2S,3R)-2-(benzyloxy)-5-(2-hexyloxyphenyl)-3-pentyl]imidazole-4-carboxamide

NMR (CDCl₃, δ): 0.8-1.0 (3H,m), 1.05 (3H,d,J=6Hz), 1.2-1.5 (6H,m), 1.6-1.9 (2H,m), 2.0-2.6 (4H,m), 3.6-3.7 (1H,m), 3.8-4.0 (3H,m), 4.38 (1H,d,J=12Hz), 4.56 (1H,d,J=12Hz), 5.37 (1H,s), 6.8-7.4 (10H,m), 7.44 (1H,s), 7.67 (1H,s)

MASS: 464 (M+H)⁺

(12) 1-[(2S,3R)-2-(benzyloxy)-5-(2,3-dichlorophenyl)-3-pentyl]imidazole-4-carboxamide

NMR (CDCl₃, δ): 1.08 (3H,d,J=6Hz), 2.0-2.5 (2H,m), 2.5-2.7 (2H,m), 3.6-4.1 (2H,m), 4.38 (1H,d,J=12Hz), 4.59 (1H,d,J=12Hz), 5.45 (1H,s), 6.9-7.4 (9H,m), 7.48 (1H,d,J=1Hz), 7.67 (1H,d,J=1Hz)

MASS: 432 (M+H)⁺

(13) 1-[(2S,3R)-2-(benzyloxy)-5-(2-phenethyloxyphenyl)-3-pentyl]imidazole-4-carboxamide

NMR (CDCl₃, δ): 0.99 (3H,d,J=6Hz), 1.9-2.6 (4H,m), 3.06 (2H,t,J=7Hz), 3.5-3.6 (1H,m), 3.8-4.6 (5H,m), 5.34 (1H,s), 6.7-7.0 (3H,m), 7.1-7.4 (13H,m), 7.62 (1H,d,J=1Hz)

MASS: 484 (M+H)⁺

(14) 1-[(2S,3R)-2-(benzyloxy)-5-(2,3-dimethylphenyl)-3-pentyl]imidazole-4-carboxamide

NMR (CDCl₃, δ): 1.06 (3H,d,J=6Hz), 2.0-2.6 (4H,m), 2.09 (3H,s), 2.26 (3H,s), 3.6-3.7 (1H,m), 3.9-4.0 (1H,m), 4.38 (1H,d,J=12Hz), 4.58 (1H,d,J=12Hz), 5.39 (1H,s), 6.7-7.4 (9H,m), 7.46 (1H,d,J=1Hz), 7.67 (1H,d,J=1Hz)

MASS: 392 (M+H)⁺

(15) 1-[(2S,3R)-2-(benzyloxy)-5-[2-(trifluoromethyl)phenyl]-3-pentyl]imidazole-4-carboxamide

NMR (CDCl₃, δ): 1.09 (3H,d,J=6Hz), 2.0-2.8 (4H,m), 3.6-3.8
(1H, m), 3.9-4.1 (1H, m), 4.39 (1H, d, J=12Hz), 4.59 (1H, d, J=12Hz), 5.40 (1H, s), 6.9-7.7 (12H, m)

**MASS:** 432 (M+H)^+

(16) 1-((2S,3R)-2-(benzyloxy)-5-[2,3-(methylenedioxy)phenyl]-3-pentyl)imidazole-4-carboxamide

**NMR** (CDCl₃, δ): 1.06 (3H, d, J=6Hz), 2.0-2.6 (4H, m), 3.6-4.0 (2H, m), 4.38 (1H, d, J=12Hz), 4.58 (1H, d, J=12Hz), 5.38 (1H, s), 5.90 (2H, s), 6.4-6.8 (3H, m), 6.96 (1H, s), 7.2-7.4 (5H, m), 7.43 (1H, d, J=1Hz), 7.65 (1H, d, J=1Hz)

**MS:** 408 (M+H)^+

(17) 1-((2S,3R)-2-(tert-butyldimethylsilyloxy)-5-(2-benzyloxyphenyl)-3-pentyl)imidazole-4-carboxamide

**NMR** (CDCl₃, δ): -0.07 (3H, s), -0.02 (3H, s), 0.84 (9H, s), 0.93 (3H, d, J=6Hz), 1.8-2.8 (4H, m), 3.7-3.9 (2H, m), 5.07 (2H, s), 5.35 (1H, s), 6.8-7.4 (11H, m), 7.61 (1H, s)

**MS:** 494 (M+H)^+

(18) 1-((2S,3R)-2-(benzyloxy)-5-(2-naphthyl)-3-pentyl)imidazole-4-carboxamide

**IR (neat):** 1662 cm⁻¹

**NMR** (CDCl₃, δ): 1.06 (3H, d, J=6Hz), 2.1-2.9 (4H, m), 3.6-3.8 (1H, m), 3.8-4.1 (1H, m), 4.37 (1H, d, J=12Hz), 4.57 (1H, d, J=12Hz), 5.45 (1H, s), 7.0 (1H, s), 7.2-7.8 (14H, m)

**MS:** 414 (M+H)^+

(19) 1-((2S,3R)-2-(benzyloxy)-6-(1-naphthyl)-3-hexyl)imidazole-4-carboxamide was prepared from the compound obtained in Preparation 1 and the compound obtained in Preparation 28(5).

**IR (neat):** 1658 cm⁻¹

**NMR** (CDCl₃, δ): 1.04 (3H, d, J=6Hz), 1.5-2.3 (4H, m), 2.9-3.2 (2H, m), 3.5-3.7 (1H, m), 3.8-4.1 (1H, m),
4.37 (1H, d, J=12Hz), 4.57 (1H, d, J=12Hz), 5.51 (1H, s), 6.97 (1H, s) 7.1-8.0 (14H, m)

MS: 428 (M+H)^+

(20) Methyl 1-[(2S,3R)-2-benzyloxy-5-(1-naphthyl)-3-pentyl]-imidazole-4-carboxylate was prepared from (2S,3S)-2-benzyloxy-5-(1-naphthyl)pentan-3-ol (obtained in Preparation 26) and methyl imidazole-4-carboxylate.

IR (neat): 2945, 1726, 1672 cm⁻¹

NMR (CDCl₃, d): 1.06 (3H, d, J=6.2Hz), 2.15-2.60 (2H, m),
2.75-3.10 (2H, m), 3.65 (1H, m), 3.91 (3H, s), 3.96 (1H, m), 4.33 (1H, d, J=11.5Hz), 4.55 (1H, d, J=11.5Hz), 7.10-7.90 (14H, m)

MASS (APCI, m/z): 429 (M+H)^+

[α]D²⁷ +13.7° (c 0.65, EtOH)

Reference Example 4

Twenty percent palladium hydroxide on carbon (30 mg) was added to a stirred solution of 1-[(2S)-2-benzyloxy-5-phenyl-3-pentyl]imidazole-4-carboxamide (obtained in Reference Example 3(1))(107 mg) in cyclohexene (5 ml) and ethanol (12.5 ml). The resulting mixture was stirred at reflux temperature for 12 hours. After cooling to room temperature, the mixture was filtered through Celite, and the insoluble material on the filter was washed with ethanol. The filtrate and washing were combined and then concentrated in vacuo. The resulting residue was purified by silica gel (3 g) chromatography eluted with chloroform/methanol (50:1) to give 1-[(2S)-2-hydroxy-5-phenyl-3-pentyl]imidazole-4-carboxamide (69.1 mg).

IR (KBr): 3338, 2969, 1658 cm⁻¹

NMR (DMSO-d₆, δ): 0.84-0.93 (3H, m), 2.00-2.50 (4H, m),
3.70-4.00 (2H, m), 4.95-5.10 (1H, m), 6.95-7.40 (7H, m), 7.66 (1H, d, J=2.2Hz), 7.72 (1H, d, J=4.1Hz)

MASS: 274 (M+H)^+
Reference Example 5

The following compounds were obtained according to a similar manner to that of Reference Example 4.

(1) 1-[(2S)-2-hydroxy-6-phenyl-3-hexyl]imidazole-4-carboxamide

IR (KBr): 3700-2800, 1660, 1594 cm⁻¹
NMR (DMSO-d₆, δ): 0.80-1.00 (3H, m), 1.15-1.55 (2H, m), 1.60-2.05 (2H, m), 2.40-2.70 (2H, m), 3.70-4.10 (2H, m), 4.95-5.10 (1H, m), 6.90-7.35 (7H, m), 7.60-7.75 (2H, m)
MASS: 288 (M+H)⁺

(2) (2S)-2-hydroxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxamide

mp: 95-98°C
IR (KBr): 3336, 1658, 1594 cm⁻¹
NMR (DMSO-d₆, δ): 0.80-1.00 (3H, m), 2.05-2.45 (2H, m), 2.60-3.15 (2H, m), 3.70-4.20 (2H, m), 5.05-5.15 (1H, m), 7.07 (1H, brs), 7.20-7.60 (5H, m), 7.70-8.00 (5H, m)
MASS: 324 (M+H)⁺

Reference Example 6

Triethylamine (1.06 g) was added dropwise to a stirred mixture of (R)-1-(tert-butyldimethylsilyl-oxy)-4-phenylbutan-2-ol (obtained in Preparation 14) (2.10 g) and methanesulfonyl chloride (1.20 g) in dichloromethane (20 ml) at ice-bath temperature. After 1 hour, the reaction mixture was partitioned between dichloromethane and water. The organic layer was washed with brine and dried over MgSO₄, and concentrated in vacuo to give the methanesulfonate (2.74 g) as an oil. This material was used for the next reaction without further purification.

