(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 March 2006 (30.03.2006)

PCT

(10) International Publication Number
WO 2006/033551 A1

(51) International Patent Classification[7]: C07D 261/02

(21) International Application Number:
PCT/KR2005/003136

(22) International Filing Date:
22 September 2005 (22.09.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10-2004-0076788
24 September 2004 (24.09.2004) KR

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CASPASE INHIBITORS CONTAINING DICARBONYLAMINO-ISOXAZOLINE

(57) Abstract: The present invention relates to a dicarbonylamino-isoxazoline derivative as an inhibitor against various caspases and a therapeutic composition for preventing inflammation and apoptosis comprising the same.

WO 2006/033551 A1

Description

CASPASE INHIBITORS CONTAINING DICARBONYLAMINO-ISOXAZOLINE

[1]

TECHNICAL FIELD

[2]

[3] The present invention relates to a dicarbonylamino-isoxazoline derivative, or pharmaceutically acceptable salt, physiologically hydrolysable ester, hydrate, solvate, or stereoisomer thereof as an inhibitor against various caspases including caspase-1[interleukin-1β-converting enzyme, ICE], caspase-3[apopain/CPP-32], caspase-8, and caspase-9, and a therapeutic composition for preventing inflammation and apoptosis comprising the same.

[4]

BACKGROUND ART

[5] Caspase is a new kind of cysteine protease in the form of $\alpha_2\beta_2$ tetramer discovered during the last 10 years. About 14 kinds thereof have been known until now. Caspase-1(ICE), one of them, is a kind of cytokine and participates in converting the inactive prointerleukin-1β to the active interleukin-1β. Interleukin-1 consists of interleukin-1α and interleukin-1β, both of which are synthesized in monocytes in the form of precursor having 31kDa. Only prointerleukin-1β is activated by ICE. The positions hydrolyzed by caspase-1 are Asp^{27}-Gly^{28} and Asp^{116}-Ala^{117}. The hydrolysis of the latter position gives interleukin-1β. Interleukin-1β has been reported to act as an important mediator in causing inflammation (1,3). Caspase-1 has been discovered for the first time in 1989, and by two independent study groups, the three dimensional structure thereof was determined by X-ray crystallographic method.

[6]

[7] Caspase-3(CPP-32) is broadly studied for its role or mechanism for action, and its three dimensional structure was determined in 1996(2). Caspase-3(apopain) activated from procaspase-3 hydrolyzes (P_4)Asp-X-X-Asp(P_1) motif, and the known substrates include poly(ADP-ribose) polymerase, U1 70,000 Mr small nuclear ribonucleoprotein, catalytic subunit of 460,000 Mr DNA-dependent protein kinase, etc. The X-ray structure of caspase-7 has been reported to be very similar to that of caspase-3(4).

[8]

[9] Caspase-8 and 9 are present in the upstream of caspase-3,6,7 and these caspases are known to participate in the apoptosis cascade. The X-ray structure of caspase-8 was determined in 1999(5), and particularly the inhibitors thereof may be advantageously

used for treating the diseases related to apoptosis.

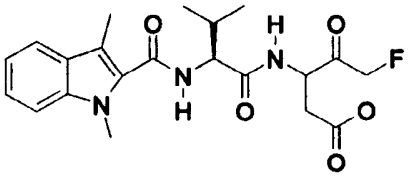
[10]

[11] Caspase inhibitors mean those compounds that inhibit the activity of caspase, and so control such symptoms as inflammation, apoptosis, etc. caused by the caspase activity. Diseases or symptoms that may be treated or attenuated by administering the inhibitors include the following: dementia, cerebral stroke, brain impairment due to AIDS, diabetes, gastric ulcer, cerebral injury by hepatitis virus, hepatitis-induced hepatic diseases, acute hepatitis, fulminant hepatic failure, sepsis, organ transplantation rejection, rheumatic arthritis, ischemic cardiac diseases, and liver cirrhosis(6).

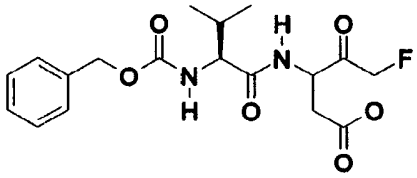
[12]

[13] Among the caspase inhibitors known until now, the most noted irreversible inhibitors are the following:

[14]



IDN-1965

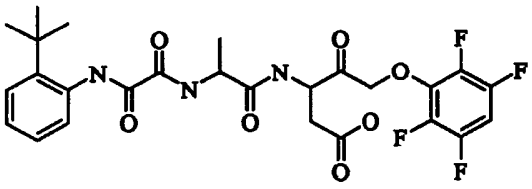


MX-1013

[15]

[16] Both the above inhibitors exhibit their activity based on the common mechanism that they irreversibly inactivate the enzyme to suppress the cell apoptosis (irreversible, broad-spectrum inhibitor). It has been reported that irreversible inhibitor has much more effective inhibitory activity than reversible inhibitor (7). Both IDN-1965 of IDUN Co. and MX-1013 of Maxim Co. are reported to show activity in cell apoptosis model for hepatic injury (8, 9). These compounds are now in the stage of preclinical test. The irreversible inhibitor IDN-6556, the structure of which has been recently reported, is now in the stage of phase II clinical test as a therapeutic agent for hepatic injury (10, 11).

[17]



IDN-6556

[18]

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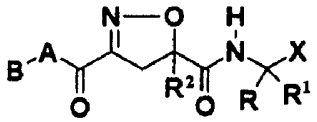
[57]

DISCLOSURE OF THE INVENTION

[58]

[59] The present inventors newly designed and synthesized some compounds which can be used as an effective inhibitor against caspases and have a distinctive structure and high selectivity for similar enzymes, and determined their binding ability and inhibitory activity for caspases. As a result, the inventors have discovered that a compound of the following formula (1) does meet such requirements, and completed the present invention.

[60]



[61] (1)

[62] in which

[63] A, B, R, R^1, R^2 and X are defined as described below.

[64]

[65] Therefore, the present invention provides the novel dicarbonylamino-isoxazoline derivative of formula (1), or pharmaceutically acceptable salt, physiologically hydrolysable ester, hydrate, solvate, or stereoisomer thereof having effective inhibitory activity against caspases.

[66]

[67] It is another object of the present invention to provide a composition, a use, or a method for inhibiting caspases, specifically a therapeutic composition, a use, or a method for preventing inflammation and apoptosis, comprising the compound of formula (1), or pharmaceutically acceptable salt, physiologically hydrolysable ester, hydrate, solvate, or stereoisomer thereof as an active ingredient together with the pharmaceutically acceptable carrier.

[68]

[69] First of all, the important terms in the present invention are defined as follows:

[70]

[71] a) Simple Alkyl Chain (SAC, below) means a hydrocarbon having 1 to 8 carbon atoms in either linear or branched isomeric form.

[72]

[73] b) Simple CycloAlkyl Chain (SCAC, below) means a cyclic radical having 3 to 10 carbon atoms.

[74]

[75] c) Aryl group (Ar, below) includes both the aromatic and heteroaromatic groups. The aromatic group means a 5 to 15-membered single or fused unsaturated cycle. One or more hydrogens may be replaced with a group(s) selected from the following: acyl, amino, carboalkoxy, carboxy, carboxyamino, cyano, halo, hydroxy, nitro, thiol, alkyl, cycloalkyl, alkoxy, aryloxy, sulfoxy, and guanido group. The heteroaromatic group means the aromatic group containing 1 to 5 hetero atoms selected from a group consisting of oxygen, sulfur, and nitrogen. Likewise, one or more hydrogens may be replaced with a group(s) selected from the following: acyl, amino, carboalkoxy, carboxy, carboxyamino, cyano, halo, hydroxy, nitro, thiol, alkyl, cycloalkyl, alkoxy, aryl, aryloxy, sulfoxy, and guanido group. Or, for example, in the case of pyridyl, an alkyl group can be added to the nitrogen atom to convert the pyridyl group to a pyridinium group having (+)-charge on the nitrogen atom.

[76]

[77] The aryl group includes phenyl, biphenyl, 1-naphthyl, 2-naphthyl, pyridinyl, N-alkyl-pyridinium, pyrimidinyl, quinolinyl, benzothienyl, indolyl, pyrazinyl, isoindolyl, isoquinolyl, qunazolinyl, quinoxalinyl, phthalazinyl, imidazolinyl, isoxazolyl, pyrazolyl, oxazolyl, thiazolyl, indolizinyl, indazolyl, benzothiazolyl, benzimidazolyl, benzofuranyl, thienyl, pyrrolyl, oxadiazolyl, thiadiazolyl, triazolyl, tetrazolyl, oxazolopyridinyl, imidazopyridinyl, isothiazolyl, cinnolinyl, carbazolyl, isochromanyl, chromanyl, tetrahydroisoquinolinyl, isoindolinyl, isobenzotetrahydrofuranyl, isobenzotetrahydro-thienyl, isobenzothienyl, benzoxazolyl, pyridopyridinyl, benzotetrahydrofuranyl, benzotetrahydrothienyl, purinyl, benzodioxolyl, triazinyl, phenoxazinyl, phenothiazinyl, pteridinyl, benzothiazolyl, imidazopyridinyl, imidazothiazolyl, dihydrobenzisoxazinyl, benzisoxazinyl, benzoxazinyl, dihydrobenzisothiopyranyl, benzopyranyl, benzothiopyranyl, coumarinyl, isocoumarinyl, chromonyl, chromanonyl, pyridinyl-N-oxide, tetrahydroquinolinyl-N-oxide, dihydroquinolinyl, dihydroquinolinonyl, dihydroisoquinolinonyl, dihydrocoumarinyl, dihydroisocoumarinyl, isoindolinonyl, benzodioxanyl, benzoxazolinonyl, pyrrolyl-N-oxide, pyrimidinyl-N-oxide, pyrazinyl-N-oxide, quinolinyl-N-oxide, indolyl-N-oxide, indolinyl-N-oxide,

isoquinolyl-N-oxide, qunazolinyl-N-oxide, quinoxalinyl-N-oxide, phthalazinyl-N-oxide, imidazolinyl-N-oxide, isoxazolyl-N-oxide, oxazolyl-N-oxide, thiazolyl-N-oxide, indolizinyl-N-oxide, indazolyl-N-oxide, benzothiazolyl-N-oxide, benzimidazolyl-N-oxide, pyrrolyl-N-oxide, oxadiazolyl-N-oxide, thiadiazolyl-N-oxide, triazolyl-N-oxide, tetrazolyl-N-oxide, etc.

[78]

[79] d) Simple Alkyl Chain substituted by Aryl (SAC-Ar, below) means a straight-chain or branched alkyl which has 1 to 8 carbon atoms and is substituted by the above mentioned aryl group.

[80]

[81] e) Natural amino acid includes the following: Glycine, Alanine, Valine, Leucine, Isoleucine, Serine, Threonine, Cysteine, Methionine, Proline, Aspartic acid, Asparagine, Glutamic acid, Glutamine, Lysine, Arginine, Histidine, Phenylalanine, Tyrosine, and Tryptophan.

[82]

[83] f) The protecting group of simple ester is a hydrocarbon having 1 to 8 carbon atoms in either linear or branched isomeric form.

[84]

[85] Further, the present specification includes the following abbreviations:

[86] N-chlorosuccinimide: NCS

[87] N-methylmorpholine: NMM

[88] O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate: HATU

[89] N,N-dimethyl formamide: DMF

[90] 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide: EDC

[91] 1-hydroxybenzotriazole hydrate: HOBt

[92] trifluoroacetic acid: TFA

[93] t-butoxycarbonyl: Boc

[94] benzyloxycarbonyl: Cbz

[95] methyl: Me

[96] ethyl: Et

[97] equivalent: eq

[98]

[99] The substituents included in the above formula (1) are specifically defined as follows.

