

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
26 March 2009 (26.03.2009)

PCT

(10) International Publication Number
WO 2009/038412 A2

(51) International Patent Classification:
C07D 233/32 (2006.01)

(21) International Application Number:
PCT/KR2008/005593

(22) International Filing Date:
22 September 2008 (22.09.2008)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
10-2007-0096584
21 September 2007 (21.09.2007) KR

(71) Applicant (for all designated States except US): **LG LIFE SCIENCES, LTD.** [KR/KR]; LG Twin Tower, 20, Yoido-dong, Youngdungpo-gu, Seoul 150-721 (KR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **KWAK, Hyo-Shin** [KR/KR]; LG Life Sciences, Ltd., Research & Development, 104-1 Monji-dong, Yuseong-gu, Daejeon 305-380 (KR). **KOO, Ki Dong** [KR/KR]; LG Life Sciences, Ltd., Research & Development, 104-1 Monji-dong, Yuseong-gu, Daejeon 305-380 (KR). **LIM, Dongchul** [KR/KR]; LG Life Sciences, Ltd., Research & Development, 104-1 Monji-dong, Yuseong-gu, Daejeon 305-380 (KR). **MIN, Kyeongsik** [KR/KR]; LG Life Sciences, Ltd., Research & Development, 104-1 Monji-dong, Yuseong-gu, Daejeon 305-380 (KR). **PARK, Heuisul** [KR/KR]; LG Life Sciences, Ltd., Research & Development, 104-1 Monji-dong, Yuseong-gu, Daejeon 305-380 (KR). **CHOI, Deog-Young** [KR/KR]; LG Life Sciences, Ltd., Research & Development, 104-1, Monji-dong, Yuseong-gu,

Daejeon 305-380 (KR). **CHOI, Jae-ung** [KR/KR]; LG Life Sciences, Ltd., Research & Development, 104-1, Monji-dong, Yuseong-gu, Daejeon 305-380 (KR). **PARK, Hyunjung** [KR/KR]; LG Life Sciences, Ltd., Research & Development, 104-1, Monji-dong, Yuseong-gu, Daejeon 305-380 (KR). **PARK, Mijeong** [KR/KR]; LG Life Sciences, Ltd. Research & Development, 104-1, Monji-dong, Yuseong-gu, Daejeon 305-380 (KR).

(74) Agent: **SOHN, Chang Kyu**; 1403, Seongji Heights 2-cha Bldg., 642-16 Yoksam 1-dong, Kangnam-gu, Seoul 135-910 (KR).

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

— without international search report and to be republished upon receipt of that report

(54) Title: BETA-SECRETASE INHIBITING COMPOUNDS

(57) Abstract: Disclosed are compounds represented by Formula (I) as defined in the specification, or pharmaceutically acceptable salts or isomers thereof, and a pharmaceutical composition for inhibiting beta-secretase activity comprising a therapeutically effective amount of the same.



WO 2009/038412 A2

BETA-SECRETASE INHIBITING COMPOUNDS

FIELD OF THE INVENTION

5 The present invention relates to a novel compound for inhibiting beta-secretase activity or a pharmaceutically acceptable salt or isomer thereof, preparation thereof, and a pharmaceutical composition comprising a therapeutically effective amount of the same.

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD), also known as senile dementia, is a neurodegenerative disease
10 which gradually progresses with age and accounts for 50 to 70% of dementia patients. The major symptoms of AD include memory loss, decline in cognitive-reasoning abilities and the like. Most Alzheimer's cases occur around age 65 and progress for about 9 years to result in the death of the patients. The number of AD patients is increasing as society develops and ages, so that it is estimated that there will be approximately 6 million patients within 10 years in the USA and this number will
15 further increase beyond that.

Likewise, the proportion of the elderly population is also increasing in Korea, and thus social problems associated with increased prevalence of senile dementia are becoming more serious. Unfortunately, no therapeutic agent has yet been developed which can treat the underlying pathogenic causes of Alzheimer's disease. Until now, acetylcholine esterase inhibitors have been

exclusively used as a general therapeutic agent. Aricept™ (Pfizer), Exelon™ (Novartis), and Reminyl™ (Janssen) are known as representative examples of the acetylcholine esterase inhibitors.

Strictly speaking, these drugs cannot be defined as therapeutic agents of Alzheimer's disease, because they do not address the root cause of the disease and result in only partial recovery from the disease in some patients (about 40-50%), and their therapeutic effects are seen for a limited time period only. In addition, the intrinsic nature of the disease requires long-term administration of the drugs, but administration of these therapeutic drugs is accompanied by various adverse side effects including liver toxicity.