NaH (60% in mineral oil, 299 mg) was added to a solution of methyl 4-imidazolcarboxylate (942 mg) in DMF (20 ml) at room
temperature. The reaction mixture was stirred for 30 minutes. The methanesulfonate prepared above was added and the resulting mixture was stirred for 37 hours at 70°C.

The reaction mixture was cooled to 10°C in an ice bath, and the insoluble material was filtered and washed thoroughly with dichloromethane. The filtrate and the washing were combined and then washed with brine. The organic layer was dried over sodium sulfate and concentrated in vacuo. The residue was purified by silica gel (50 g) column chromatography eluting with toluene/ethyl acetate (20:1) to give methyl (S)-1-[1-(tert-butyldimethylsiloxyl)-4-phenyl-2-butyl]imidazole-4-carboxylate (1.52g).

IR (neat): 2950, 2933, 2857, 1725, 1675, 1189, 1122 cm⁻¹

NMR (CDCl₃, δ): -0.06 (3H, s), -0.05 (3H, s), 0.84 (9H, s), 2.10-2.25 (2H, m), 2.35-2.75 (2H, m), 3.70-3.80 (2H, m), 3.91 (3H, s), 4.00 (1H, m), 7.05-7.38 (5H, m), 7.51 (1H, s), 7.69 (1H, s)

MASS: 359 (M+H)⁺

Reference Example 7

A solution of 28% NaOMe in methanol (772 mg) was added to an ice cooled solution of aminoguanidine hydrochloride (332 mg) in methanol (5 ml). After 10 minutes, methyl (S)-1-[1-(tert-butyldimethylsiloxyl)-4-phenyl-2-butyl]-imidazole-4-carboxylate (obtained in Reference Example 6) (389 mg) in methanol (2 ml) was added to the mixture and the resulting mixture was stirred at reflux for 22 hours. After cooling, the insoluble material was removed and then the filtrate was evaporated. The residue was diluted with water and the solution was acidified to pH 4 with 6N HClaq. The resulting mixture was washed with CHCl₃. The aqueous layer was purified by HP-20 (50 cc) column chromatography eluting with water/2-propanol (9:1) and lyophilized to give (S)-2-[4-(5-amino-1,2,4-triazol-3-yl)-1-imidazoly1]-4-phenylbutan-1-ol (107 mg).

mp: 80°C (decompose)

IR (KBr): 3700-2700, 1641, 1602, 1238, 1058 cm⁻¹
NMR (DMSO-\(d_6\), \(\delta\)) : 1.95-2.60 (4H, m), 3.64 (2H, brs), 4.10 (1H, m), 5.02 (1H, brs), 5.40 (2H, br), 7.10-7.35 (6H, m), 7.55 (1H, s), 7.70 (1H, s)

MASS: 299 (M+H)

Reference Example 8

A solution of 28% NaOMe in methanol (583 mg) was added to an ice cooled solution of guanidine hydrochloride (307 mg) in DMF (5 ml). After 10 minutes, methyl (S)-1-[1-(tert-butyl-dimethylsilyloxy)-4-phenyl-2-butyl]imidazole-4-carboxylate (obtained in Reference Example 6) (250 mg) in DMF (2 ml) was added to the mixture and the resulting mixture was stirred at 100°C for 5 hours. After cooling, the reaction mixture was poured into water (30 ml) and the solution was washed with ethyl acetate. The aqueous layer was purified by HP-20 (40 cc) column chromatography eluting with water/2-propanol (9:1) and lyophilized to give (S)-1-[1-hydroxy-4-phenyl-2-butyl]imidazole-4-carbonylguanidine (55.4 mg)

mp: 111-113°C

IR (KBr): 3700-2700, 1639, 1592, 1517, 1405 \text{cm}^{-1}

NMR (DMSO-\(d_6\), \(\delta\)) 1.90-2.60 (4H, m), 3.62 (2H, d, J=5.0), 4.07 (1H, m), 5.02 (1H, brs), 7.00-8.00 (11H, m)

MASS: 302 (M+H)

Reference Example 9

To an ice cooled solution of 1-[1-(tert-butyldimethylsilyloxy)-4-(4-methylphenyl)-2-butyl]imidazole-4-carboxamide (obtained in Reference Example 3(4))(194 mg, 0.50 mmol) in THF (5 ml) was added dropwise 1.0M Bu₄NF in THF (1.0 ml). After the addition was completed, the reaction mixture was stirred at ice-bath temperature for 1h. 25% AcONH₄ (4 ml) was added, and the resulting mixture was stirred for several minutes and then extracted with chloroform. The organic layer was washed with brine, dried (sodium sulfate) and concentrated in vacuo. The residue was purified by silica gel (5g) column chromatography eluting with chloroform/methanol (20:1) to give 1-[1-
hydroxy-4-(4-methylphenyl)-2-butyl]imidazole-4-carboxamide (44.9mg, 32.9%) as a white solid.

mp: 138-141°C

IR (KBr): 3320, 3193, 2852, 1693, 1668, 1606 cm⁻¹

NMR (DMSO-d₆, δ): 1.90-2.15 (2H, m), 2.20-2.50 (5H, m), 3.61 (2H, t, J=5.4Hz), 4.08 (1H, m), 5.00 (1H, t, J=5.3Hz), 6.90-7.15 (5H, m), 7.27 (1H, br s), 7.69 (1H, s), 7.74 (1H, s)

MASS: 274 (M+H)⁺

Reference Example 10
The following compound was prepared by a similar procedure to that of Reference Example 9.

(1) 1-[(1-Hydroxy-4-[3-(ethoxycarbonyl)phenyl]-2-butyl]-imidazole-4-carboxamide

mp: 92-95°C

IR (KBr): 3322, 3193, 2954, 1720, 1662, 1604, 1278 cm⁻¹

NMR (DMSO-d₆, δ): 1.32 (3H, t, J=7.1Hz), 2.00-2.20 (2H, m), 2.35-2.55 (2H, m), 3.64 (2H, br), 4.31 (2H, q, J=7.1Hz), 5.03 (1H, br s), 7.03 (1H, br s), 7.26 (1H, br s), 7.40-7.50 (2H, m), 7.65-7.85 (4H, m)

MASS: 332 (M+H)⁺

(2) 1-[(1-Hydroxy-4-[3-(methoxycarbonylmethyl)phenyl]-2-butyl]imidazole-4-carboxamide

mp: 138.5-141.0°C

IR (KBr): 3600-3000, 2951, 1738, 1651, 1583, 1267 cm⁻¹

NMR (DMSO-d₆, δ): 1.90-2.20 (2H, m), 2.20-2.50 (2H, m), 3.50-3.75 (7H, m), 4.10 (1H, m), 5.01 (1H, t, J=5.3Hz), 6.90-7.35 (6H, m), 7.70 (1H, s), 7.75 (1H, s)

MASS: 332 (M+H)⁺

(3) 1-[(2S,3R)-2-hydroxy-5-(2-benzylxoyphenyl)-3-pentyl]-imidazole-4-carboxamide

NMR (CDCl₃, δ): 1.02 (3H, d, J=6Hz), 1.9-2.8 (5H, m),

3.8-4.0 (2H, m), 5.07 (2H, s), 5.38 (1H, s), 6.8-7.4 (1H, m), 7.66 (1H, d, J=1Hz)

MS: 380 (M+H)^+

[\alpha]_D^{27} = +16.2^\circ \ (c \ 1.0, \ EtOH)

Reference Example 11

Sodium methoxide (39 mg, 0.72 mmol) was added to a stirred solution of 1-{1-hydroxy-4-[3-(ethoxycarbonyl)phenyl]-2-butyl}-imidazole-4-carboxamide (obtained in Reference Example 10(1)) (60 mg, 0.18 mmol) in formamide (1.5ml), and the reaction mixture was stirred at 110°C for 3 h. After cooling, the reaction mixture was poured into water (5 ml). The residue was purified by HP-20 (16 cc) column chromatography eluting with water/2-propanol (9:1) and lyophilized to give 1-[4-(3-carbamoylphenyl)-1-hydroxy-2-butyl]imidazole-4-carboxamide (39.2 mg, 71.6%) as an amorphous solid.

IR (KBr): 3700-2800, 1660, 1592, 1402 cm\(^{-1}\)

NMR (DMSO-\(d_6\), \(\delta\)): 2.09 (2H, m), 2.30-2.65 (2H, m), 3.64 (2H, t, J=5.4Hz), 4.14 (1H, m), 5.04 (1H, t, J=5.3Hz), 7.05 (1H, br s), 7.20-7.50 (4H, m), 7.65-7.85 (4H, m), 7.93 (1H, br s)

MASS: 332 (M+H)^+

Reference Example 12

The following compound was prepared by a similar procedure to that of Reference Example 1.