[100]

[101] I) R represents H, simple alkyl chain (-SAC), s imple cycloalkyl chain (-SCAC), aryl group (-Ar), or simple alkyl chain substituted by aryl (-SAC-Ar),

[102]

[103] II) R^1represents -SAC, -SCAC, -Ar, -SAC-Ar, or a side chain residue of all the natural amino acids; and the compound of formula (1) may exist in a specific di-astereomeric form, or mixtures thereof when the carbon to which R^1 is attached becomes a stereocenter due to the R^1 group; the compound of formula (1) may have a protecting group in an ester form ($-CO_2R^3$ wherein R^3 is -SAC) or a sulfonamide form ($-CONHSO_2R^4$ wherein R^4 is -SAC), or may exist in the form of pharmaceutically acceptable salt, when R^1 is a side chain residue of an amino acid containing carboxyl moiety; or the compound of formula (1) may also exist in the form of pharmaceutically acceptable salt when R^1 is a side chain residue of an amino acid containing a base moiety,

[104]

[105] III) R^2 represents -SAC, -SCAC, -Ar, -SAC-Ar, or a side chain residue of all the natural amino acids; and the compound of formula (1) may exist in a specific di-astereomeric form, or mixtures thereof when the carbon to which R^2 is attached becomes a stereocenter due to the R^2 group; the compound of formula (1) may have a protecting group in an ester form ($-CO_2R^5$ wherein R^5 is -SAC) or a sulfonamide form ($-CONHSO_2R^6$ wherein R^6 is -SAC), or may exist in the form of pharmaceutically acceptable salt, when R^2 is a side chain residue of an amino acid containing carboxyl moiety; or the compound of formula (1) may also exist in the form of pharmaceutically acceptable salt when R^2 is a side chain residue of an amino acid containing a base moiety, or R^2 further represents H; $-(CH_2)_nOR^7$ wherein R^7 is -SAC, -SCAC, -Ar, or -SAC-Ar, and n = 1 or 2; or $-(CH_2)_nOC(=O)R^8$ wherein R^8 is -SAC, -SCAC, -Ar, or -SAC-Ar, and n = 1 or 2,

[106]

[107] IV) A represents $-(NR^9)_n-$ wherein R^9 is H, -SAC, -SCAC, -Ar, or -SAC-Ar, and n=0-1,

[108]

[109] V) B represents H, -SAC, -SCAC, -Ar, or -SAC-Ar, or

[110]

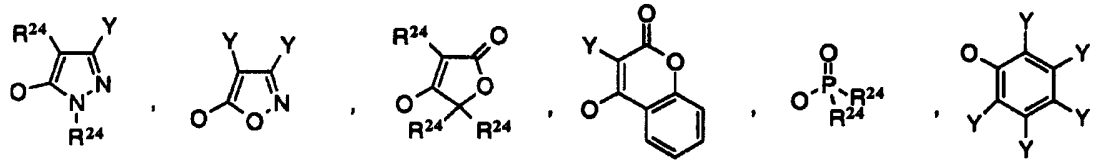
[111] VI) Rand R^1 may form a cycle together with the carbon atom to which they are attached, where $-R-R^1-$ is $-(CH_2)_n-$, $-(CH_2)_n-O-(CH_2)_m-$, or $-(CH_2)_n-NR^{10}-(CH_2)_m-$ wherein n+m<9 and R^{10} is -SAC, -SCAC, -Ar, -SAC-Ar, -C(=O)-SAC, -C(=O)-SCAC, -C(=O)-Ar, or -C(=O)-SAC-Ar,

[112]

[113] VII) X represents $-C(=O)CH_2OR^{11}$ wherein R^{11} is -SAC, -SCAC, -Ar, or -SAC-Ar; $-C(=O)CH_2OC(=O)R^{12}$ wherein R^{12} is -SAC, -SCAC, -Ar, or -SAC-Ar; $-CH=CH-CO_2R^{13}$ wherein R^{13} is -SAC, -SCAC, -Ar, or -SAC-Ar; $-CH=CH-SO_2R^{14}$ wherein R^{14} is -

SAC, -SCAC, -Ar, or -SAC-Ar; -C(=O)CH=CH_2; -$COCHN_2$; or -$COCH_2$-W wherein W is -F, -Cl, -Br, -I, -$NR^{15}R^{16}$ (R^{15} and R^{16} each are -SAC, -SCAC, -Ar, or -SAC-Ar, or together may form 3- to 6-membered saturated or unsaturated cyclic group), -SR^{17} (R^{17} is -SAC, -SCAC, -Ar, or -SAC-Ar), or is the following formula:

[114]



[115]

[116] wherein

[117] Y is H, -OH, -OR^{18} (R^{18} = -SAC or -SCAC), -C(=O)R^{19} (R^{19}= -H, -SAC, or -SCAC), -F, -Cl, -Br, -I, -CN, -N_3, -CO_2H, -CF_3, -CO_2R^{20} (R^{20} = -SAC or -SCAC), -C(=O)NHR^{21} (R^{21} = -SAC or -SCAC), or -C(=O)$NR^{22}R^{23}$ (R^{22} and R^{23} each are -SAC, -SCAC, -Ar, or -SAC-Ar), and

[118] R^{24} is H or -SAC.

[119]

[120] The preferred compounds among the compound of formula (1) above are those wherein

[121] I) R represents H;

[122] II) R^1 represents -CH_2COOH, -CH_2COOR^3 (R^3 = -SAC), or -$CH_2CONHSO_2R^4$ (R^4 = -SAC);

[123] III) R^2 represents H, -SAC, -Ar, or -$(CH_2)_nOR^7$ (R^7 = -SAC, -SCAC, -Ar, or -SAC-Ar, and n = 1 or 2);

[124] IV) A represents -$(NR^9)_n$- (R^9 is H, -SAC, -SCAC, -Ar, or -SAC-Ar, and n=0-1),

[125] V) B represents H, -SAC, -SCAC, -Ar, or -SAC-Ar, or

[126] VI) X represents -$COCHN_2$, -$COCH_2F$, -$COCH_2Cl$, -$COCH_2Br$, -$COCH_2I$, -$COCH_2OAr$, -$COCH_2OCOAr$, or -$COCH_2SR^{17}$ (R^{17} is -SAC, -SCAC, -Ar, or -SAC-Ar).

[127]

[128] The more preferred compounds are those selected from the group consisting of the following:

[129] (3*S*)-3-{[(3-benzoyl-5-ethyl-4,5-dihydro-5-isoxazolyl)carbonyl] amino}-5-(2,6-dichlorobenzoyl)oxy]-4-oxopentanoic acid(1);

[130] (3S)-3-({[5-ethyl-3-(1-naphthoyl)-4,5-dihydro-5-isoxazolyl] carbonyl}amino)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid(2);

[131] 3-({[5-Ethyl-3-(1-naphthoyl)-4,5-dihydro-5-isoxazolyl] carbonyl}amino)-5-fluoro-4-oxopentanoic acid(3);

[132] 3-({[5-Ethyl-3-(1-isoquinolinylcarbonyl)-4,5-dihydro-5-isoxazolyl]carbonyl}

amino)-5-fluoro-4-oxopentanoic acid(4);

[133] (3S)-3-[({5-ethyl-3-[(1-naphthylamino)carbonyl]-4,5-dihydro-5-isoxazolyl} carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid(5);

[134] 3-[({5-Ethyl-3-[(1-naphthylamino)carbonyl]-4,5-dihydro-5-isoxazolyl}carbonyl) amino]-5-fluoro-4-oxopentanoic acid(6);

[135] (3*S*)-3-[({3-[([1,1'-biphenyl]-2-ylamino)carbonyl] -5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid(7);

[136] 3-[({3-[([1,1'-Biphenyl]-2-ylamino)carbonyl]-5-ethyl-4,5-dihydro-5-isoxazolyl} carbonyl)amino]-5-fluoro-4-oxopentanoic acid(8);

[137] 3-{[(5-Ethyl-3-{[(2'-methyl[1,1'-biphenyl]-2-yl)amino] carbonyl}-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-fluoro-4-oxopentanoic acid(9);

[138] 3-[({3-[3,4-Dihydro-1(2*H*)-quinolinylcarbonyl]-5-ethyl-4,5-dihydro-5-isoxazolyl} carbonyl)amino]-5-fluoro-4-oxopentanoic acid(10);

[139] (3S)-3-[({3-[3,4-dihydro-2(1*H*)-isoquinolinylcarbonyl] -5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy) pentanoic acid(11);

[140] 3-{[(3-{[2-(*tert*-Butyl)anilino]carbonyl}-5-ethyl-4,5-dihydro-5-isoxazolyl) carbonyl]amino}-5-fluoro-4-oxopentanoic acid(12);

[141] 3-{[(3-{[2-(*tert*-Butyl)anilino]carbonyl}-5-isopropyl-4,5-dihydro-5-isoxazolyl) carbonyl]amino}-5-fluoro-4-oxopentanoic acid(13); and

[142] 3-{[(3-{[2,5-Di(*tert*-butyl)anilino] carbonyl}-5-isopropyl-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-fluoro-4-oxopentanoic acid(14).

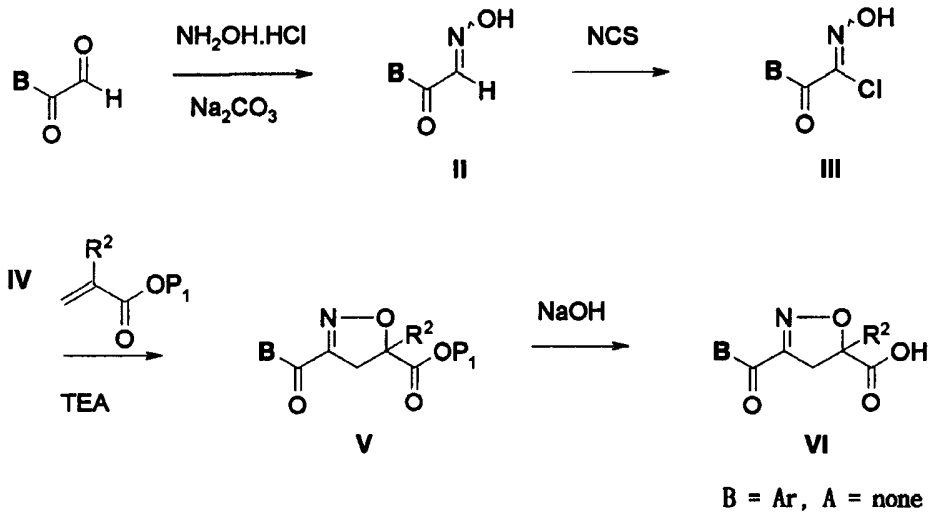
[143]

[144] The processes for preparation of the novel dicarbonylamino-isoxazoline derivative of formula (1) showing an inhibitory activity against caspases are depicted in the following Reaction Schemes 1 to 5. However, those illustrated in the following Reaction Schemes represent only the typical processes used in the present invention. The manipulation order may be changed with no limit, and so the processes are not restricted to those explained below.

[145]

[146] <u>Reaction Scheme 1</u>

[147]



[148] in which

[149] P^1 represents simple alkyl chain.

[150] VI a) B=Phenyl, R^2=Et

[151] b) B=1-Naphthyl, R^2=Et

[152] c) B=1-Isoquinolinyl, R^2=Et

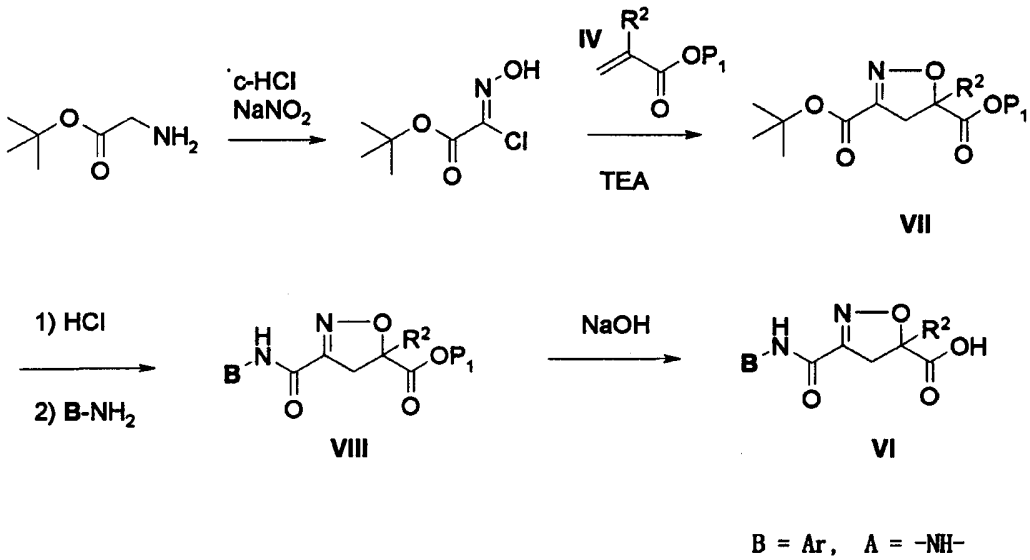
[153]

[154] In Reaction Scheme 1 above, a glyoxal derivative is reacted with hydroxylamine-hydrochloride and sodium carbonate in a solvent mixture of alcohol-water to give an oxime derivative (II) (a mixture of *syn* and *anti* oximes). The resulting oxime derivative (II) is treated by NCS (N-chlorosuccinimide) in dimethylformamide solution to give a hydroxamoyl chloride derivative (III). Thus obtained hydroxamoyl chloride derivative (III) is reacted with an acrylate derivative (IV) (see the following Reaction Scheme 5) to give an isoxazoline derivative (V), which is then hydrolyzed, if needed, to give a deprotected isoxazoline derivative (VI). If appropriate, the oxime derivative (II), the acrylate derivative (IV), and NaOCl may react together in a reaction vessel (*in situ*) to directly give the isoxazoline derivative (V).

[155]

[156] Reaction Scheme 2

[157]



[158] **VI** e) B = 1-Naphthyl, A = NH, R^2 = Et

[159] f) B = [1,1'-Biphenyl]-2-yl, A = NH, R^2 = Et

[160] g) B = (2'-Methyl[1,1'-biphenyl]-2-yl), A = NH, R^2 = Et

[161] h) B-A = 3,4-Dihydro-1(2*H*)-quinolinyl, R^2 = Et

[162] i) B-A = 3,4-Dihydro-2(1*H*)-isoquinolinyl, R^2 = Et

[163] j) B = 2-t-Butyl-1-phenyl, A = NH, R^2 = Et,

[164] k) B = 2-t-Butyl-1-phenyl, A = NH, R^2 = i-Pr

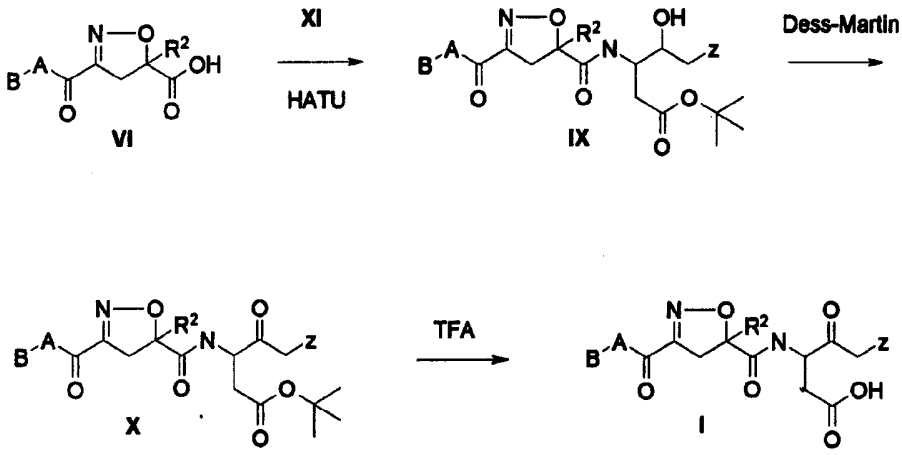
[165]

[166] In Reaction Scheme 2, glycine t-butyl ester · HCl synthesized according to a known process (*J. of Chemical Society, PT* 1, **1997**, 3005) is used to give *tert*-butyl 2-chloro-2-(hydroxyimino)acetate, which is then reacted with the acrylate derivative (IV) to give an isoxazoline derivative (VII). Thus obtained isoxazoline derivative (VII) is deprotected and fused with an amine group to give an isoxazoline derivative (VIII), which may be hydrolyzed to give the deprotected isoxazoline derivative (VI).

[167]

[168] Reaction Scheme 3

[169]



[170]

[171] **I** a) B = Phenyl, A = None, R^2 = Et, Z = OCO-Ph(2,6-dichloro)

[172] b) B = 1-Naphthyl, A = None, R^2 = Et, Z = OPh (2,3,5,6-tetrafluoro)

[173] c) B = 1-Naphthyl, A = None, R^2 = Et, Z = F

[174] d) B = 1-Isoquinolinyl, A = None, R^2 = Et, Z = F

[175] e) B = 1-Naphthyl, A = NH, R^2 = Et, Z = OPh (2,3,5,6-tetrafluoro)

[176] f) B = 1-Naphthyl, A = NH, R^2 = Et, Z = F

[177] g) B = [1,1'-Biphenyl]-2-yl, A = NH, R^2 = Et, Z = OPh (2,3,5,6-tetrafluoro)

[178] h) B = [1,1'-Biphenyl]-2-yl, A = NH, R^2 = Et, Z = F

[179] i) B = (2'-Methyl[1,1'-biphenyl]-2-yl), A = NH, R^2 = Et, Z = F

[180] j) B-A = 3,4-Dihydro-1(2*H*)-quinolinyl, R^2 = Et, Z = F

[181] k) B-A = 3,4-Dihydro-2(1*H*)-isoquinolinyl, R^2 = Et, Z = OPh (2,3,5,6-tetrafluoro)

[182] l) B = 2-t-Butyl-1-phenyl, A = NH, R^2 = Et, Z = F

[183] m) B = 2-t-Butyl-1-phenyl, A = NH, R^2 = i-Pr, Z = F

[184]

[185] As depicted in Reaction Scheme 3 above, the carboxylic acid derivative (VI) is coupled with an aspartic acid derivative (XI) (see the following Reaction Scheme 4) to give a compound (IX), which is then subjected to Dess-Martin periodene oxidation reaction, and deprotection reaction, if needed, to give the desired compound of formula (1).

[186]

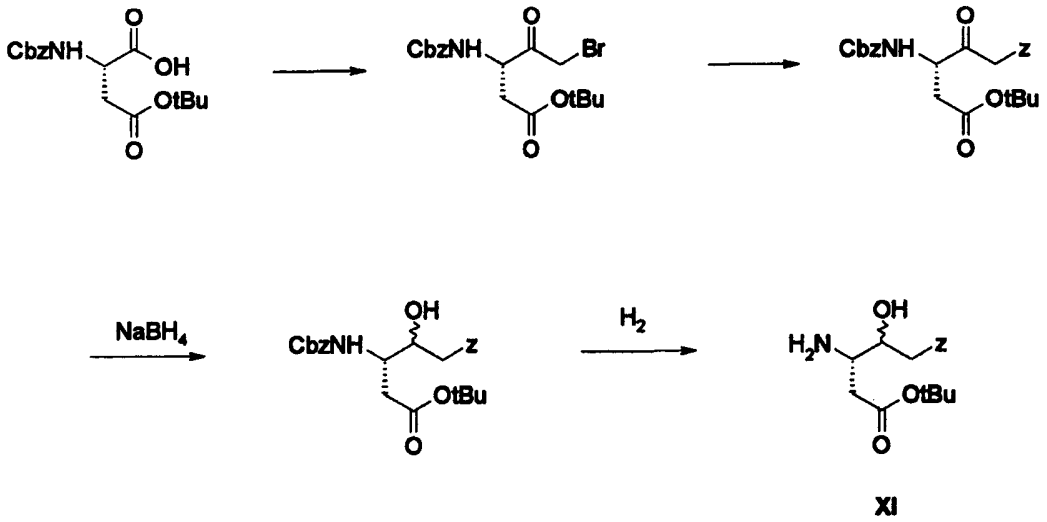
[187] The functional group Z in the compound (I) of Reaction Scheme 3 may be formed first by synthesizing the compound (XI) already having the desired Z group as depicted in the following Reaction Scheme 4, and by coupling the compound (XI) with the carboxylic acid compound (VI) on the left side (see WO 00/23421). Or, the desired Z group may be introduced later according to the process of Reaction Scheme 4 after the carboxylic acid compound (VI) is combined with an aspartic acid (β-t-Bu) methyl ester

and hydrolyzed. When Z is F, the racemic compound may be prepared according to a method known in *Tetrahedron Letters*, **1994**, *35*(52), 9693-9696.

[188]

[189] Reaction Scheme 4

[190]



XI

[191]

[192] **XI** a) Z = OCOPh (2,6-dichloro)

[193] b) Z = OPh (2,3,5,6-tetrafluoro)

[194] c) Z = F (racemic)

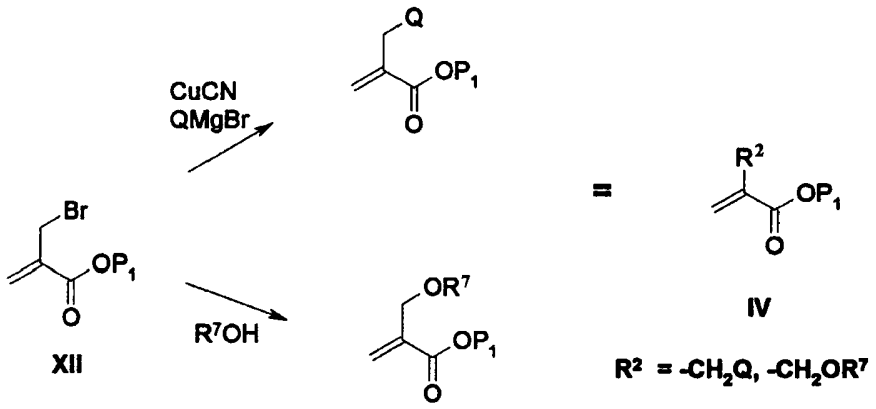
[195]

[196] The acrylate derivative (IV) used as a reactant in the above Reaction Schemes 1 and 2 may be prepared via two (2) pathways. That is, the compound (IV) can be easily prepared from a known compound (XII) (*Synthesis*, **1982,** p 924) as depicted in the following Reaction Scheme 5, or in case the compound (IV) is methyl (ethyl) 2-i-propylacrylate, the compound (IV) can be prepared from dimethyl(diethyl)malonate (*J. Chemical Society Perkin Trans. 1* **1997**, 1559-1570).

[197]

[198] Reaction Scheme 5

[199]



[200]

[201] **IV** a) P_1 = Et, R^2= Me (Commercially available)

[202] b) P_1 = Et, R^2 = Et

[203] c) P_1 = Me, R^2 = i-Pr

[204] d) P_1 = Et, R^2 = n-Pr

[205] e) P_1 = Et, R^2 = n-Bu

[206] f) P_1 = Et, R^2 = i-Bu

[207] g) P_1 = Et, R^2 = CH_2Ph

[208] h) P_1 = Et, R^2 = CH_2OPh

[209] i) P_1 = Et, R^2 = CH_2OMe

[210]

[211] The compound of formula (1) according to the present invention has a broad spectrum of inhibitory activity against caspases as demonstrated by the results of the following Experiments, and so has an effect for preventing inflammation and apoptosis. Thus, the present invention provides a composition for inhibiting caspases, specifically a therapeutic composition for preventing inflammation and apoptosis, comprising the compound of formula (1), or pharmaceutically acceptable salt, physiologically hydrolysable ester, hydrate, solvate, or stereoisomer thereof as an active ingredient together with the pharmaceutically acceptable carrier. Specifically, the composition of the present invention has a therapeutic effect for dementia, cerebral stroke, brain impairment due to AIDS, diabetes, gastric ulcer, cerebral injure by hepatitis, hepatitis-induced hepatic diseases, acute hepatitis, fulminant hepatic failure, sepsis, organ transplantation rejection, rheumatic arthritis, cardiac cell apoptosis due to ischemic cardiac diseases, or liver cirrhosis.

[212]

[213] Caspase inhibitor, particularly the compound of formula (1), may be formulated into various pharmaceutical forms for administration purpose. To prepare the pharmaceutical composition according to the present invention, an effective amount of the caspase inhibitor, particularly the compound of formula (1), or pharmaceutically

acceptable salt, physiologically hydrolysable ester, hydrate, solvate, or stereoisomer thereof, is mixed with a pharmaceutically acceptable carrier that may take a wide variety of forms depending on the formulation to be prepared.

[214]

[215] The caspase inhibitor compound may be formulated as a parenteral injection, or percutaneous or oral preparation, depending on its application purpose. It is especially advantageous to formulate the composition in a unit dosage form for ease of administration and uniformity of dosage.

[216]

[217] For the oral preparation, any usual pharmaceutical carrier may be used. For example, water, glycols, oils, alcohols and the like may be used for such oral liquid preparations as suspensions, syrups, elixirs and solutions; or starches, sugars, kaolin, lubricants, binders, disintegrating agents and the like may be used for such solid preparations as powders, pills, capsules and tablets. Due to their ease of administration, tablets and capsules are the most advantageous dosage unit forms. It is also desirable for tablets and pills to be formulated into enteric-coated preparation.

[218]

[219] For the parenteral preparation, sterile water is usually used as the carrier, though other ingredients such as solubility aids may be used. Injections, for example, sterilized aqueous or oily suspension for injection, can be prepared according to the known procedure using suitable dispersing agent, wetting agent, or suspending agent. Solvents that can be used for preparing injections include water, Ringer's fluid, and isotonic NaCl solution, and also sterilized fixing oil may be conveniently used as the solvent or suspending media. Any non-stimulative fixing oil including mono- or di-glyceride may be used for this purpose. Fatty acid such as oleic acid may also be used for injections.

[220]

[221] For the percutaneous administration, the carrier may include a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives having no significant skin irritation. Said additives may facilitate the administration through the skin and/or may assist preparation of a desired composition. These percutaneous preparations are administered via various manners, e.g., as a transdermal patch, a spot-on, or an ointment.

[222]

[223] When the caspase inhibitor, specifically the compound of formula (1), is used for clinical purpose, it is preferable to administer to the subject patient in an amount ranging from 0.1 to 100 □ per □ of body weight a day. The total daily dosage may be administered once or over several times. However, specific administration dosage for an individual patient can be varied with specific compound used, body weight, gender,

hygienic condition, or diet of subject patient, time or method of administration, excretion rate, mixing ratio of agent, severity of disease to be treated, etc.