(1) 1-{1-hydroxy-4-[3-(2-hydroxyethyl)phenyl]-2-butyl}-imidazole-4-carboxamide

IR (KBr): 3700-3000, 2927, 2861, 1658, 1595, 1414, 1055 cm\(^{-1}\)

NMR (DMSO-\(d_6\), \(\delta\)): 1.90-2.20 (2H, m), 2.20-2.50 (2H, m), 2.68 (2H, t, J=7.1Hz), 3.50-3.70 (4H, m), 4.10 (1H, m), 4.61 (1H, t, J=5.2Hz), 5.01 (1H, t, J=5.4Hz), 6.90-7.35 (6H, m), 7.70 (1H, s), 7.74 (1H, s)

MASS: 304 (M+H)^+
(2) 1-[1-Hydroxy-4-phenyl-2-butyl]imidazole-4-carbonitrile
mp: 111-115°C
IR (KBr): 3500-3000, 2943, 2867, 2237, 1078 cm⁻¹
NMR (DMSO-d₆, δ): 1.95-2.60 (4H, m), 3.55-3.70 (2H, m),
4.18 (1H, m), 5.06 (1H, t, J=5.4Hz), 7.05-7.35 (5H, m), 7.93 (1H, s), 8.25 (1H, s)
MASS: 242 (M+H)⁺

Reference Example 13
A mixture of methyl (S)-1-[1-(tert-butyldimethylsilyloxy)-4-phenyl-2-butyl]imidazole-4-carboxylate (obtained in Reference Example 6) (300 mg, 0.77 mmol) and hydrazine monohydrate (5 ml) in DMF (3 ml) was stirred at 100°C for 2 h.

After cooling, the reaction mixture was poured into water (10 ml) and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated in vacuo. The residue was purified by silica gel (10 g) column chromatography eluting with chloroform/methanol (100:1) to give (S)-1-[1-(tert-butyldimethylsilyloxy)-4-phenyl-2-butyl]imidazole-4-carboxyrazide (274 mg, 91.5%).
IR (neat): 3700-3000, 2933, 2858, 1646, 1568, 1466, 1252,
1120 cm⁻¹
NMR (CDCl₃, δ): -0.07 (3H, s), -0.05 (3H, s), 0.83 (9H, s), 2.05-2.75 (4H, m), 3.65-4.10 (5H, m), 7.00-7.40 (5H, m), 7.43 (1H, s), 7.63 (1H, s)
MASS: 389 (M+H)⁺

Reference Example 14
A powder of NaOMe (417 mg, 7.72 mmol) was added to an ice cooled solution of hydroxylamine hydrochloride (536 mg, 7.72 mmol) in methanol (5 ml). After 30 minutes, methyl (S)-1-[1-(tert-butyldimethylsilyloxy)-4-phenyl-2-butyl]imidazole-4-carboxylate (obtained in Reference Example 6) (389 mg, 1.0 mmol) in methanol (2 ml) was added to the mixture and the resulting
mixture was stirred at reflux for 3 day. After cooling, the insoluble material was removed and then the filtrate was evaporated. The residue was diluted with water and the solution was acidified to pH 4 with 1N HClaq. The resulting mixture was washed with CHCl3. The aqueous layer was purified by HP-20 (40 cc) column chromatography eluting with water/2-propanol (9:1) and lyophilized to give (S)-1-[1-hydroxy-4-phenyl-2-butyl]-imidazole-4-carboxyhydroxamic acid (92.5 mg, 43.5%) as an amorphous solid.

IR (neat): 3700-2700, 1645, 1566, 1238, 1141 cm⁻¹

NMR (DMSO-d₆, δ): 1.90-2.60 (4H, m), 3.63 (2H, m), 4.10 (1H, m), 5.01 (1H, br s), 7.05-7.40 (5H, m), 7.70 (1H, s), 7.76 (1H, s), 8.72 (1H, br s), 10.62 (1H, br s)

MASS: 276 (M+H)⁺

Reference Example 15

A powder of NaOMe (67.2 mg, 1.24 mmol) was added to a solution of hydroxylamine hydrochloride (86.4 mg, 1.24 mmol) in methanol (2 ml) at room temperature. After 30 minutes, 1-(1-hydroxy-4-phenyl-2-butyl)imidazole-4-carbonitrile (obtained in Reference Example 12(2)) (100 mg, 0.41 mmol) was added to the mixture and the resulting mixture was stirred at reflux for 2 h. After cooling, the insoluble material was removed and then the filtrate was evaporated. The residue was purified by silica gel (5 g) column chromatography eluting with chloroform/methanol (20:1) and concentrated in vacuo. The residue was triturated with isopropyl ether to give N-hydroxy-1-[1-hydroxy-4-phenyl-2-butyl]imidazole-4-carboximidamide (86.3 mg, 76.0%) as an amorphous solid.

IR (KBr): 3700-2800, 1649, 1604, 1496 cm⁻¹

NMR (DMSO-d₆, δ): 1.90-2.60 (4H, m), 3.62 (2H, t, J=4.9Hz), 4.09 (1H, m), 5.04 (1H, t, J=5.2Hz), 6.06 (2H, br s), 7.10-7.40 (5H, m), 7.58 (1H, s), 7.75 (1H, s), 9.41 (1H, br s)

MASS: 275 (M+H)⁺
Reference Example 16

A mixture of 1-(1-hydroxy-4-phenyl-2-butyl)imidazole-4-carbonitrile (obtained in Reference Example 12(2))(100 mg, 0.41 mmol), ammonium chloride (111 mg, 2.07 mmol) and sodium azide (135 mg, 2.07 mmol) in DMF (4 ml) was stirred at 100 °C for 8 h.

After cooling, the reaction mixture was poured into water (30 ml) and the solution was washed with CHCl₃. The aqueous layer was purified by HP-20 (16 cc) column chromatography eluting with water/2-propanol (9:1) and lyophilized to give 1-(1-hydroxy-4-phenyl-2-butyl)-4-(5-tetrazolyl)imidazole (63.3 mg, 53.8%) as an amorphous solid.

IR (KBr): 3700-2700, 1651, 1612, 1496, 1458, 1250 cm⁻¹
NMR (DMSO-d₆, δ): 1.95-2.60 (4H, m), 3.66 (2H, m), 4.09 (1H, m), 5.03 (1H, br), 7.05-7.35 (5H, m), 7.55 (1H, s), 7.68 (1H, s), 9.41 (1H, br s)

MASS: 285 (M+H)⁺

Reference Example 17

A suspension of imidazole-4-carboxamide (obtained in Preparation 1)(207 mg) in DMF (3 ml) was treated with sodium hydride (60% in mineral oil, 87 mg) at ice-bath temperature and the mixture was stirred at room temperature for 20 min. A solution of (2S,3S)-2-benzyl oxy-5-(1-naphthyl)-3-pentyl methanesulfonate (obtained in Preparation 27)(0.62 mg) in DMF (5 ml) was added and the mixture was stirred at 80 °C for 48 h. After cooling, the mixture was filtered to remove the insoluble material. The filtrate was poured into water and extracted with ethyl acetate. The extract was washed with water and brine, dried and concentrated in vacuo. Flash chromatography (dichloromethane:methanol = 50:1) gave 1-[(2S,3R)-2-benzyl oxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxamide (221 mg, 34.4%) as an oil.

IR (neat): 3458, 3332, 3184, 1666, 1593 cm⁻¹
NMR (CDCl₃, δ): 1.04 (3H, d, J=6.3Hz), 2.15-2.60 (2H, m), 2.80-3.10 (2H, m), 3.64 (1H, m), 3.98 (1H, m), 4.53 (1H, d, J=11.6Hz), 4.55 (1H, d, J=11.6Hz), 5.49 (1H,
bs), 7.00 (1H, bs), 7.15-7.90 (14H, m)
MASS (APCI, m/z): 414 (M+H)'
[α]_D^{27} +23.7° (c 0.5, EtOH)

5 Reference Example 18

The following compounds were obtained according to the procedure of Reference Example 17.

(1) 1-[(2S,3S)-2-benzyloxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxamide
IR (neat): 3460, 3330, 3182, 1668, 1593 cm⁻¹
NMR (CDCl₃, d): 1.03 (3H, d, J=6.2Hz), 2.15-2.55 (2H, m), 2.74-3.03 (2H, m), 3.66 (1H, m), 3.86 (1H, m), 4.26 (1H, d, J=11.6Hz), 4.56 (1H, d, J=11.6Hz), 5.52 (1H, bs), 7.02 (1H, bs), 7.12-7.50 (10H, m), 7.71-7.88 (4H, m)
MASS (APCI, m/z): 414 (M+H)'
[α]_D^{26} -21.1° (c 0.5, EtOH)

20 (2) 1-[(2S,3R)-2-(tert-butyl(dimethyl)silyloxy)-5-phenyl-3-pentyl]imidazole-4-carboxamide
IR (neat): 3465, 3332, 3188, 2935, 1672, 1599 cm⁻¹
NMR (CDCl₃, d): 0.19 (3H, s), 0.10 (3H, s), 0.88 (9H, s), 0.98 (3H, d, J=6.1Hz), 2.09 (1H, m), 2.26-2.45 (2H, m), 2.62 (1H, m), 3.77 (1H, m), 3.88 (1H, m), 5.47 (1H, bs), 6.98 (1H, bs), 7.06 (2H, d, J=6.4Hz), 7.20-7.33 (7H, m), 7.38 (1H, d, J=1.1Hz), 7.62 (1H, d, J=1.1Hz)
MS (APCI, m/z): 388 (M+H)'
[α]_D^{27} +29.3° (c 0.5, EtOH)

Reference Example 19

The following compound was prepared by a similar procedure to that of Reference Example 9.