[224]

[225] The present invention will be more specifically explained by the following examples. However, it should be understood that these examples are intended to illustrate the present invention but not in any manner to limit the scope of the present invention. In the following examples, the compounds of Examples 3 to 14 were prepared according to the same procedure as Example 1 or 2.

[226]

[227] **Preparation 1**

[228] **2-Oxo-2-phenylacetaldehyde oxime**

[229] Phenyl glyoxal hydrate (5.09g, 33.5mmol) was dissolved in ethanol (60 □)-water (30 □), hydroxylamine hydrochloride (2.80g, 1.2eq) and anhydrous sodium carbonate (Na_2CO_3, 2.13g, 0.6eq) were added thereto at 0℃, and the mixture was stirred for one hour at room temperature. Saturated aqueous sodium chloride solution (100 □) was added thereto, and the mixture was extracted twice with ethyl acetate (300 □). The extract was washed with dilute aqueous sodium bicarbonate solution ($NaHCO_3$, 100 □ x 2), dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to give the title yellow oxime compound in a stoichiometric yield (5.06g).

[230] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.35(bs, 1H), 8.05(m, 3H), 7.59(t, 1H), 7.48(m, 2H)

[231]

[232] **Preparation 2**

[233] ***N*-hydroxy-2-oxo-2-phenylethanimidoyl chloride**

[234] The oxime compound prepared in Preparation 1 (33.5 mmol) was dissolved in dimethylformamide (100 □), and N-chlorosuccinimide (4.70g, 1.05eq) was added thereto. This solution was stirred for one hour in a water bath of about 40℃, and the volatile solvent was removed by distillation under reduced pressure. The residue was dissolved in ethyl acetate-hexane (1:1, 150 □), washed with water (100 □ x 3), dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to give the title compound (3.86g, 99%). This compound was used in the next reaction without further purification.

[235] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.75(s, 1H), 7.99(d, 2H), 7.62(t, 1H), 7.47(t, 2H)

[236]

[237] **Preparation 3**

[238] **Ethyl 2-ethylacrylate**

[239] To CuCN (26.9g, pre-dried under vacuum) was added about 500 □ of anhydrous tetrahydrofuran under nitrogen atmosphere. The mixture was maintained at -78℃, and 100 □ of methyl magnesium bromide (3.0M in ethylether) was slowly added thereto,

with stirring by mechanical stirrer. The thick mixture was stirred for about 30 minutes at -78°C, and ethyl 2-bromomethylacrylate (28.9g, 150 mmol, Synthesis: Villieras, J. and Rambaud, M. Synthesis, 1982, *914*) dissolved in about 30 □ of anhydrous tetrahydrofuran was slowly added thereto. The reaction mixture was slowly warmed to room temperature over 2 hours. Saturated aqueous ammonium chloride solution (~50 □) was slowly added thereto to complete the reaction. The reaction mixture was filtered through a cellit to remove the precipitate, and washed with ethylether. The organic layer was washed with water and saturated aqueous sodium bicarbonate solution (300 □ x 2), dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to give transparent liquid (26.7g, stoichiometric yield). This liquid was identified to be the title compound having about 75% weight purity by ^{1}H-NMR ($CDCl_3$, 500MHz) analysis.

[240] ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.12(1H, s), 5.50(1H, s), 4.20(2H, q, J = 7.3 Hz), 2.31(2H, qt), 1.28(3H, t, J = 7.3 Hz), 1.07(3H, t, J = 7.8Hz)

[241]

[242] **Preparation 4**

[243] **Ethyl 3-benzoyl-5-ethyl-4,5-dihydro-5-isoxazolecarboxylate**

[244] Hydroxamoyl chloride prepared in Preparation 2 (1.84, 10.0mmol) and ethyl 2-ethylacrylate prepared in Preparation 3 (1.54g, 1.2eq) were dissolved in anhydrous ether (100 □) under nitrogen atmosphere, and maintained at -78°C. Triethylamine (2.79 □ , 2.02g, 2.0eq) was added thereto. The mixture was stirred overnight while slowly warming it to room temperature. Water (100 □) was added thereto, and the mixture was extracted with ethyl acetate (100 □ x 2), washed with water (100 □), dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was separated by column chromatography (10% ethyl acetate-hexane) to give the title compound (1.05g, 38%), which was then analyzed by ^{1}H-NMR to be a mixture of about 1:1 di-astereomers.

[245] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.20(d, 2H), 7.59(t, 1H), 7.47(t, 2H), 4.28(m, 2H), 3.78(d, 1H), 3.30(d, 1H), 2.06(m, 2H), 1.32(t, 3H), 1.00(t, 3H)

[246]

[247] **Preparation 5**

[248] **3-Benzoyl-5-ethyl-4,5-dihydro-5-isoxazolecarboxylic acid**

[249] The compound prepared in Preparation 4 (260mg) was dissolved in distilled tetrahydrofuran (10 □), and 1N aqueous sodium hydroxide solution (1.42 □ , 1.5eq) was added thereto. After about 3 hours, the solution was neutralized by 1N aqueous hydrochloric acid solution, and distilled under reduced pressure to remove most of the tetrahydrofuran. The residue was dissolved in excess ethyl acetate (50 □), washed with aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to give the title compound in a stoichiometric yield. This compound

was used in the next reaction without further purification.

[250] Mass : M+H 248

[251]

[252] **Preparation 6**

[253] **(3*S*)-3-{[(benzyloxy)carbonyl]amino}-5-(*tert*-butoxy)-2-hydroxy-5-oxopentyl 2,6-dichlorobenzoate**

[254] To N-benzyloxycarbonyl-β-t-butyl-aspartic acid (5.03g, 15.6mmol) and NMM (1.90 □ , 17.1mmol) was added anhydrous tetrahydrofuran (60 □) under nitrogen atmosphere, which was then maintained at -15℃. Isobutylchloroformate (2.12 □ , 16.3mmol) was added thereto, and the mixture was stirred for about 20 minutes. While maintaining the reaction temperature at 0℃, diazomethane-ether solution (synthesized from 2.0eq of 1-methyl-3-nitro-1-nitroso-guanidine, 60 □) was added thereto to give diazoketone derivative (~30 minutes). 30% HBr/AcOH (6.42 □ , 2.0eq) was added thereto to give bromomethylketone derivative (30-60 minutes), which was then extracted with ethyl acetate, washed with saturated aqueous sodium bicarbonate solution (x2) and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to give the bromomethylketone derivative (6.4g).

[255]

[256] The bromomethylketone derivative (4.36g) and 2,6-dichlorobenzoic acid (2.28g, 1.1eq) were dissolved in dimethylformamide (18 □), KF (1.58g, 2.5eq) was added thereto, and the mixture was stirred for 2 hours. The mixture was concentrated under reduced pressure, extracted with ethyl acetate, washed with saturated aqueous sodium bicarbonate solution (x2) and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated again under reduced pressure to give 2,6-dichlorobenzoyloxymethylketone derivative. This compound was dissolved in methanol (20 □) and reacted by adding $NaBH_4$(412mg)-methanol solution (40 □) (-10℃-r.t., 2 hours). The reaction was stopped by acetic acid, and distillation under reduced pressure was carried out to remove methanol. The residue was extracted with ethyl acetate (50 □ x 2), washed with water and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was separated-purified by column chromatography (ethyl acetate-hexane, 1:5) to give the title compound (4.80g, 86%) in a diastereomeric form.

[257] ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.3-7.2(m, 8H), 5.9(m, 1H), 5.2(m, 4H), 4.7(m, 1H), 2.9(m, 1H), 2.7(m, 1H), 1.4(s, 9H)

[258]

[259] **Preparation 7**

[260] **(3*S*)-3-amino-5-(*tert*-butoxy)-2-hydroxy-5-oxopentyl 2,6-dichlorobenzoate**

[261] The compound prepared in Preparation 6 was dissolved in methanol (300 □), Pd/C

(10%, 1.50g) was added thereto, and benzyloxycarbonyl group was removed (Pd/C) for 3 hours in a hydrogen balloon to give the title compound (100%).

[262] ^{1}H-NMR (400MHz, DMSO-d_6) δ 8.2(br, 2H), 7.6-7.5(m, 3H), 6.1(m, 1H), 4.4-3.9(m, 3H), 3.0-2.6(m, 2H), 1.4(s, 9H)

[263]

[264] **Preparation 8**

[265] **(3*S*)-3-{[(3-benzoyl-5-ethyl-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-(*tert*-butoxy)-2,5-dioxopentyl 2,6-dichlorobenzoate**

[266] A mixture of the carboxylic acid derivative prepared in Preparation 5 (VIa, 80mg, 0.324mmol), the amino alcohol derivative prepared in Preparation 7 (147mg, 1.1eq), and HATU (160mg, 1.3eq) was cooled to 0℃, triethylamine (0.14 □ , 3.0eq) in DMF (5 □) solvent was added thereto, and the resulting mixture was reacted for 5 hours. The solvent was distilled off under reduced pressure, and the residue was extracted with ethyl acetate (30 □ x 2), washed with water, aqueous sodium bicarbonate solution, and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (20-30% ethyl acetate-hexane) to give Compound [IX(a)] (172mg, 87%). To this compound and Dess-Martin reagent (356mg, 3.0eq) was added anhydrous dichloromethane (4 □), which was then stirred for 1 hour at room temperature. Isopropyl alcohol (1 □) was added thereto to stop the reaction. The solid was removed by filtration through cellite under reduced pressure, and the filtrate was extracted with ethyl acetate (20 □ x 2). The extract was washed with water, saturated aqueous sodium bicarbonate solution, and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was primarily purified by column chromatography (20-25% ethyl acetate-hexane) to give the title compound (138mg, 82%) in a di-astereomeric form.

[267] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.15(m, 2H), 7.70(m, 1H), 7.59(m, 1H), 7.45(m, 2H), 7.34-7.27(m, 3H), 5.18-5.02(m, 2H), 4.95(m, 1H), 3.72(d, 1H), 3.35(two d, 1H), 2.97-2.79(m, 2H), 2.17(m,1H), 1.99(m, 1H), 1.45 & 1.38(two s, 9H), 1.06 & 1.01(two t, 3H)

[268]

[269] **Example 1**

[270] **(3*S*)-3-{[(3-benzoyl-5-ethyl-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-[(2,6-dichlorobenzoyl)oxy]-4-oxopentanoic acid**

[271] The compound prepared in Preparation 8 (118mg) was dissolved in dichloromethane (4 □), and trifluoroacetic acid (2 □) was added thereto at 0℃. While slowly warming the mixture to room temperature, it was stirred for 2 hours, concentrated under reduced pressure, and purified by Prep-TLC (10% methanol-

dichloromethane) to give the title compound (91mg, 85%, white powder).

[272] ^{1}H-NMR (400MHz, DMSO-d_6) δ 8.71(bs, 1H), 8.03(d, 2H), 7.69(m, 1H), 7.61-7.36(m, 5H), 5.16(m, 2H), 4.82(m, 1H), 3.60(m, 2H), 3.11-2.70(m, 2H), 2.06-1.91(m,2H), 0.89(m, 3H)

[273]

[274] **Preparation 9**

[275] **2-(1-Naphthyl)-2-oxo-acetaldehyde oxime**

[276] To 1'-acetonaphthone (5.11g, 30.0mmol) and $CuBr_2$ (8.04g, 1.2eq) was added ethyl acetate (100 □), which was then refluxed over one day. $CuBr_2$ (0.3eq) was added thereto further, and the mixture was refluxed again for 3 hours until 1'-acetonaphthone was removed. The solid was removed by filtration through cellite, and the organic layer was washed 2~3 times with water, washed with aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to give 7.7g of crude compound.

[277]

[278] The compound obtained above (2.38g, 9.55mmol) was dissolved in DMSO (20 □), and stirred for one day at room temperature. The reaction mixture was dissolved in ethyl acetate (100 □), washed with water (100 □ x 3) and aqueous sodium chloride solution, and purified by column chromatography (50% ethyl acetate-hexane) to give glyoxal (1.0g, Purity 50%). The glyoxal was dissolved in ethanol (20 □)-water (10 □), hydroxylamine hydrochloride (415mg, 1.1eq) and anhydrous sodium carbonate (Na_2CO_3, 320mg, 0.55eq) were added thereto at 0°C, and the mixture was stirred for 1 hour at room temperature. Saturated aqueous sodium chloride solution (30 □) was added thereto, and the mixture was extracted twice with ethyl acetate (60 □), dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to give the title yellow oxime compound (530mg, 49%).

[279] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.84(d, 1H), 8.25(bs, 1H), 8.09(s, 1H), 8.03(d, 1H), 7.91(d, 1H), 7.86(d, 1H), 7.62-7.51(m, 3H)

[280]

[281] **Preparation 10**

[282] ***N*-hydroxy-2-(1-naphthyl)-2-oxoethanimidoyl chloride**

[283] The oxime compound prepared in Preparation 9 (530mg, 2.66mmol) was dissolved in dimethylformamide (10 □), and N-chlorosuccinimide (373mg, 1.05eq) was added thereto. This solution was stirred for 1 hour in a water bath of about 40°C, and the volatile solvent was removed by distillation under reduced pressure. The residue was dissolved in ethyl acetate-hexane (1:1, 50 □), washed with water (20 □ x 2) and saturated aqueous sodium chloride solution (30 □), dried (anhydrous $NaSO_4$), and concentrated under reduced pressure to give the title compound (621mg) in a stoi-

chiometric yield. This compound was used in the next reaction without further purification.

[284]

[285] **Preparation 11**

[286] **Ethyl 5-ethyl-3-(1-naphthoyl)-4,5-dihydro-5-isoxazolecarboxylate**

[287] The hydroxamoyl chloride prepared in Preparation 10 (2.66mmol) and ethyl 2-ethylacrylate (374mg, 1.1eq) were dissolved in anhydrous ether (30 □) under nitrogen atmosphere, maintained at 0℃, and triethylamine (0.74 □ , 538mg, 2.0eq) was added thereto. While slowly warming the mixture to room temperature, it was stirred for 2 hours. Water (30 □) was added thereto, and the mixture was extracted with ethyl acetate (50 □ x 2), washed with saturated aqueous sodium bicarbonate solution and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (10% ethyl acetate-hexane) to give the title compound (500mg, 57%).

[288] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.42(d, 1H), 8.08(d, 1H), 8.05(d, 1H), 7.91(d, 1H), 7.62-7.52(m, 3H), 4.32(m, 2H), 3.87(d, 1H), 3.39(d, 1H), 2.11(m, 2H), 1.36(t, 3H), 1.04(t, 3H)

[289]

[290] **Preparation 12**

[291] **5-Ethyl-3-(1-naphthoyl)-4,5-dihydro-5-isoxazolecarboxylic acid**

[292] The compound prepared in Preparation 11 (500mg) was dissolved in distilled tetrahydrofuran (10 □), and 1N aqueous sodium hydroxide solution (1.69 □ , 1.5eq) was added thereto. After about 0.5 hour, the solution was neutralized by 1N aqueous hydrochloric acid solution, and distilled under reduced pressure to remove most of the tetrahydrofuran. The residue was dissolved in excess ethyl acetate (50 □), washed with aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (30% EA/Hex-10% to 20% MeOH/CH_2Cl_2) to give the title compound (440mg, 96%) as yellow powder.

[293] Mass : M+H 298

[294]

[295] Preparation 13

[296] ***tert*-Butyl (3*S*)-3-{[(benzyloxy)carbonyl] -amino}-4-hydroxy-5-(2,3,5,6-tetrafluorophenoxy)pentanoate**

[297] To N-benzyloxycarbonyl-β-t-butyl-aspartic acid (18.5g, 55.7mmol) and NMM (6.78 □ , 1.1eq) was added anhydrous tetrahydrofuran (180 □) under nitrogen atmosphere, and the mixture was maintained at -15℃. Then, isobutylchloroformate (7.63 □ , 1.05eq) was added thereto, and the mixture was stirred for about 20 minutes.

While maintaining the reaction temperature at 0℃, diazomethane-ether solution (synthesized from 2.0eq of 1-methyl-3-nitro-1-nitroso-guanidine, 60 □) was added thereto to give diazoketone derivative (~3 minutes). 30% HBr/AcOH (23.3 □ , 2.0eq) was added thereto to give bromomethylketone derivative (30-60 minutes), which was then extracted with ethyl acetate, washed with saturated aqueous sodium bicarbonate solution (x2) and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to give the bromomethylketone derivative (23g) in a stoichiometric yield.

[298]

[299] The bromomethylketone derivative (23g, 55.7mmol) and 2,3,5,6-tetrafluorophenol (10.2g, 1.1eq) were dissolved in dimethylformamide (150 □), KF (8.14g, 2.5eq) was added thereto, and the mixture was stirred for 2 hours at room temperature. The mixture was concentrated under reduced pressure, extracted with ethyl acetate, washed with saturated aqueous sodium bicarbonate solution (x2) and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated again under reduced pressure to give 2,3,5,6-tetrafluorophenoxymethylketone derivative. This compound was dissolved in methanol (150 □) and reacted by slowly adding $NaBH_4$(4.24g) (0℃-r.t., 2 hours). The reaction was stopped by acetic acid, and distillation under reduced pressure was carried out to remove methanol. The residue was extracted with ethyl acetate (200 □ x 2), washed with water and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was separated-purified by column chromatography (15% ethyl acetate/hexane) to give the title compound (20.2g, 74%) in a diastereomeric form.

[300] Mass : M+H 488

[301]

[302] **Preparation 14**

[303] ***tert*-Butyl (3*S*)-3-amino-4-hydroxy-5-(2,3,5,6-tetrafluorophenoxy)-pentanoate (XIb)**

[304] The compound prepared in Preparation 13 was dissolved in methanol (300 □), Pd/C (10%, 1.50g) was added thereto, and benzyloxycarbonyl group was removed (Pd/C) for 3 hours in a hydrogen balloon to give the title compound (95%).

[305] ^{1}H-NMR (400MHz, DMSO-d_6) δ 8.2(br, 2H), 7.6-7.5(m, 1H), 5.9(m, 1H), 4.3-4.1(m, 3H), 3.6(m, 1H), 2.7(m, 1H), 1.4(s, 9H)

[306]

[307] **Preparation 15**

[308] ***tert*-Butyl (3*S*)-3-({[5-ethyl-3-(1-naphthoyl)-4,5-dihydro-5-isoxazolyl] carbonyl}-amino)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoate**

[309] A mixture of the carboxylic acid derivative prepared in Preparation 12 (140mg,

0.471mmol), the aminoalcohol derivative prepared in Preparation 14 (182mg, 1.1eq) and HATU (233mg, 1.3eq) was cooled to 0℃, triethylamine (0.20 □ , 3.0eq) in DMF (5 □) solvent was added thereto, and the resulting mixture was reacted for 5 hours. The solvent was distilled off under reduced pressure. The residue was extracted with ethyl acetate (30 □ x 2), washed with water, aqueous sodium bicarbonate solution, and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography to give Compound[IX(b)] (248mg, 83%). To this compound and Dess-Martin reagent (482mg, 3.0eq) was added anhydrous dichloromethane (8 □), which was then stirred for 1 hour at room temperature. Isopropyl alcohol (1 □) was added thereto to stop the reaction. The solid was removed by filtration through cellite under reduced pressure. The residue was extracted with ethyl acetate (30 □ x 2), washed with water, saturated aqueous sodium bicarbonate solution, and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was primarily purified by column chromatography (20-25% ethyl acetate-hexane) to give the title compound (150mg, 65%) in a diastereomeric form.

[310] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.35(t, 1H), 7.97(m, 2H), 7.82(d, 1H), 7.52-7.42(m, 4H), 6.70(m, 1H), 5.08-4.90(m, 3H), 3.72(two d, 1H), 3.34(two d, 1H), 2.96-2.72(m, 2H), 2.11(m, 1H), 1.95(m, 1H), 1.34 & 1.28(two s, 9H), 0.97(two t, 3H)

[311]

[312] **Example 2**

[313] **(3*S*)-3-({[5-ethyl-3-(1-naphthoyl)-4,5-dihydro-5-isoxazolyl] carbonyl}amino)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid**

[314] The compound prepared in Preparation 15 (150mg, 0.238mmol) was dissolved in dichloromethane (4 □), and trifluoroacetic acid (2 □) was added thereto at 0℃. While slowly warming the mixture to room temperature, it was stirred for 1 hour, concentrated under reduced pressure, and purified by Prep-TLC (10% methanol-dichloromethane) to give the title compound (109mg, 80%, white powder).

[315] ^{1}H-NMR (500MHz, DMSO-d_6) δ 8.70(two d, 1H), 8.20(m, 2H), 8.05(m, 1H), 7.98(m, 1H), 7.61(m,4H), 5.24(m, 2H), 4.81(two q, 1H), 3.67(two d, 1H), 3.47(two d, 1H), 2.84(m, 1H), 2.65(m, 1H), 2.00(m, 2H), 0.91(m, 3H)

[316] Mass : M+H 575

[317]

[318] **Preparation 16**

[319] ***tert*-Butyl 3-({[5-ethyl-3-(1-naphthoyl)-4,5-dihydro-5-isoxazolyl]carbonyl} amino)-5-fluoro-4-oxopentanoate**

[320] A mixture of the carboxylic acid derivative prepared in Preparation 12 (170mg, 0.572mmol), the aminoalcohol derivative (**XIc**, 135mg, 1.1eq), and HATU (283mg,

1.3eq) was cooled to 0°C, triethylamine (0.24 □ , 3.0eq) in DMF (5 □) solvent was added thereto, and the mixture was reacted for 5 hours. The solvent was distilled off under reduced pressure. The residue was extracted with ethyl acetate (30 □ x 2), washed with water, aqueous sodium bicarbonate solution, and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (30-40% ethyl acetate-hexane) to give Compound [IX(c)] (200mg, 72%). To this compound and Dess-Martin reagent (520mg, 3.0eq) was added anhydrous dichloromethane (6 □), which was then stirred for 1 hour at room temperature. Isopropyl alcohol (1 □) was added thereto to stop the reaction. The solid was removed by filtration through cellite under reduced pressure. The filtrate was extracted with ethyl acetate (30 □ x 2), washed with water, saturated aqueous sodium bicarbonate solution, and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (30% ethyl acetate-hexane) to give the title compound (186mg, 93%) in a diastereomeric form.

[321] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.41(m, 1H), 8.04(m, 2H), 7.90(d, 1H), 7.65-7.52(m, 4H), 5.20-4.94(m, 3H), 3.78(m, 1H), 3.41(two d, 1H), 3.03-2.7(m, 2H), 2.19(m, 1H), 2.01(m, 1H), 1.42 & 1.35(two s, 9H), 1.04(two t, 3H)

[322]

[323] **Example 3**

[324] **3-({[5-Ethyl-3-(1-naphthoyl)-4,5-dihydro-5-isoxazolyl] carbonyl}amino)-5-fluoro-4-oxopentanoic acid**

[325] The compound prepared in Preparation 16 (180mg, 0.371mmol) was reacted according to the same procedure as Example 2 to give the title compound (quantitative yield).

[326] ^{1}H-NMR (500MHz, DMSO-d_6) δ 8.65(m, 1H), 8.21(m, 2H), 8.02(m, 2H), 7.63(m, 3H), 5.25(m, 2H), 4.79(m, 1H), 3.68(m, 1H), 3.46(m, 1H), 2.83(m, 1H), 2.63(m, 1H), 2.00(m, 2H), 0.90(m, 3H)

[327]

[328] **Preparation 17**

[329] **2-Diazo-1-(1-isoquinolinyl)-1-ethanone**

[330] To 1-isoquinolinecarboxylic acid (1.73g, 10.0mmol) and NMM (1.50 □ , 1.4eq) was added anhydrous tetrahydrofuran (10 □) under nitrogen atmosphere. While maintaining the mixture at 0°C, isobutylchloroformate (1.36 □ , 1.05eq) was added thereto and the mixture was stirred for about 2 hours. While maintaining the reaction mixture at 0°C, diazomethane-ether solution (synthesized from 2.0eq of 1-methyl-3-nitro-1-nitroso-guanidine, 20 □) was added thereto to give diazoketone derivative (1 hour), which was then extracted with ethyl acetate, washed with water,

saturated aqueous sodium bicarbonate solution, and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (10-15% EA/Hex) to give the title diazo derivative (1.50g, 76%, yellow powder).

[331] ^{1}H-NMR (500MHz, $CDCl_3$) δ 9.29(d, 1H), 8.51(d, 1H), 7.88-7.83(m, 2H), 7.76-7.69 (m, 2H), 6.71(s, 1H)

[332]

[333] **Preparation 18**

[334] **2-(1-Isoquinolinyl)-2-oxoacetaldehyde oxime**

[335] The diazo derivative prepared in Preparation 17 (670mg, 3.40mmol) was dissolved in acetone (5 □), dimethyldioxirane-acetone solution (0.1M, 30 □) was added thereto, and the mixture was stirred for 2~3 minutes at room temperature. Water was added thereto, and the mixture was distilled under reduced pressure to remove acetone. The residue was extracted with ethyl acetate, washed with aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was dissolved in ethanol-water (2:1, 20 □), hydroxylamine hydrochloride (236mg, 1.0eq) and anhydrous sodium carbonate (Na_2CO_3, 180mg, 0.5eq) were added thereto at 0°C, and the mixture was stirred for 30 minutes at room temperature. Saturated aqueous sodium chloride solution (30 □) was added thereto. The mixture was extracted twice with ethyl acetate (60 □), dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (20-30% EA/Hex) to give the title oxime derivative (306mg, 45%) having yellow color.