30 (1) (S)-1-[1-hydroxy-4-phenyl-2-butyl]imidazole-4-
carbohydrazide

NMR (DMSO-d_6, δ): 2.00-2.55 (4H, m), 3.63 (2H, t, J=5.2Hz), 4.10 (1H, m), 4.33 (2H, br), 5.01 (1H, t, J=5.3Hz), 7.10-7.35 (5H, m), 7.70 (1H, s), 7.77 (1H, s), 8.97 (1H, br s)

MASS: 275 (M+H)^+

(2) 1-[(2S,3R)-2-hydroxy-5-phenyl-3-pentyl]imidazole-4-carboxamide

IR (KBr): 3336, 1658, 1593 cm^{-1}

NMR (DMSO-d_6, δ): 0.87 (3H, d, J=6.0Hz), 2.00-2.40 (4H, m), 3.75-3.95 (2H, m), 5.08 (1H, d, J=4.8Hz), 7.07 (1H, bs), 7.10-7.30 (6H, m), 7.72 (1H, s), 7.74 (1H, s)

MS (APCI, m/z): 274(M+H)^+

[α]_D^{26} +43.5° (c 0.4, EtOH)

Reference Example 20

A mixture of methyl 1-[(2S,3R)-2-benzyloxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxylate (obtained in Reference Example 3(20)) (160 mg) in ammonium hydroxide (10 ml) and DMF (5 ml) was heated at 100°C for 8 h in a sealed tube and then concentrated in vacuo. Flash chromatography (dichloromethane:methanol = 20:1) gave 1-[(2S,3R)-2-benzyloxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxamide (144 mg, 93.3%) as an oil.

Reference Example 21

1-[(2S,3R)-2-benzyloxy-5-(1-naphthyl)-3-pentyl]-imidazole-4-carboxamide (obtained in Reference Example 17 or 20) (5.07 g) was dissolved in a mixture of ethanol (300 ml) and cyclohexene (150 ml) and then palladium hydroxide (20% on carbon, 5.0 g) was added. The mixture was heated under reflux for 3 days. After cooling, the catalyst was filtered and washed with ethanol. The combined filtrate and washings were concentrated in vacuo. Flash chromatography (dichloromethane : methanol = 10 : 1) gave 1-[(2S,3R)-2-hydroxy-5-(1-naphthyl)-3-pentyl]imidazole-4-
carboxamide (2.71 g, 68.4%) as a foam.

IR (KBr): 3334, 1666, 1593 cm⁻¹

NMR (DMSO-d₆, d): 0.88 (3H, d, J=6.2Hz), 2.10-2.40 (2H, m),
2.60-2.95 (2H, m), 3.83 (1H, m), 4.05 (1H, m), 5.09
(1H, d, J=4.9Hz), 7.10 (1H, bs), 7.25 (1H, d, J=6.3Hz),
7.34 (1H, bs), 7.42 (1H, t, J=7.6Hz), 7.49-7.54 (2H, m),
7.76-7.94 (5H, m)

MASS (APCI, m/z): 324 (M+H)⁺

[α]D²⁷ +29.2° (c 0.5, EtOH)

Reference Example 22

1-[(2S,3S)-2-hydroxy-5-(1-naphthyl)-3-pentyl]-
imidazole-4-carboxamide was prepared from the compound obtained
in Reference Example 18(1) according to a similar procedure to

Reference Example 21.

IR (KBr): 3334, 1658, 1593 cm⁻¹

NMR (DMSO-d₆, d): 0.91 (3H, d, J=6.3Hz), 2.10-2.30 (2H, m),
2.60-3.05 (2H, m), 3.95 (1H, m), 4.13 (1H, m), 5.05
(1H, d, J=4.1Hz), 7.06 (1H, bs), 7.25-7.55 (5H, m),
7.75-7.95 (5H, m)

MASS (APCI, m/z): 324 (M+H)⁺

[α]D²⁷ -22.4° (c 0.25, EtOH)

Reference Example 23

The following compound was prepared by a similar procedure
to that of Reference Example 4.

(1) 1-[(2S,3R)-2-hydroxy-5-(2-methylphenyl)-3-pentyl]-
imidazole-4-carboxamide

mp: 60-62°C

NMR (CDCl₃, δ): 1.11 (3H,d,J=6Hz), 2.0-2.6 (5H,m), 2.20
(3H,s), 3.8-4.1 (2H,m), 5.47 (1H,s), 6.9-7.2 (5H,m),
7.46 (1H,d,J=1Hz), 7.73 (1H,d,J=1Hz)

MASS: 288 (M+H)⁺

[α]D²⁸ = +110.5° (c 0.50, EtOH)
(2) 1-[(2S,3R)-2-hydroxy-5-(2-methoxyphenyl)-3-pentyl]-imidazole-4-carboxamide

NMR (CDCl₃, δ): 1.09 (3H,d,J=6Hz), 2.0-2.7 (5H,m), 3.81 (3H,s), 3.9-4.0 (2H,m), 5.40 (1H,s), 6.8-7.3 (5H,m), 7.46 (1H,d,J=1Hz), 7.72 (1H,d,J=1Hz)

MASS: 304 (M+H)⁺

[α]₀°₂₅ = +110.0° (c 0.50, EtOH)

(3) 1-[(2S,3R)-2-hydroxy-5-(2-hexyloxyphenyl)-3-pentyl]-imidazole-4-carboxamide

NMR (CDCl₃, δ): 0.8-1.0 (3H,m), 1.09 (3H,d,J=6Hz), 1.2-1.5 (6H,m), 1.7-1.9 (3H,m), 2.0-2.7 (4H,m), 3.8-4.0 (4H,m), 5.35 (1H,s), 6.8-7.3 (5H,m), 7.45 (1H,s), 7.69 (1H,s)

MASS: 374 (M+H)⁺

[α]₀°₂₅ = +22.9° (c 0.50, EtOH)

(4) 1-[(2S,3R)-2-hydroxy-5-(2-hydroxyphenyl)-3-pentyl]-imidazole-4-carboxamide

NMR (DMSO-d₆, δ): 0.87 (3H,d,J=6Hz), 1.9-2.4 (4H,m), 3.7-4.0 (2H,m), 5.05 (1H,d,J=5Hz), 6.6-7.3 (6H,m), 7.71 (2H,s), 9.29 (1H,s)

MASS: 290 (M+H)⁺

(5) 1-[(2S,3R)-2-hydroxy-5-(2,3-dimethylphenyl)-3-pentyl]-imidazole-4-carboxamide

NMR (CDCl₃, δ): 1.10 (3H,d,J=6Hz), 2.0-2.6 (5H,m), 2.11 (3H,s), 2.26 (3H,s), 3.9-4.0 (2H,m), 5.43 (1H,s), 6.8-7.1 (4H,m), 7.47 (1H,d,J=1Hz), 7.72 (1H,d,J=1Hz)

MASS: 302 (M+H)⁺

[α]₀°₂₅ = +26.7° (c 0.50, EtOH)

(6) 1-[(2S,3R)-2-hydroxy-5-[2-(trifluoromethyl)phenyl]-3-pentyl]imidazole-4-carboxamide

NMR (CDCl₃, δ): 1.13 (3H,d,J=6Hz), 2.0-2.4 (3H,m), 2.5-2.8 (2H,m), 3.9-4.1 (2H,m), 5.42 (1H,s), 6.9-7.8 (7H,m)
MASS: 342 (M+H)⁺
[α]₀₂⁵ = -0.70° (c 0.50, EtOH)

(7) Methyl 1-[(2S,3R)-2-hydroxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxylate
NMR (CDCl₃, δ): 1.09 (3H, d, J=6Hz), 1.9-2.6 (3H, m), 2.8-3.2 (2H, m), 3.92 (3H, s), 3.9-4.1 (2H, m), 7.1-7.9 (9H, m)
MASS: 339 (M+H)⁺

(8) 1-{(2S,3R)-2-hydroxy-5-[2,3-(methylenedioxy)phenyl]-3-pentyl}imidazole-4-carboxamide
NMR (CDCl₃, δ): 1.11 (3H, d, J=6Hz), 2.1-2.7 (5H, m), 3.8-4.1 (2H, m), 5.44 (1H, s), 5.92 (2H, s), 6.5-6.8 (3H, m), 6.99 (1H, s), 7.44 (1H, d, J=1Hz), 7.70 (1H, d, J=1Hz)
MS: 318 (M+H)⁺
[α]₀²⁷ = +29.3° (c 0.50, EtOH)