[336] ^{1}H-NMR (500MHz, $CDCl_3$) δ 10.28(bs, 1H), 8.69(d, 1H), 8.62(s, 1H), 8.57(d, 1H), 7.89(d, 1H), 7.84(d, 1H), 7.77-7.69(m, 2H)

[337]

[338] **Preparation 19**

[339] **Ethyl 5-ethyl-3-(1-isoquinolinylcarbonyl)-4,5-dihydro-5-isoxazolecarboxylate**

[340] The compound of Preparation 18 was reacted according to the same procedure as Preparations 10 and 11 to give the title compound.

[341] ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.63(d, 1H), 8.26(d, 1H), 7.90(d, 1H), 7.82(d, 1H), 7.74(t, 1H), 7.66(t, 1H), 4.31(m, 2H), 3.93(d, 1H), 3.39(d, 1H), 2.09(qt, 2H), 1.34(t, 3H), 1.02(t, 3H)

[342]

[343] **Preparation 20**

[344] **t-Butyl 3-({[5-ethyl-3-(1-isoquinolinylcarbonyl)-4,5-dihydro-5-isoxazolyl] carbonyl}amino)-5-fluoro-4-oxopentanoate**

[345] The compound of Preparation 19 was reacted according to the same procedure as Preparations 12 and 16 to give the title compound.

[346] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.61(m, 1H), 8.39(m, 1H), 7.91(d, 1H), 7.84(d, 1H), 7.75(t, 1H), 7.70-7.56(m, 2H), 5.29-5.00(m, 2H), 4.92(m, 1H), 3.86-3.80(two d, 1H), 3.47-3.43(two d, 1H), 3.04-2.76(m, 2H), 2.23-2.15(m, 1H), 2.08-1.98(m, 1H), 1.41-1.36(two s, 9H), 1.03(two t, 3H)

[347]

[348] **Example 4**

[349] **3-({[5-Ethyl-3-(1-isoquinolinylcarbonyl)-4,5-dihydro-5-isoxazolyl]carbonyl} amino)-5-fluoro-4-oxopentanoic acid**

[350] The compound prepared in Preparation 20 (108mg, 0.223mmol) was dissolved in dichloromethane (4 □), and trifluoroacetic acid (2 □) was added thereto at 0℃. While slowly warming the mixture to room temperature, it was stirred for 1 hour, concentrated under reduced pressure, and purified by Prep-TLC (15% methanol-dichloromethane) to give the title compound (68mg, 72%, a little yellow powder).

[351] ^{1}H-NMR (500MHz, DMSO-d_6) δ 8.60(m, 1H), 8.23(m, 1H), 8.13-8.06(m, 2H), 7.87(t, 1H), 7.76(t, 1H), 5.20(bs, 2H), 4.72(m, 1H), 3.75-3.69(two d, 1H), 3.53-3.46(two d, 1H), 2.83-2.65(two bs, 2H), 2.06-1.90(two m, 2H), 0.92-0.84(two t, 3H)

[352] MS ; M+MeOH+H 462

[353]

[354] **Preparation 21**

[355] ***tert*-Butyl 2-chloro-2-(hydroxyimino)acetate**

[356] Glycine t-butyl ester HCl salt (12.0g, 71.6mmmol) synthesized according to a method known in *J. of Chemical Society*, *PT* 1, **1997**, 3005 was dissolved in water (60 □), c-HCl solution (5.97 □ , 1.0eq) was added thereto once at 0℃, and immediately $NaNO_2$ solution (4.95g in 15 □ water, 1.0eq) was added thereto. Again, each 1.0eq of c-HCl and $NaNO_2$ solutions was added thereto. The mixture was stirred for about 10 minutes at 0℃, extracted with ethyl acetate (50 □ x 3), washed with aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to give the title compound (6.3g, 49%).

[357]

[358] **Preparation 22**

[359] **3-(*tert*-Butyl) 5-ethyl 5-ethyl-4,5-dihydro-3,5-isoxazoledicarboxylate**

[360] Ethyl 2-ethylacrylate (3.0g, 23.4mmol, 1.0eq) and triethylamine (6.52 □ , 2.0eq) were dissolved in anhydrous chloroform (40 □) under nitrogen atmosphere, and maintained at 0℃. The hydroxamoyl chloride prepared in Preparation 21 (4.82g, 1.15eq) was dissolved in chloroform (20 □), and this solution was slowly added thereto over 1 hour. While slowly warming the mixture to room temperature, it was stirred overnight. Water (50 □) was added thereto, and the mixture was extracted with ethyl

acetate (100 □ x 2), washed with water (100 □), dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was separated by column chromatography (10% ethyl acetate-hexane) to give the title compound (3.4g, 53%).

[361] ^{1}H-NMR (500MHz, $CDCl_3$) δ 4.26(m, 2H), 3.60(d, 1H), 3.06(d, 1H), 2.00(m, 2H), 1.54(s, 9H), 1.30(t, 3H), 0.95(t, 3H)

[362]

[363] **Preparation 23**

[364] **Ethyl 5-ethyl-3-[(1-naphthylamino)carbonyl]-4,5-dihydro-5-isoxazole carboxylate)**

[365] The compound prepared in Preparation 22 (400mg, 1.47mmol) was dissolved in 4.0 N HCl(g)/EtOAc (30 □), stirred for 2 hours, and concentrated under reduced pressure. To the resulting compound were added 1-naphthylamine (211mg, 1.0eq) and HATU (727mg, 1.3eq). Triethylamine (0.61 □ , 3.0eq) dissolved in DMF (5 □) was added thereto, and the mixture was stirred for one day. The solvent was distilled off under reduced pressure. The residue was extracted with ethyl acetate (30 □ x 2), washed with water, aqueous sodium bicarbonate solution, and aqueous sodium chloride solution, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (10-15% ethyl acetate-hexane) to give the title compound (460mg, 92%).

[366] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.85(bs, 1H), 8.07(d, 1H), 7.90(d, 1H), 7.87(d, 1H), 7.58-7.48(m, 3H), 4.32(m, 2H), 3.78(d, 1H), 3.30(d, 1H), 2.07(m, 2H), 1.35(t, 3H), 1.03(t, 3H)

[367]

[368] **Preparation 24**

[369] ***tert*-Butyl (3*S*)-3-[({5-ethyl-3-[(1-naphthylamino)carbonyl] -4,5-dihydro-5-isoxazolyl}carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoate**

[370] The compound of Preparation 23 was reacted according to the same procedure as Preparations 12 and 15 to give the title compound.

[371] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.82(d, 1H), 8.07(m, 1H), 7.88(m, 2H), 7.73(m, 2H), 7.54(m, 3H), 6.79(two m, 1H), 5.16-4.96(m, 3H), 3.75(dd, 1H), 3.33(d, 1H), 3.07-2.81(m, 2H), 2.18(m,1H), 2.01(m, 1H), 1.43 & 1.41(two s, 9H), 1.03(two t, 3H)

[372] Mass M+Na 668

[373]

[374] **Example 5**

[375] **(3*S*)-3-[({5-ethyl-3-[(1-naphthylamino)carbonyl]-4,5-dihydro-5-isoxazolyl} carbonyl)-amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid**

[376] The compound prepared in Preparation 24 (178mg, 0.276mmol) was dissolved in

dichloromethane (4 □), and trifluoroacetic acid (2 □) was added thereto at 0℃. While slowly warming the mixture to room temperature, it was stirred for 1 hour, concentrated under reduced pressure, and purified by Prep-TLC (7.5% methanol-dichloromethane) to give the title compound (quantitative yield).

[377] ^{1}H-NMR (500MHz, CD_3OD) δ 8.13-8.05(m, 2H), 7.96(d, 1H), 7.78(m, 1H), 7.70-7.61(m, 3H), 7.29-7.11(m, 1H), 3.82-3.46(m, 2H), 3.10-2.92(m, 2H), 2.23(m, 1H), 2.15(m, 1H), 1.16(m, 3H)

[378]

[379] **Example 6**

[380] **3-[({5-Ethyl-3-[(1-naphthylamino)carbonyl]-4,5-dihydro-5-isoxazolyl} carbonyl)amino]-5-fluoro-4-oxopentanoic acid**

[381] The compound of Preparation 23 was reacted according to the same procedure as Preparations 12, 16 and Example 1 to give the title compound.

[382] ^{1}H-NMR (500MHz, DMSO-d_6) δ 10.53(d, 1H), 8.61(bs, 1H), 7.98-7.83(m, 3H), 7.62-7.52(m, 4H), 5.40-4.45(bs, 2H), 4.81-4.70(m, 1H), 3.65-3.58(two set of d, 1H), 3.38(m, 1H), 2.95-2.68(m, 2H), 2.05-1.90(m, 2H), 0.94-0.80(m, 3H)

[383] Mass M+H 444.1

[384]

[385] **Preparation 25**

[386] **Ethyl 3-[([1,1'-biphenyl]-2-ylamino)carbonyl]-5-ethyl-4,5-dihydro-5-isoxazole carboxylate**

[387] The compound of Preparation 22 was reacted according to the same procedure as Preparations 23 using 2-aminobiphenyl instead of 1-naphtylamine to give the title compound.

[388] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.57(d, 1H), 8.42(d, 1H), 7.57-7.15(m, 8H), 4.25(m, 2H), 3.68(d, 1H), 3.18(d, 1H), 1.99(m, 2H), 1.30(t, 3H), 0.94(t, 3H)

[389]

[390] **Preparation 26**

[391] ***tert*-Butyl (3*S*)-3-[({3-[([1,1'-biphenyl]-2-ylamino)carbonyl] -5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoate**

[392] The compound of Preparation 25 was reacted according to the same procedure as Preparations 12 and 15 to give the title compound.

[393] ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.51(s, 1H), 8.39(m, 1H), 7.55-7.22(m, 9H), 6.78(m, 1H), 5.08-4.90(m,3H), 3.61(two d, 1H), 3.21(two d, 1H), 3.03-2.78(m, 2H), 2.08(m, 1H), 1.89(m, 1H), 1.39(two s, 9H), 0.95(two t, 3H)

[394]

[395] **Example 7**

[396] **(3*S*)-3-[({3-[([1,1'-biphenyl]-2-ylamino)carbonyl] -5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid**

[397] The compound prepared in Preparation 26 (106mg, 0.158mmol) was dissolved in dichloromethane (4 □), and trifluoroacetic acid (2 □) was added thereto at 0°C. While slowly warming the mixture to room temperature, it was stirred for 1 hour, concentrated under reduced pressure, and purified by Prep-TLC (7.5% methanol-dichloromethane) to give the title compound (quantitative yield).

[398] ^{1}H-NMR (400MHz, DMSO-d_6) δ 9.51(m, 1H), 8.61(br, 1H), 7.74(m, 1H), 7.51(m, 1H), 7.42-7.30(m, 8H), 5.18(br, 2H), 4.75(m, 1H), 3.42(two d, 1H), 3.13(two d, 1H), 2.91-2.49(m, 2H), 1.98-1.77(m, 2H), 0.82(two t, 3H)

[399] Mass : M+H 616

[400]

[401] **Example 8**

[402] **3-[({3-[([1,1'-Biphenyl]-2-ylamino)carbonyl] -5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-5-fluoro-4-oxopentanoic acid**

[403] The compound of Preparation 25 was reacted according to the same procedure as Preparations 12, 16 and Example 1 to give the title compound.

[404] ^{1}H-NMR (400MHz, DMSO-d_6) δ 9.54(m, 1H), 8.57-8.51(two set of d, 1H), 7.75(m, 1H), 7.47-7.31(m, 8H), 5.22-5.08(m, 2H), 4.76-4.67(m, 1H), 3.46-3.12(m, 2H), 2.95-2.53(m, 2H), 1.90-1.81(m, 2H), 0.88-0.80(m, 3H)

[405] Mass M+H 444.1

[406]

[407] **Preparation 27**

[408] **Ethyl 5-ethyl-3-{[(2'-methyl[1,1'-biphenyl]-2-yl)amino] carbonyl}-4,5-dihydro-5-isoxazolecarboxylate**

[409] The compound of Preparation 22 was reacted according to the same procedure as Preparation 23 using 2-iodoaniline instead of 1-naphtylamine (yield 25%) and then Suzuki coupling (2-tolylboronic acid, Na_2CO_3, $PdCl_2(PPh_3)_2$, 110°C, DMF, 81%) to give the title compound.