(9) 1-[(2S,3R)-2-hydroxy-5-(2-naphthyl)-3-pentyl]imidazole-4-carboxamide
IR (KBr): 3340, 1658 cm⁻¹
NMR (CDCl₃, δ): 1.10 (3H, d, J=6Hz), 2.1-2.4 (3H, m), 2.4-2.7 (1H, m), 2.7-2.9 (1H, m), 3.8-4.1 (2H, m), 5.46 (1H, s), 7.00 (1H, s), 7.2-7.9 (9H, m)
MS: 324 (M+H)⁺
[α]₀²⁶ = +55.4° (c 0.50, EtOH)

(10) 1-[(2S,3R)-2-hydroxy-6-(1-naphtyl)-3-hexyl]imidazole-4-carboxamide
IR (KBr): 3340, 1658 cm⁻¹
NMR (CDCl₃, δ): 1.08 (3H, d, J=6Hz), 1.5-2.2 (5H, m), 3.06 (2H, t, J=8Hz), 3.8-4.0 (2H, m), 5.48 (1H, s), 6.98 (1H, s), 7.2-8.0 (9H, m)
MS: 338 (M+H)⁺
Reference Example 24

A mixture of 1-[(2S,3R)-2-(benzyloxy)-5-(2-chlorophenyl)-3-pentyl]imidazole-4-carboxamide (obtained in Reference Example 3(9)) (40 mg) and iodo(trimethyl)silane (0.02 ml) in chloroform (1 ml) was stirred at room temperature for 2 hours. The mixture was poured into methanol and the whole was evaporated in vacuo. The residue was taken up in ethyl acetate, washed with water, aqueous sodium bisulfite and sodium bicarbonate, successively, and dried. The residue left after evaporation of solvent was purified by column chromatography on silica gel, eluting with a mixture of dichloromethane and methanol (20:1) to give a white powder of 1-[(2S,3R)-2-hydroxy-5-(2-chlorophenyl)-3-pentyl]imidazole-4-carboxamide (6.1 mg).

NMR (CDCl₃, δ): 1.12 (3H, d, J=6Hz), 1.9-2.4 (3H, m), 2.5-2.7 (2H, m), 3.9-4.1 (2H, m), 5.40 (1H, s), 6.9-7.4 (5H, m), 7.49 (1H, d, J=1Hz), 7.72 (1H, d, J=1Hz)

MASS: 308 (M+H)+

[α]D²⁰ = +17.9° (c 0.50, EtOH)

Reference Example 25

1-[(2S,3R)-2-Hydroxy-5-(2,3-dichlorophenyl)-3-pentyl]imidazole-4-carboxamide was prepared by a similar procedure to that of Reference Example 24 from the compound obtained in Reference Example 3(12).

mp: 70-75°C

NMR (CDCl₃, δ): 1.13 (3H, d, J=6Hz), 1.98 (1H, d, J=5Hz), 2.1-2.4 (2H, m), 2.6-2.8 (2H, m), 3.9-4.1 (2H, m), 5.39 (1H, s), 6.9-7.4 (4H, m), 7.49 (1H, d, J=1Hz), 7.72 (1H, d, J=1Hz)

MASS: 342 (M+H)+

[α]D²⁰ = +9.30° (c 0.50, EtOH)

Reference Example 26

Methyl 1-[(2S,3R)-2-(benzyloxy)-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxylate was prepared by a similar procedure to that of Reference Example 6 from methyl 4-
imidazolecarboxylate and the compound obtained in Preparation 27.

NMR (CDCl₃, δ): 1.06 (3H, d, J=6Hz), 2.1-2.6 (2H, m), 2.7-3.1 (2H, m), 3.6-3.7 (1H, m), 3.97 (3H, s), 3.9-4.1 (1H, m), 4.33 (1H, d, J=11Hz), 4.56 (1H, d, J=11Hz), 7.1-7.91 (14H, m)

MASS: 429 (M+H)⁺

Reference Example 27

1-[(2S,3R)-2-hydroxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxylguanidine acetic acid salt was prepared by a similar procedure to that of Reference Example 8 from the compound obtained in Reference Example 23(7).

NMR (DMSO-d₆, δ): 0.90 (3H, d, J=6Hz), 1.88 (3H, s), 2.1-2.5 (2H, m), 2.6-3.0 (2H, m), 3.8-4.2 (2H, m), 5.15 (1H, br s), 7.2-8.0 (9H, m)

MASS: 366 (M+H)⁺

[α]₀ᵇ²⁶ = +17.5° (c 0.50, EtOH)

Reference Example 28

A mixture of 1-[(2S,3R)-2-hydroxy-5-(2-hydroxyphenyl)-3-pentyl]imidazole-4-carboxamide (obtained in Reference Example 23(4))(4.1 mg), 1-bromo-3-phenylpropane (7 mg), and potassium carbonate (4 mg) in N,N-dimethylformamide (0.5 ml) was stirred overnight at room temperature. The mixture was taken up in ethyl acetate, washed twice with water, dried, and evaporated. The residue was purified by column chromatography on silica gel, eluting with a mixture of dichloromethane and methanol (20:1) to give a colorless gummy oil of 1-[(2S,3R)-2-hydroxy-5-[2-(3-phenylpropoxy)phenyl]-3-pentyl]imidazole-4-carboxamide (4.9 mg).

NMR (CDCl₃, δ): 1.08 (3H, d, J=6Hz), 2.0-2.9 (9H, m), 3.9-4.0 (4H, m), 5.39 (1H, s), 6.7-7.4 (10H, m), 7.45 (1H, s), 7.71 (1H, s)

MS: 408 (M+H)⁺
Reference Example 29

A mixture of methyl 1-[(2S,3R)-2-hydroxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxylate (obtained in Reference Example 23(7))(25 mg) and methylamine (40 % in water; 1 ml) in tetrahydrofuran (3 ml) was heated in a steel sealed tube at 120°C overnight. The mixture was taken up in dichloromethane, washed with water, dried, and evaporated. The residue was purified by column chromatography on silica gel, eluting with a mixture of dichloromethane and methanol (30:1) to give a white powder of N-methyl-1-[(2S,3R)-2-hydroxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxamide (18.6 mg).

NMR (CDCl₃, δ): 1.07 (3H, d, J=6Hz), 2.1-2.5 (3H, m), 2.7-3.1 (2H, m), 3.01 (3H, d, J=7Hz), 3.8-4.0 (2H, m), 7.0-7.9 (10H, m)

MS: 338 (M+H)⁺

[α]D₂⁷ = +24.7° (c 0.50, EtOH)

Reference Example 30

A mixture of methyl 1-[(2S,3R)-2-benzyloxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxylate (obtained in Reference Example 3(20))(97 mg) and sodium hydroxide (12 mg) in ethanol (2 ml) and water (0.2 ml) was stirred at room temperature overnight. The solvent was evaporated and the residue was taken up in a mixture of ethyl acetate and water. The aqueous layer was separated, acidified to pH 3 with hydrochloric acid, and extracted with ethyl acetate. The extract was dried and evaporated to give a pale brown powder of 1-[(2S,3R)-2-benzyloxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxylic acid (84.5 mg).

NMR (CDCl₃, δ): 1.07 (3H, d, J=6Hz), 2.2-2.6 (2H, m), 2.8-3.2 (2H, m), 3.5-3.7 (1H, m), 3.9-4.1 (1H, m), 4.34 (1H, d, J=12Hz), 4.55 (1H, d, J=12Hz), 7.1-7.9 (14H, m)

MS: 415 (M+H)⁺
Reference Example 31
A mixture of 1-[(2S,3R)-2-benzylxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxylic acid (obtained in Reference Example 30) (55 mg), methanesulfonamide (12.7 mg), 4-dimethylaminopyridine (24.3 mg), and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (51.2 mg) in N,N-dimethylformamide (2 ml) was stirred at room temperature for three days. Ethyl acetate and water were added, and the whole was acidified to pH 3 with hydrochloric acid. The organic layer was dried and evaporated. The residue was purified by column chromatography on silica gel, eluting with a mixture of dichloromethane and methanol (20:1) to give a pale yellow gummy oil of N-methylsulfonyl-1-[(2S,3R)-2-benzylxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxamide (18 mg).

NMR (CDCl₃, δ): 1.05 (3H, d, J=6Hz), 2.2-2.5 (2H, m), 2.8-3.1 (2H, m), 3.40 (3H, s), 3.6-3.7 (1H, m), 3.9-4.1 (1H, m), 4.2-4.6 (2H, m), 7.0-7.9 (14H, m)
MS: 490 (M-H)⁻

Reference Example 32
N-methylsulfonyl-1-[(2S,3R)-2-hydroxy-5-(1-naphthyl)-3-pentyl]imidazole-4-carboxamide was prepared from the compound obtained in Reference Example 31 according to the procedure of Reference Example 4.