[410] ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.43(m, 1H), 8.14(s, 1H), 7.40-7.15(m, 7H), 4.25(m, 2H), 3.64(m, 1H), 3.14(m, 1H), 2.10(s, 3H), 1.94(m, 2H), 1.27(m, 3H), 0.92(m, 3H)

[411]

[412] **Preparation 28**

[413] ***tert*-Butyl 3-{[(5-ethyl-3-{[(2'-methyl[1,1'-biphenyl]-2-yl)amino] carbonyl}-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-fluoro-4-oxopentanoate**

[414] The compound of Preparation 27 was reacted according to the same procedure as

Preparations 12 and 16 to give the title compound.

[415] ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.43(m, 1H), 8.10(s, 1H), 7.42-7.13(m, 8H), 5.13-4.82(m, 3H), 3.59(m, 1H), 3.16(m, 1H), 3.01-2.70(m, 2H), 2.11(two s, 3H), 2.07(m, 1H), 1.87(m, 1H), 1.43(two s, 9H), 0.93(two t, 3H)

[416]

[417] **Example 9**

[418] **3-{[(5-Ethyl-3-{[(2'-methyl[1,1'-biphenyl]-2-yl)amino] carbonyl}-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-fluoro-4-oxopentanoic acid**

[419] The compound prepared in Preparation 28 (61mg, 0.113mmol) was dissolved in dichloromethane (4 □), and trifluoroacetic acid (2 □) was added thereto at 0℃. While slowly warming the mixture to room temperature, it was stirred for 1 hour, concentrated under reduced pressure, and the resulting white-yellow solid was filtered and washed with hexane, to give the title compound (quantitative yield).

[420] ^{1}H-NMR (400MHz, DMSO-d_6) δ 8.82(m, 1H), 8.54(m, 1H), 8.01(m, 1H), 7.44-7.14 (m, 7H), 5.21-5.05(m, 2H), 4.70(m, 1H), 3.37(m, 1H), 3.13(m, 1H), 2.81-2.51(m, 2H), 2.05(two s, 3H), 1.92-1.76(m, 2H), 0.80(two t, 3H)

[421] Mass : M+H 484

[422]

[423] **Preparation 29**

[424] **Ethyl 3-[3,4-dihydro-1(2*H*)-quinolinylcarbonyl] - 5-ethyl-4,5-dihydro-5-isoxazolecarboxylate**

[425] The compound of Preparation 22 was reacted according to the same procedure as Preparation 23 using 1,2,3,4-tetrahydroquinoline instead of 1-naphtylamine to give the title compound.

[426] ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.26-7.10(m, 4H), 4.28(m, 2H), 4.02-3.78(m, 2H), 3.57(m, 1H), 3.13(m, 1H), 2.79(m, 2H), 2.07-1.94(m, 4H), 1.31(t, 3H), 0.92(t, 3H)

[427]

[428] **Preparation 30**

[429] ***tert*-Butyl 3-[({3-[3,4-dihydro-1(2*H*)-quinolinylcarbonyl] - 5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-5-fluoro-4-oxopentanoate**

[430] The compound of Preparation 29 was reacted according to the same procedure as Preparations 12 and 16 to give the title compound.

[431] ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.54(m, 1H), 7.27-7.07(m, 4H), 5.16-4.80(m, 3H), 3.97-3.78(m, 2H), 3.61-2.78(m, 4H), 2.14-1.77(m, 4H), 1.43(two s, 9H), 0.97(two t, 3H)

[432]

[433] **Example 10**

[434] **3-[({3-[3,4-Dihydro-1(2*H*)-quinolinylcarbonyl] -**

5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-5-fluoro-4-oxopentanoic acid

[435] The compound prepared in Preparation 30 (42mg, 0.086mmol) was dissolved in dichloromethane (4 □), and trifluoroacetic acid (2 □) was added thereto at 0℃. While slowly warming the mixture to room temperature, it was stirred for 1 hour, concentrated under reduced pressure, and purified by trituration with dichloromethane/ hexane to give the title compound (quantitative yield).

[436] ^{1}H-NMR (400MHz, DMSO-d_6) δ 8.54(m, 1H), 7.23-7.11(m, 4H), 5.23-5.09(m, 2H), 4.70(m, 1H), 3.80-3.75(m, 2H), 3.21(m, 1H), 2.78-2.60(m, 4H), 1.94-1.90(m, 4H), 0.94(m, 3H)

[437] Mass : M+H 434

[438]

[439] **Preparation 31**

[440] **Ethyl 3-[3,4-dihydro-2(1*H*)-isoquinolinylcarbonyl] - 5-ethyl-4,5-dihydro-5-isoxazolecarboxylate**

[441] The compound of Preparation 22 was reacted according to the same procedure as Preparation 23 using 1,2,3,4-tetrahydroisoquinoline instead of 1-naphtylamine to give the title compound.

[442] ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.21-7.09(m, 4H), 5.03(s, 1H), 4.80(dd, 1H), 4.27(m, 2H), 4.18-3.78(m, 2H), 3.71(dd, 1H), 3.31(dd, 1H), 2.93(m, 2H), 2.02(m, 2H), 1.32(m, 3H), 0.98(m, 3H)

[443]

[444] **Preparation 32**

[445] ***tert*-Butyl (3*S*)-3-[({3-[3,4-dihydro-2(1*H*)-isoquinolinylcarbonyl] - 5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoate**

[446] The compound of Preparation 31 was reacted according to the same procedure as Preparations 12 and 15 to give the title compound.

[447] ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.13(m, 1H), 7.82-7.68(m, 2H), 7.51-7.34(m, 4H), 6.85(m, 1H), 6.82-6.58(m, 1H), 5.11-4.86(m, 5H), 3.56(two d, 1H), 3.12(two d, 1H), 2.99-2.75(m, 2H), 2.11(m, 1H), 1.91(m, 1H), 1.43(two s, 9H), 0.97(two t, 3H)

[448]

[449] **Example 11**

[450] **(3*S*)-3-[({3-[3,4-dihydro-2(1*H*)-isoquinolinylcarbonyl] - 5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid**

[451] The compound prepared in Preparation 32 (50mg, 0.079mmol) was dissolved in dichloromethane (4 □), and trifluoroacetic acid (2 □) was added thereto at 0℃. While slowly warming the mixture to room temperature, it was stirred for 1 hour, con-

centrated under reduced pressure, and purified by Prep-TLC (7.5% methanol-dichloromethane) to give the title compound (quantitative yield).

[452] ^{1}H-NMR (500MHz, CD_3OD) δ 7.20-6.98(m, 5H), 5.17-4.71(m, 5H), 3.94-3.85 (m, 2H), 3.52(two d, 1H), 3.33(two d, 1h), 3.05-2.78(m, 4H), 2.09(m, 1H), 1.97(m, 1H), 1.00(two t, 3H)

[453] Mass : M+H 580

[454]

[455] **Example 12**

[456] **3-{[(3-{[2-(*tert*-Butyl)anilino] carbonyl}-5-ethyl-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-fluoro-4-oxopentanoic acid**

[457] The title compound was obtained according to the same procedure as Preparations 23 (2-tert-butylaniline instead of 1-naphthylamine), 12, and 16, and Example 1.

[458] ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.57(d, 1H), 7,87-7.74(m, 1H), 7.45-7.33(m, 2H), 7.22-7.18(m, 1H), 5.00-4.70(m, 3H), 3.73-3.70(m, 1H), 3.31(d, 1H), 3.06-2.82(m, 2H), 2.13(m, 1H), 1.99(m, 1H), 1.45(s, 9H), 1.01(m, 3H)

[459] Mass : M+H 450

[460]

[461] **Example 13**

[462] **3-{[(3-{[2-(*tert*-Butyl)-anilino] carbonyl}-5-isopropyl-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-fluoro-4-oxopentanoic acid**

[463] The title compound was obtained according to the same procedure as Preparations 22 [methyl 2-i-propylacrylate instead of ethyl 2-ethylacrylate (*J. Chemical Society Perkin Trans. 1* **1997**, 1559-1570)], 23 (2-tert-butylaniline instead of 1-naphthylamine), 12, and 16, and Example 1.

[464] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.50(d, 1H), 7.83(m, 1H), 7.41(d, 1H), 7.17(m, 1H), 4.79-4.42(m, 3H), 3.66-3.61(two d, 1H), 3.38(d, 1H), 3.09-2.73(m, 2H), 2.31(m, 1H), 1.43(s, 9H), 1.02(m, 6H)

[465] Mass : M+H 464

[466]

[467] **Example 14**

[468] **3-{[(3-{[2,5-Di(*tert*-butyl)anilino] carbonyl}-5-isopropyl-4,5-dihydro-5-isoxazolyl)carbonyl]-amino}-5-fluoro-4-oxopentanoic acid**

[469] The title compound was obtained according to the same procedure as Preparations 22 [methyl 2-i-propylacrylate instead of ethyl 2-ethylacrylate (*J. Chemical Society Perkin Trans. 1* **1997**, 1559-1570)], 23 (2,5-di-tert-butylaniline instead of

1-naphthylamine), 12, and 16, and Example 1.

[470] ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.49(d, 1H), 7.89(s, 1H), 7.33(d, 1H), 7.18(dd, 1H), 4.77-4.45(m, 3H), 3.66-3.61(dd, 1H), 3.38(d, 1H), 3.09-2.75(two m, 2H), 2.31(m, 1H), 1.41(s, 9H), 1.31(s, 9H), 1.02(m, 6H)

[471] Mass : M+H 520

[472]

[473] **Experiment 1**

[474] **Determination of the caspase inhibitory effect**

[475] Caspase-1 and caspase-8 known as cysteine proteases in the form of $\alpha_2\beta_2$ were expressed, purified, and activated by modifying a method known in Thornberry, N. A. et al, *Nature*, **1992**, *356*, 768. Thornberry, N. A. *Methods in Enzymology*, **1994**, *244*, 615. Walker, N. P. C. et al. *Cell*, **1994**, *78*, 343, and caspase-9 was also purified by a similar method, and the inhibitory activity against them was tested. Briefly describing, p10 and p20 subunits (Thornberry, N. A. et al, *Nature*, **1992**, *356*, 768) were expressed in *E.coli* and purified by nickel column and anionic exchange chromatography to give caspase-1, caspase-8 and caspase-9. The fluorescent substrates AcYVAD-AFC for caspase-1, AcDEVD-AFC for caspase-8, and AcLEHD-AFC for caspase-9, were used for determining specific activity of the synthesized inhibitors. The enzyme reaction was carried out at 25 ℃ with various concentrations of the inhibitors in a buffer solution containing 20mM HEPES(pH 7.40), 10%(w/v) sucrose, 0.1%(w/v) CHAPS, 100mM NaCl, 1mM EDTA, and 10mM DTT in the presence of 25μM AcYVAD-AFC for 10nM caspase-1, 25μM AcDEVD-AFC for 2.1nM caspase-8, and 150μM AcLEHD-AFC for 200nM caspase-9. The inhibitory constants K_i and K_{obs} of the inhibitors were determined by measuring the reaction velocity with the time lapse using a fluorescent spectrometer and by obtaining the initial rate constant. K_i was calculated from the Lineweaver Burk Plot, and K_{obs} from the following Equation 1.

[476]

[477] Equation 1

[478] $K_{obs} = -\ln (1-A_t/A_{oo})/t$

[479] in which

[480] A_t means cleavage rate (%) at time t, and

[481] A_{oo} means the maximum cleavage rate (%).

[482]

[483] Spectra MAX GeminiXS Fluorescent Spectrometer of Molecular Device Co. was used at the excitation wavelength of 400nm and the emission wavelength of 505nm.

[484]

[485] The *in vitro* inhibitory activity of the inhibitors was determined by subjecting Jurkat cell (ATCC TIB-152) to apoptosis using anti-Fas monoclonal antibody (Upstate

Biotech 05-201) and by detecting the color change according to the WST-1 method (TAKARA MK400) known in Francoeur A.M. and Assalian A. (1996) Biochemica **3**, 19-25 to observe the amount of alive Jurkat cells when the cells were treated by the inhibitor. Spectra MAX 340 Spectrometer of Molecular Device Co. was used at the absorbance wavelength of 440nm.

[486]

[487] Table 1

Example No.	Caspase-8 $K_{obs}/[I]$ ($M^{-1}min^{-1}$)	Caspase-9 $K_{obs}/[I]$ ($M^{-1}min^{-1}$)	Jurkat cell IC_{50} (μM)
1	7.1 E3	-	29.6
2	1.0 E5	3.2 E2	2.8
3	2.3 E5	1.3 E2	0.93
4	1.5E5	-	0.73
5	2.4 E6	5.1 E4	2.58
6	5.7 E6	5.7 E4	0.13
7	2.4 E6	6.6 E4	0.20
8	1.2 E6	2.6 E4	0.1
9	2.1 E6	5.6 E4	0.16
10	1.4 E5	-	8.3
11	2.9 E5	3.0 E2	2.0
12	1.3 E7	6.4 E4	0:1
13	2.0 E7	1.9 E3	0.1
14	8.4 E6		0.1

[488]

[489] **Experiment 2**

[490] **Therapeutic effect for LPS-induced acute hepatitis in mouse**

[491] Step 1) Preparation of blood sample

[492] Male Balb/c mice (6 weeks, Charles River Laboratory, Osaka , Japan) were kept under the conditions of 22℃, 55% of relative humidity, and light-darkness cycle of 12 hours. Food and water were supplied ad libitum. In pyrogen-free saline were dissolved LPS (lipopolysaccaride) and D-galactosamine in concentrations of 0.4 □ / □ and 280 □ / □ , respectively, and their 1:1 mixture was injected to each mouse in the amount of 5 □ / □ . Immediately after the injection of LPS and D-galactosamine, vehicle (a mixture of

PEG400 : ethanol : Tween80 = 15 : 7.5 : 2.5 was diluted by five times with phosphate buffer) wherein the test compound is dissolved or the vehicle alone was intraperitoneally injected into the mice. After 8 hours from the drug injection, blood samples were obtained from their hearts.