NMR (CDCl₃+CD₂OD, δ): 0.97 (3H, d, J=6Hz), 2.0-2.3 (2H, m), 2.7-3.1 (2H, m), 3.06 (3H, s), 3.8-4.1 (2H, m), 7.1-7.9 (9H, m)
MS: 402 (M+H)⁺

Preparation 30
2-Methylthiophenylmethylmagnesium chloride was prepared from magnesium turnings (654 mg) and 2-(chloromethyl)thioanisole (1.55 g) in ether (29 ml) by the method of J. Am. Chem. Soc. (1943) 65, 295. A solution of lithium chloride (38 mg) and copper(II) chloride (60 mg) in THF (2.8 ml) was added dropwise to the ethereal solution of the Grignard reagent followed by addition of a
solution of (2RS,3S)-3-(benzylxyloxy)-1,2-epoxybutane (0.8 g) in ether (8 ml) below -60°C. The mixture was stirred at -70°C for 1 h, and then allowed to warm to room temperature and stirred overnight. After cooling, the mixture was quenched with saturated aqueous ammonium chloride solution (22 ml). The organic layer was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo to give an oil. Column chromatography (CH₂Cl₂) over silica gel gave a pale brown oil of (2S,3S)-2-benzyloxy-5-(2-methylthiophenyl)pentan-3-ol (0.65 g, 45.8%).

NMR (CDCl₃, δ): 1.20(3H,d, J=6Hz), 1.6-1.9(2H,m), 2.46(3H,s), 2.66(1H,d, J=3Hz), 2.7-3.1(2H,m), 3.4-3.6(2H,m), 4.44(1H,d, J=11Hz), 4.67(1H,d, J=11Hz), 7.0-7.4(9H,m).

MS: 339(M+Na)⁺.

**Reference Example 33**

To an ice-cooled solution of (2S,3S)-2-benzyloxy-5-(2-methylthiophenyl)pentan-3-ol (0.64 g) in dichloromethane (12 ml) was added methanesulfonyl chloride (0.234 ml) followed by triethylamine (0.421 ml). The mixture was stirred at 4°C for 40 min. The mixture was washed with water and brine, dried and concentrated in vacuo to give 2-[(3S,4S)-4-benzyloxy-3-methanesulfonyloxypentyl]-thioanisole (0.92 g) as an oil.

A suspension of imidazole-4-carboxamide (313 mg) in DMF (3.8 ml) was treated with sodium hydride (60% in mineral oil, 129 mg) at ice-bath temperature and the mixture was stirred at room temperature for 20 min. A solution of 2-[(3S,4S)-4-benzyloxy-3-methanesulfonyloxypentyl]thioanisole (0.92 g) in DMF (7.5 ml) was added and the mixture was stirred at 85°C for 3 days. The mixture was poured into water and extracted with ethyl acetate. The extract was washed with water, dried and concentrated in vacuo. Column chromatography (dichloromethane:methanol = 30:1) gave 1-[(2S,3R)-2-benzyloxy-5-(2-methylthiophenyl)-3-pentyl]imidazole-4-
carboxamide (96.4 mg, 11.6%) as a pale yellow oil.

NMR (CDCl₃, δ): 1.07(3H, d, J=6Hz), 2.0-2.4(2H, m),
2.44(3H, s), 2.5-2.7(2H, m), 3.6-3.8(1H, m), 3.9-
4.1(1H, m), 4.39(1H, d, J=12Hz), 4.58(1H, d, J=12Hz),
5.37(1H, s), 6.9-7.2(10H, m), 7.50(1H,d,J=1Hz),
7.69(1H,d,J=1Hz).

MS (APCI, m/z): 410(M+H)+.

Reference Example 34

A mixture of 1-[(2S,3R)-2-(benzyloxy)-5-(2-methyl-
thiophenyl)-3-pentyl]imidazole-4-carboxamide (133 mg) and
iodotrimethylsilane (0.16 ml) in chloroform (5 ml) was stirred
at room temperature for 2 hours. The mixture was poured into cold
methanol and the whole was evaporated in vacuo. The residue was
taken up in ethyl acetate, washed with water, aqueous sodium
bisulfite and sodium bicarbonate, successively, and dried. The
residue left after evaporation of solvent was purified by column
chromatography on silica gel, eluting with a mixture of
dichloromethane and methanol (20:1) to give a white powder of

1-[(2S,3R)-2-hydroxy-5-(2-methylthiophenyl)-3-
pentyl]imidazole-4-carboxamide (62 mg).

NMR (CDCl₃, δ): 1.11(3H,d,J=6Hz), 2.0-2.7(5H,m),
2.45(3H,s), 3.9-4.0(2H,m), 5.41(1H,s), 6.9-
7.3(5H,m), 7.50(1H,d,J=1Hz), 7.73(1H,d,J=1Hz).

MS: 320(M+H)+.

[α]_D^{22} = +28.0 ° (c 1.0, EtOH).

Reference Example 35

A mixture of 1-[(2S,3R)-2-hydroxy-5-(2-
methylthiophenyl)-3-pentyl]imidazole-4-carboxamide (7.1 mg)
and m-chloroperbenzoic acid (75%; 5.12 mg) in dichloromethane
(1 ml) was stirred at 5°C for 2 hours. The mixture was purified
by column chromatography on silica gel, eluting with a mixture
of dichloromethane and methanol (5:1) to give a colorless oil
of

1-[(2S,3R)-2-hydroxy-5-(2-methyl-sulfinylphenyl)-3-
pentyl]imidazole-4-carboxamide (5.4 mg).
NMR (CDCl$_3$, $\delta$): 1.0-1.2(3H,m), 2.1-2.9(8H,m), 3.8-4.1(2H,m), 5.50(1H,s), 6.9-7.5(5H,m), 7.7-8.0(2H,m).

MS: 336(M+H)$^+$.  

Reference Example 36

A mixture of 1-{[(2S,3R)-2-hydroxy-5-(2-methylthiophenyl)-3-pentyl]imidazole-4-carboxamide (6.0 mg) and m-chloroperbenzoic acid (75%; 13 mg) in dichloromethane (1 ml) was stirred at room temperature overnight. The mixture was purified by column chromatography on silica gel, eluting with a mixture of dichloromethane and methanol (10:1) to give a colorless oil of 1-{[(2S,3R)-2-hydroxy-5-(2-methylsulfonylphenyl)-3-pentyl]imidazole-4-carboxamide (4.6 mg).

NMR (CDCl$_3$, $\delta$): 1.13(3H,d,J=6Hz), 2.0-3.0(5H,m), 3.03(3H,s), 3.9-4.2(2H,m), 5.60(1H,s), 7.08(1H,s), 7.2-8.1(6H,m).

MS: 352(M+H)$^+$.  

Reference Example 37

A mixture of 1-{[(2S,3R)-2-hydroxy-5-(2-hydroxyphenyl)-3-pentyl]imidazole-4-carboxamide (20 mg), 3-(dimethylamino)propyl chloride hydrochloride (32.7 mg) and potassium carbonate (38 mg) in N,N-dimethylformamide (3 ml) was stirred overnight at 60°C. The mixture was evaporated. The residue was purified by column chromatography on silica gel, eluting with a mixture of dichloromethane, triethylamine, and methanol (20:1:4) and the product was treated with HCl in EtOAc to give a pale brown oil of 1-{[(2S,3R)-2-hydroxy-5-{2-[(3-dimethylaminoproproxy)phenyl]-3-pentyl]imidazole-4-carboxamide dihydrochloride (21.2 mg).

MS: 375(M+H)$^+$.  

$[\alpha]_{D}^{22} = +2.0$ ° (c 0.50, EtOH).
Preparation 31

A mixture of 1-[(2S,3R)-2-hydroxy-5-(2-hydroxyphenyl)-3-pentyl]imidazole-4-carboxamide (0.11 g), 1-bromo-6-phthalimido-hexane (0.47 g) and potassium carbonate (0.42 g) in N,N-dimethylformamide (5 ml) was stirred overnight at room temperature. The mixture was taken up in ethyl acetate, washed three times with water, dried, and evaporated. The residue was purified by column chromatography on silica gel, eluting with a mixture of dichloromethane and methanol (20:1) to give a white powder of 1-{(2S,3R)-2-hydroxy-5-[2-(6-phthalimidohexyloxy)phenyl]-3-pentyl}imidazole-4-carboxamide (219 mg).

NMR (CDCl₃, δ): 1.08(3H,d,J=6Hz), 1.3-1.9(8H,m), 2.0-2.7(5H,m), 3.6-4.0(6H,m), 5.40(1H,s), 6.7-7.3(5H,m), 7.46(1H,d,J=1Hz), 7.7-7.9(5H,m).

Reference Example 38

A mixture of 1-{(2S,3R)-2-hydroxy-5-[2-(6-phthalimido-hexyloxy)phenyl]-3-pentyl}imidazole-4-carboxamide (217 mg), hydrazine hydrate (0.04 ml), MeOH (2.9 ml) and tetrahydrofuran (2.9 ml) was stirred overnight at room temperature and evaporated. The residue was purified by column chromatography on silica gel, eluting with a mixture of dichloromethane, acetic acid and methanol (15 : 1 : 5) and the product was treated with HCl in EtOAc to give a pale brown powder of 1-{(2S,3R)-2-hydroxy-5-[2-(6-aminohexyloxy)-phenyl]-3-pentyl}imidazole-4-carboxamide dihydrochloride (120 mg).

NMR (DMSO-d₆, δ): 0.98(3H,d,J=6Hz), 1.2-1.8(8H,m), 2.0-2.6(4H,m), 2.7-2.9(2H,m), 3.3-4.3(4H,m), 6.7-7.2(4H,m), 7.8-8.4(6H,m), 9.08(1H,s).

MS: 389(M+H)+.

[α]D²¹ = +8.0 ° (c 0.50, EtOH).

Reference Example 39

A mixture of 1-[(2S,3R)-2-hydroxy-5-(2-hydroxyphenyl)-3-pentyl]imidazole-4-carboxamide (10 mg), 2,2,2-trifluoroethyl
tosylate (27.4 mg) and potassium carbonate (14.9 mg) in N,N-
dimethylformamide (1 ml) was stirred at 130°C for 13 hours. The
mixture was taken up in ethyl acetate, washed three times with
water, dried, and evaporated. The residue was purified by column
chromatography on silica gel, eluting with a mixture of
dichloromethane and methanol (10:1) to give a pale brown powder
of 1-{(2S,3R)-2-hydroxy-5-[2-(2,2,2-trifluoroethoxy)phenyl]-
3-pentyl}imidazole-4-carboxamide (7.0 mg).

NMR (CDCl₃, δ): 1.10(3H,d,J=6Hz), 2.0-2.7(5H,m), 3.8-
4.1(2H,m), 4.37(2H,q,J=8Hz), 5.41(1H,s),
6.78(1H,d,J=8Hz), 6.9-7.3(4H,m), 7.45(1H,s),
7.68(1H,s).

MS: 372(M+H)+.

[α]₀²³ = +15.6 ° (c 0.125, EtOH).

Reference Example 40

The following compound was prepared by a similar manner to
Preparation 31.
1-{(2S,3R)-2-hydroxy-5-[2-(3-(4-chlorophenyl)propoxy)-
phenyl]-3-pentyl}imidazole-4-carboxamide

NMR (CDCl₃, δ): 1.08(3H,d,J=6Hz), 2.0-2.8(9H,m), 3.8-
4.0(4H,m), 5.33(1H,s), 6.7-7.3(9H,m), 7.45(1H,s),
7.70(1H,s).

MS: 442(M+H)+.

[α]₀²¹ = +19.4 ° (c 0.30, EtOH).

Preparation 32

The following compound was prepared by a similar manner to
Preparation 30.
(2S,3S)-2-benzyloxy-5-(6-chloro-1-naphthyl)pentan-3-ol

NMR (CDCl₃, δ): 1.17(3H,d,J=6Hz), 1.8-2.0(2H,m),
2.71(1H,d,J=3Hz), 3.0-3.6(4H,m), 4.42(1H,d,J=11Hz),
4.67(1H,d,J=11Hz), 7.3-8.1(11H,m).

MS: 377(M+Na)+.
Reference Example 41
The following compound was prepared by a similar manner to Reference Example 33.
1-[(2S,3R)-2-benzyloxy-5-(6-chloro-1-naphthyl)-3-pentyl]-imidazole-4-carboxamide
NMR (CDCl₃, δ): 1.04(3H, d, J=6Hz), 2.1-2.6(2H, m), 2.7-3.0(2H, m), 3.6-3.7(1H, m), 3.9-4.0(1H, m), 4.35(1H, d, J=11Hz), 4.56(1H, d, J=11Hz), 5.46(1H, s), 6.9-7.9(14H, m).
MS: 448(M+H)⁺.

Reference Example 42
The following compound was prepared by a similar manner to Reference Example 34.
1-[(2S,3R)-2-hydroxy-5-(6-chloro-1-naphthyl)-3-pentyl]-imidazole-4-carboxamide
NMR (CDCl₃, δ): 1.09(3H,d,J=6Hz), 2.2-2.5(3H,m), 2.7-3.1(2H,m), 3.8-4.1(2H,m), 5.46(1H,s), 7.01(1H,s), 7.1-7.9(8H,m).
MS: 358(M+H)⁺.
[α]D²¹ = +15.9° (c 0.50, EtOH).

Preparation 33
The following compound was prepared by a similar manner to Preparation 30.
(2S,3S)-2-benzyloxy-5-(7-chloro-1-naphthyl)pentan-3-ol
NMR (CDCl₃, δ): 1.18(3H,d,J=6Hz), 1.8-2.0(2H,m), 2.69(1H,d,J=4Hz), 3.0-3.7(4H,m), 4.44(1H,d,J=11Hz), 4.68(1H,d,J=11Hz), 7.2-8.1(11H,m).
MS: 377(M+Na)⁺.

Reference Example 43
The following compound was prepared by a similar manner to Reference Example 33.
1-[(2S,3R)-2-benzyloxy-5-(7-chloro-1-naphthyl)-3-pentyl]-imidazole-4-carboxamide
NMR (CDCl₃, δ): 1.04(3H, d, J=6Hz), 2.1-2.6(2H, m), 2.7-3.1(2H, m), 3.6-3.7(1H, m), 3.9-4.0(1H, m), 4.36(1H, d, J=11Hz), 4.56(1H, d, J=11Hz), 5.40(1H, s), 6.99(1H, s), 7.1-7.9(13H, m).

MS: 448(M+H)⁺.

Reference Example 44

The following compound was prepared by a similar manner to Reference Example 2.

1-[(2S,3R)-2-hydroxy-5-(7-chloro-1-naphthyl)-3-pentyl]-imidazole-4-carboxamide

NMR (CDCl₃, δ): 1.10(3H, d, J=6Hz), 2.0-2.6(3H, m), 2.7-3.1(2H, m), 3.9-4.0(2H, m), 5.43(1H, s), 7.01(1H, s), 7.1-7.9(8H, m).

MS: 358(M+H)⁺.

[α]₀²¹ = +38.1° (c 0.10, EtOH).

Reference Example 45

To a stirred mixture of 1-naphthol (48.5 mg, 0.336 mmol), 1-[(2S,3R)-2-benzyloxy-5-hydroxy-3-pentyl]imidazole-4-carboxamide (85 mg, 0.280 mmol), and triphenylphosphine (88.2 mg, 0.336 mmol) in tetrahydrofuran (5 ml) was added dropwise diethyl azodicarboxylate (58.6 mg, 0.336 mmol) at ice-bath temperature. After the mixture was stirred overnight at room temperature, the solvent was removed in vacuo. The residue was purified by silica gel (10 g) chromatography eluting with chloroform/methanol (40:1) to give a mixture (60.6 mg) of 1-[(2S,3R)-2-benzyloxy-5-(1-naphthoxy)-3-pentyl]imidazole-4-carboxamide and by-products. This material was used without further purification.

MS: 430(M+H)⁺.

Reference Example 46

To a solution of 1-[(2S,3R)-2-benzyloxy-5-(1-naphthoxy)-3-pentyl]imidazole-4-carboxamide (56.5 mg, 0.132 mmol) in cyclohexene (3 ml) and ethanol (6 ml) was added 20%
palladium hydroxide on carbon (30 mg). The resulting mixture was stirred at reflux for 22h.

After cooling to room temperature, the mixture was filtered through Celite and washed with ethanol. The filtrate was concentrated in vacuo and then the residue was purified by silica gel (1.4 g) chromatography eluted with chloroform/methanol (50:1 to 10:1) to give 1-[(2S,3R)-2-hydroxy-5-(1-naphthyloxy)-3-pentyl]imidazole-4-carboxamide (24.8 mg, 55.6%).

IR (KBr, cm⁻¹): 3600-2800, 1658, 1589, 1267, 1099

NMR (DMSO-d₆, δ): 0.97(3H, d, J=6.2Hz), 2.30-2.80(2H, m), 3.70-4.50(4H, m), 5.22(1H, d, J=5.0Hz), 6.84(1H, d, J=7.3Hz), 7.03(1H, brs), 7.15-7.60(5H, m), 7.60-8.20(4H, m).

MS: 340(M+H)⁺

[α]D²⁴ = +94.10°(C = 0.50, EtOH).

Reference Example 47

To a stirred mixture of 1-[(2S,3R)-2-benzyloxy-5-hydroxy-3-pentyl]imidazole-4-carboxamide (85 mg, 0.28 mmol) and methanesulfonyl chloride (64 mg, 0.56 mmol) in dichloromethane (5 ml) was added dropwise triethylamine (57 mg, 0.56 mmol) at ice-bath temperature. After 1h, the reaction mixture was partitioned between dichloromethane and water. The organic layer was washed with brine, dried (sodium sulfate), and concentrated in vacuo to give 1-[(2S,3R)-2-benzyloxy-5-methanesulfonyloxy-3-pentyl]imidazole-4-carboxamide (106 mg, 99%) as an oil. This material was used immediately without further purification.