[493]

[494] Step 2: Determination of the activity of plasma aminotransferase

[495] The plasma ALT activity was determined for the blood samples obtained in Step 1 using ALT assay kit (Asan Pharm. Co., Seoul , Korea) according to the manufacturer's instruction. The results appeared that the injection of LPS and D-galactosamine sharply increases the ALT activity in plasma, and the test compounds inhibit the increased enzyme activity in a dose-dependent manner. Based on these results, ED_{50} values of the test compounds were calculated using Prism software of GraphPad Co.

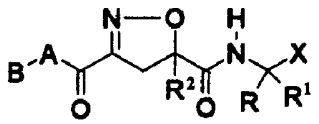
[496]

[497] Table 2

Example No.	ED_{50} (mg/kg)	95% Confidence intervals
3	0.075	0.045 ~ 0.126
8	0.405	0.048 ~ 3.512

Claims

[1] 1. A compound of the following formula (1):

[2] 

(1)

in which

I) R represents H, -SAC, -SCAC, -Ar, or -SAC-Ar,

II) R^1 represents -SAC, -SCAC, -Ar, -SAC-Ar, or a side chain residue of all the natural amino acids; and the compound of formula (1) may exist in a specific di-astereomeric form or mixtures thereof when the carbon to which R^1 is attached becomes a stereocenter due to the R^1 group; or the compound of formula (1) may have a protecting group in an ester form of $-CO_2R^3$ wherein R^3 is -SAC or a sulfonamide form of $-CONHSO_2R^4$ wherein R^4 is -SAC, or may exist in the form of pharmaceutically acceptable salt, when R^1 is a side chain residue of an amino acid containing carboxyl moiety; or the compound of formula (1) may also exist in the form of pharmaceutically acceptable salt when R^1 is a side chain residue of an amino acid containing a base moiety,

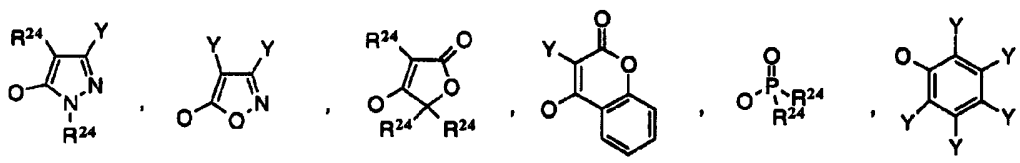
III) R^2 represents -SAC, -SCAC, -Ar, -SAC-Ar, or a side chain residue of all the natural amino acids; and the compound of formula (1) may exist in a specific di-astereomeric form or mixtures thereof when the carbon to which R^2 is attached becomes a stereocenter due to the R^2 group; the compound of formula (1) may have a protecting group in an ester form of $-CO_2R^5$ wherein R^5 is -SAC or a sulfonamide form of $-CONHSO_2R^6$ wherein R^6 is -SAC, or may exist in the form of pharmaceutically acceptable salt, when R^2 is a side chain residue of an amino acid containing carboxyl moiety; or the compound of formula (1) may also exist in the form of pharmaceutically acceptable salt when R^2 is a side chain residue of an amino acid containing a base moiety, or R^2 further represents H; $-(CH_2)_nOR^7$ wherein R^7 is -SAC, -SCAC, -Ar, or -SAC-Ar, and n = 1 or 2; or $-(CH_2)_nOC(=O)R^8$ wherein R^8 is -SAC, -SCAC, -Ar, or -SAC-Ar, and n = 1 or 2,

IV) A represents $-(NR^9)_n-$ wherein R^9 is H, -SAC, -SCAC, -Ar, or -SAC-Ar, and n=0 or 1,

V) B represents H, -SAC, -SCAC, -Ar, or -SAC-Ar, or

VI) R and R^1 may form a cycle together with the carbon atom to which they are attached, where $-R-R^1-$ is $-(CH_2)_n-$, $-(CH_2)_n-O-(CH_2)_m-$, or $-(CH_2)_n-NR^{10}-(CH_2)_m-$ wherein n+m<9 and R^{10} is -SAC, -SCAC, -Ar, -SAC-Ar, -C(=O)-SAC, -C(=O)-SCAC, -C(=O)-Ar, or -C(=O)-SAC-Ar,

VII) X represents -C(=O)CH_2OR^{11} wherein R^{11} is -SAC, -SCAC, -Ar, or -SAC-Ar; -C(=O)$CH_2OC(=O)R^{12}$ wherein R^{12} is -SAC, -SCAC, -Ar, or -SAC-Ar; -CH=CH-CO_2R^{13} wherein R^{13} is -SAC, -SCAC, -Ar, or -SAC-Ar; -CH=CH-SO_2R^{14} wherein R^{14} is -SAC, -SCAC, -Ar, or -SAC-Ar; -C(=O)CH=CH_2; -$COCHN_2$; or -$COCH_2$-W wherein W is -F, -Cl, -Br, -I, -$NR^{15}R^{16}$ (R^{15} and R^{16} each are -SAC, -SCAC, -Ar, or -SAC-Ar, or together may form 3- to 6-membered saturated or unsaturated cyclic group), -SR^{17} (R^{17} is -SAC, -SCAC, -Ar, or -SAC-Ar), or is the following formula:

[3] 

wherein

is H, -OH, -OR^{18} (R^{18} = -SAC or –SCAC), -C(=O)R^{19} (R^{19} = -H, -SAC, or -SCAC), -F, -Cl, -Br, -I, -CN, -N_3, -CO_2H, -CF_3, -CO_2R^{20} (R^{20} = -SAC or -SCAC), -C(=O)NHR^{21} (R^{21} = -SAC or -SCAC), or -C(=O)$NR^{22}R^{23}$ (R^{22} and R^{23} each are -SAC, -SCAC, -Ar, or -SAC-Ar), and

R^{24} is H or –SAC, or pharmaceutically acceptable salt, physiologically hydrolysable ester, hydrate, solvate, or stereoisomer thereof.

[4] 2. The compound according to claim 1 wherein

I) R represents H;

II) R^1 represents -CH_2COOH, -CH_2COOR^3 (R^3 = -SAC), or -CH2CONHSO_2R^4 (R^4 = -SAC);

III) R^2 represents H, -SAC, -Ar, or -$(CH_2)_nOR^7$ (R^7 = -SAC, -SCAC, -Ar, or -SAC-Ar, and n = 1 or 2);

IV) A represents -$(NR^9)_n$- (R^9 is H, -SAC, -SCAC, -Ar, or -SAC-Ar, and n=0 or 1),

V) B represents H, -SAC, -SCAC, -Ar, or -SAC-Ar, or

VI) X represents -$COCHN_2$, -$COCH_2F$, -$COCH_2Cl$, -$COCH_2Br$, -$COCH_2I$, -$COCH_2OAr$, -$COCH_2OCOAr$, or -$COCH_2SR^{17}$ (R^{17} is -SAC, -SCAC, -Ar, or -SAC-Ar), or pharmaceutically acceptable salt, physiologically hydrolysable ester, hydrate, solvate, or stereoisomer thereof.

[5] 3. The compound according to claim 1 which is selected from the group consisting of the following:

(3S)-3-{[(3-benzoyl-5-ethyl-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-(2,6-dichlorobenzoyl)oxy]-4-oxopentanoic acid(1);

(3S)-3-({[5-ethyl-3-(1-naphthoyl)-4,5-dihydro-5-isoxazolyl]carbonyl}amino)-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid(2);

3-({[5-Ethyl-3-(1-naphthoyl)-4,5-dihydro-5-isoxazolyl]carbonyl}amino)-5-fluoro-4-oxopentanoic acid(3);
3-({[5-Ethyl-3-(1-isoquinolinylcarbonyl)-4,5-dihydro-5-isoxazolyl]carbonyl}amino)-5-fluoro-4-oxopentanoic acid(4);
(3S)-3-[({5-ethyl-3-[(1-naphthylamino)carbonyl]-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid(5);
3-[({5-Ethyl-3-[(1-naphthylamino)carbonyl]-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-5-fluoro-4-oxopentanoic acid(6);
(3S)-3-[({3-[([1,1'-biphenyl]-2-ylamino)carbonyl]-5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid(7);
3-[({3-[([1,1'-Biphenyl]-2-ylamino)carbonyl]-5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-5-fluoro-4-oxopentanoic acid(8);
3-{[(5-Ethyl-3-{[(2'-methyl[1,1'-biphenyl]-2-yl)amino]carbonyl}-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-fluoro-4-oxopentanoic acid(9);
3-[({3-[3,4-Dihydro-1(2*H*)-quinolinylcarbonyl]-5-ethyl-4,5-dihydro-5-isoxazolyl} carbonyl)amino]-5-fluoro-4-oxopentanoic acid(10);
(3S)-3-[({3-[3,4-dihydro-2(1*H*)-isoquinolinylcarbonyl]-5-ethyl-4,5-dihydro-5-isoxazolyl}carbonyl)amino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)pentanoic acid(11);
3-{[(3-{[2-(*tert*-Butyl)anilino]carbonyl}-5-ethyl-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-fluoro-4-oxopentanoic acid(12);
3-{[(3-{[2-(*tert*-Butyl)anilino]carbonyl}-5-isopropyl-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-fluoro-4-oxopentanoic acid(13); and
3-{[(3-{[2,5-Di(*tert*-butyl)anilino]carbonyl}-5-isopropyl-4,5-dihydro-5-isoxazolyl)carbonyl]amino}-5-fluoro-4-oxopentanoic acid(14), or pharmaceutically acceptable salt, physiologically hydrolysable ester, hydrate, solvate, or stereoisomer thereof.

[6] 4. A therapeutic composition for preventing inflammation and apoptosis comprising the compound of formula (1), or pharmaceutically acceptable salt, physiologically hydrolysable ester, hydrate, solvate, or stereoisomer thereof as defined in claim 1 as an active ingredient together with the pharmaceutically acceptable carrier.

[7] 5. The composition according to claim 4 for the treatment of dementia, cerebral stroke, brain impairment due to AIDS, diabetes, gastric ulcer, cerebral injury by hepatitis, hepatitis-induced hepatic diseases, acute hepatitis, fulminant hepatic failure, sepsis, organ transplantation rejection, rheumatic arthritis, cardiac cell apoptosis due to ischemic cardiac diseases, or liver cirrhosis.

[8] 6. The composition according to claim 4 for the treatment of acute hepatitis or

liver cirrhosis.

[9] 7. The composition according to claim 4 for the treatment of rheumatic arthritis.

[10] 8. The composition according to any one of claims 4 to 7 which is formulated as an oral preparation, an injection, or a patch.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2005/003136

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07D 261/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC:C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean patents and applications since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
Medline, CAPLUS(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Manuela Rodriquez et al., "Ionic Liquid as a Suitable Phase for Multistep Parallel Synthesis of an Array of Isoxazolines", Organic letters, 2003, Vol.5, No. 22, pp. 4029-4031 see the whole documenet	1-3
X	Dario Conti et al., "1,3-Cycloaddition of nitrile oxides in ionic liquids. An easier route to 3-carboxy isoxazolones, potential constrained glutamic acid analogues", Tetrahedron Letters, 2003, 44, pp. 5327-5330 see the whole documenet	1-3
X	KR 1999-0079268 A1(LG CHEMICAL LTD.) 05 NOVEMBER 1999 see the whole documenet	1-8
X	KR 2001-0045347 A1(LG CHEMICAL LTD.) 05 JUNE 2001 see the whole documenet	1-8

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 DECEMBER 2005 (23.12.2005)	**23 DECEMBER 2005 (23.12.2005)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office 920 Dunsan-dong, Seo-gu, Daejeon 302-701, Republic of Korea Facsimile No. 82-42-472-7140	Authorized officer KIM, Hee Sue Telephone No. 82-42-481-5605

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2005/003136

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KR 1999-0079268 A1	05.11.1999	NONE	
KR 2001-0045347 A1	05.06.2001	EP 01212309 B1	18.08.2004
		CA 2388564 A1	29.03.2001
		US 6747050 B1	08.06.2004