Under N₂, to a solution of 2-naphthol (49 mg, 0.34 mmol) in DMF (3 ml) was added potassium carbonate (93 mg, 0.67 mmol) at room temperature. The reaction mixture was stirred for 30 minutes. The methanesulfonate prepared above was added and the resulting mixture was stirred for 6h at 60-70°C. The reaction mixture was poured into water (30 ml) and extracted with ethyl acetate. The organic layer was washed with brine, dried (sodium sulfate) and evaporated in vacuo. The residue was purified by
silica gel (4.8 g) chromatography eluting with chloroform/methanol (40:1) to give 1-[(2S,3R)-2-benzylxoy-5-(2-naphthyloxy)-3-pentyl]imidazole-4-carboxamide (51 mg, 42.4%).

IR (KBr, cm⁻¹): 3600-2800, 1666, 1595, 1261.
NMR (CDCl₃, δ): 1.19(3H, d, J=6.3Hz), 2.10 - 2.75(2H, m), 3.60-3.90(2H, m), 4.08(1H, m), 4.30-4.70(3H, m), 5.36(1H, brs), 6.80 - 7.15 (3H, m), 7.20-7.50(8H, m), 7.60-7.80(4H, m).

MS: 430(M+H)⁺.

[α]₀²⁴.₇ = +59.30°(C = 0.50, EtOH).

Reference Example 48

This compound was prepared by a similar procedure to that of Reference Example 14.

1-[(2S,3R)-2-hydroxy-5-(2-naphthyloxy)-3-pentyl]imidazole-4-carboxamide (28.6mg, 79.3%).

IR (KBr, cm⁻¹): 3600-2800, 1655, 1597, 1261.
NMR (DMSO-d₆, δ): 0.94(3H, d, J=6.2Hz), 2.20-2.65(2H, m), 3.65-4.55(4H, m), 5.21(1H, d, J=4.2Hz), 6.90-7.50(6H, m), 7.60-7.90(5H, m).

MS: 340(M+H)⁺.

[α]₀²⁴.₂ = +71.20°(C = 0.50, EtOH).

Reference Example 49

A solution of 1-[(2S,3R)-2-hydroxy-5-(2,3-dichlorophenyl)-3-pentyl]imidazole-4-carboxamide prepared in Reference Example 25 (74 mg) and 4N hydrogen chloride in ethyl acetate (0.2 ml) in methanol (5 ml) was evaporated in vacuo. The residue was crystallized from acetone to give a pale yellow powder of

1-[(2S,3R)-2-hydroxy-5-(2,3-dichlorophenyl)-3-pentyl]imidazole-4-carboxamide hydrochloride (74 mg).

NMR (DMSO-d₆, δ): 0.98(3H,d,J=6Hz), 2.0-2.9(4H,m), 3.8-4.0(1H,m), 4.2-4.4(1H,m), 7.2-7.6(3H,m), 7.79(1H,s), 8.18(1H,s), 8.30(1H,s), 9.03(1H,s).
MS: 342(M+H)^+.

2. Adenosine Deaminase (ADA) Enzyme Assay

The reaction velocity (V) was measured by a change in absorbance at 265 nm (A_{265}) resulting from the deamination of adenosine. Human ADA was expressed and purified from ADA-deficient bacterial strain. Reaction mixtures in a total volume of 200 μl contained 25 mU/ml of ADA and varying concentrations of adenosine and Test Compounds A and B as used in Examples 1 and 2 in 10 mM phosphate buffer saline (pH 7.4). The reaction was started by addition of ADA to a mixture of adenosine and the test compound. The reaction was allowed to proceed at room temperature with recording decrease in A_{265} for 5 minutes in SPECTRAmax 250 (Molecular Devices, USA) to automatically calculate Vmax. The inhibition constant (Ki) value of the test compound was determined by Dixon plot. The results are shown in the table below.

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Ki (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.9</td>
</tr>
<tr>
<td>B</td>
<td>3.4</td>
</tr>
</tbody>
</table>
CLAIMS

1. A product comprising an IL-2 inhibitor and an adenosine deaminase inhibitor for simultaneous, separate or sequential combination use in suppressing an immune reaction.

2. The product according to claim 1, wherein said adenosine deaminase inhibitor is an imidazole derivative.

3. The product according to claim 2, wherein said imidazole derivative is represented by the formula (II):

\[
\text{(II)}
\]

[wherein } R^{31} \text{ is hydrogen, hydroxy, protected hydroxy, or aryl optionally substituted with suitable substituent(s);} 

R^{32} \text{ is hydrogen or lower alkyl; } 
R^{33} \text{ is hydroxy or protected hydroxy; } 
R^{34} \text{ is cyano, (hydroxy)iminoamino(lower)alkyl, carboxy, protected carboxy, heterocyclic group optionally substituted with amino, or carbamoyl optionally substituted with suitable substituent(s); and } 
-A- \text{ is } -Q- \text{ or } -O-Q-, \text{ wherein } Q \text{ is single bond or lower alkylene, provided that when } R^{32} \text{ is lower alkyl, then } R^{31} \text{ is hydroxy, protected hydroxy, or aryl optionally substituted with suitable substituent(s)}, \text{ or } 

4. The product according to any one of claims 1 to 3, wherein said IL-2 inhibitor is a compound represented by the formula (I):
wherein each of adjacent pairs of $R^1$ and $R^2$, $R^3$ and $R^4$, and $R^5$ and $R^6$ independently
(a) is two adjacent hydrogen atoms, but $R^2$ may also be an alkyl group or
(b) may form another bond formed between the carbon atoms to which they are attached;

$R^7$ is a hydrogen atom, a hydroxy group, a protected hydroxy group, or an alkoxy group, or an oxo group together with $R^1$;

$R^8$ and $R^9$ are independently a hydrogen atom or a hydroxy group;

$R^{10}$ is a hydrogen atom, an alkyl group, an alkyl group substituted by one or more hydroxy groups, an alkenyl group, an alkenyl group substituted by one or more hydroxy groups, or an alkyl group substituted by an oxo group;

$X$ is an oxo group, (a hydrogen atom and a hydroxy group), (a hydrogen atom and a hydrogen atom), or a group represented by the formula $-\text{CH}_2\text{O}-$;

$Y$ is an oxo group, (a hydrogen atom and a hydroxy group), (a hydrogen atom and a hydrogen atom), or a group represented by the formula $\text{N}-\text{NR}^{12}_2$ or $\text{N}-\text{OR}^{13}$;

$R^{11}$ and $R^{12}$ are independently a hydrogen atom, an alkyl group, an aryl group or a tosyl group;

$R^{13}$, $R^{14}$, $R^{15}$, $R^{16}$, $R^{17}$, $R^{18}$, $R^{19}$, $R^{22}$, and $R^{23}$ are independently a hydrogen atom or an alkyl group;

$R^{24}$ is an optionally substituted ring system which may contain
one or more heteroatoms;
n is an integer of 1 or 2;
in addition to the above definitions, Y, R¹⁰, and R²³, together with
the carbon atoms to which they are attached, may represent a
saturated or unsaturated 5- or 6-membered nitrogen, sulfur and/or
oxygen containing heterocyclic ring optionally substituted by
one or more groups selected from the group consisting of an alkyl,
a hydroxy, an alkoxy, a benzyl, a group of the formula -CH₂Se(C₆H₄),
and an alkyl substituted by one or more hydroxy groups.] or its
salt.
5. The product according to claim 4, wherein said IL-2
inhibitor is tacrolimus or its hydrate.
6. The product according to any one of claims 1 to 3, wherein
said IL-2 inhibitor is cyclosporins.
7. The product according to claim 6, wherein said IL-2
inhibitor is cyclosporin A.
8. Use of an IL-2 inhibitor in combination with an adenosine
deaminase inhibitor for manufacturing a medicament for
simultaneous, separate or sequential use of suppressing the
immune reaction.
9. A method for suppressing the immune reaction by
administering an effective amount of an IL-2 inhibitor in
combination with an effective amount of an adenosine deaminase
inhibitor to a human or an animal.
10. A pharmaceutical composition for increasing an effect
caused by an IL-2 inhibitor, the composition comprising an
adenosine deaminase inhibitor.
11. Use of an adenosine deaminase inhibitor for manufacturing
a medicament for increasing an effect caused by an IL-2 inhibitor.
12. A method for increasing an effect caused by an IL-2
inhibitor by administering an effective amount of an adenosine
deaminase inhibitor to a human or an animal.
13. A pharmaceutical composition for suppressing an immune
reaction comprising an IL-2 inhibitor, for using with an adenosine
deaminase inhibitor simultaneously, separately or sequentially.
14. A pharmaceutical composition comprising an IL-2 inhibitor
and an adenosine deaminase inhibitor.

15. A commercial package comprising the pharmaceutical composition of any one of claims 10, 13, and 14, and a written matter associated therewith, wherein the written matter states that the pharmaceutical composition can or should be used for suppressing the immune reaction.
Figure 1

Last drug administration (day 14)

<table>
<thead>
<tr>
<th>Number of surviving grafts</th>
<th>Days after transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
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<tr>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
</tr>
</tbody>
</table>

FK506 + ADA inhibitor
MST: 20 days

FK506 alone
MST: 11 days

p<0.001
(Mann-Whitney Test)