Therefore, there is an urgent need for development of a novel therapeutic agent that can halt and reverse the progression of Alzheimer's and related diseases. As an approach to find a pathogenic cause responsible for Alzheimer's disease, long-term studies have been undertaken on familial early-onset Alzheimer's disease patients. As a result, it was revealed that mutations in some genes are primarily responsible for the pathogenesis of Alzheimer's disease.

As the common physiological results of the above-mentioned genetic pathogenic factors, the production of beta amyloid consisting of 42 amino acid residues (hereinafter, sometimes referred to as "Ab42") was found to increase. Based on this finding, it is assumed that the production of Ab42 is a main causative agent of Alzheimer's disease pathogenesis. Therefore, if a method capable of decreasing the production of Ab42 is developed, it will be a direct and effective therapeutic measure capable of blocking a pathogenic mechanism of Alzheimer's disease.

Beta-amyloid proteins are produced from a high-molecular weight amyloid precursor protein (APP) found in neuronal cells through serial cleavage events by 3 types of proteases

(secretases). This process takes place at the Golgi apparatus of neuronal cells, wherein APP and secretases are anchored in the Golgi membrane. The N-terminus of beta-amyloid (Ab) corresponds to the 99th amino acid from the C-terminus of APP, and this site is cleaved by beta-secretase (hereinafter, sometimes referred to as "beta-site APP cleaving enzyme" or "BACE"). The C-terminus of the membrane-bound beta-amyloid (Ab) is cleaved by gamma-secretase to generate a beta-amyloid (Ab) protein which is then secreted extracellularly from neuronal cells. Alternatively, APP may also be cleaved at different sites via an alternative pathway; for example, where the middle site of Ab (between 16th and 17th amino acid residues from the N-terminus) is cleaved by alpha-secretase, sAPP alpha having a high molecular weight is produced and secreted. This alternate pathway precludes the formation of beta-amyloid.

Cleavage of APP by the action of BACE and gamma-secretase may result in a variety of beta amyloid (Ab) proteins having different lengths, usually a 40-amino acid fragment (Ab40) and a 42-amino acid fragment (Ab42). In comparison with Ab40, Ab42 tends to easily aggregate and accelerates the formation of amyloid plaques in the brains of diseased patients, thereby resulting in gradual necrosis of the surrounding neuronal cells. This is assumed to be a major pathogenic mechanism of Alzheimer's disease.

Ab40 and Ab42 are produced in a ratio of about 9:1 under normal conditions. However, it is known that where levels of two amyloid proteins Ab40 and Ab42 are increased or where levels of Ab42 are selectively increased by mutations of Presenilin 1 and 2 genes, the onset of Alzheimer's disease is further accelerated and the symptoms of the disease are more severe. Therefore, it can be said that lowering of Ab42 production is the most important factor for development of anti-Alzheimer drugs. For this purpose, there is a need for development of beta- or gamma-secretase inhibitors.

Therefore, many multinational pharmaceutical companies have been investing heavily in research and development in the secretase inhibitor field, especially in the area of beta- or gamma-secretase inhibitors capable of suppressing the production of 42-amino acid beta-amyloid fragments, which form plaques assumed to be an underlying pathological cause of Alzheimer's disease.

5 Unfortunately, there are no noteworthy results of studies in terms of the development of a therapeutic agent that is capable of arresting a mechanism of beta-amyloid production to directly prevent the progression of the concerned disease.

The beta- and gamma-secretases are known as aspartic proteases and found in the membrane-bound form. However, no gene coding for gamma-secretase has yet been identified.

10 Further, it is known that substrates for gamma-secretase are not limited to APP, but that the enzyme also participates in the cleavage of Notch proteins, which are known to play crucial roles in regulating cell fate decision during differentiation processes. In particular, gene-knockout animals from which a gamma-secretase gene has been deleted died in utero, and recent clinical tests on gamma-secretase inhibitors showed significant drug toxicity. For these reasons, gamma-secretase

15 inhibitors are not likely to be promising drug candidates. As a result, it has not yet been confirmed whether gamma-secretase inhibitors can be developed as safe anti-Alzheimer medications.

On the other hand, in case of BACE, the gene thereof was identified through various methods by many pharmaceutical companies and the *in vivo* activity of beta-secretase was reported in 1999. Furthermore, the X-ray crystal structure of beta-secretase was determined and a peptide

20 inhibitor having high affinity for beta-secretase was also known in the art. As well, it was reported that a gene-knockout animal with deletion of the BACE gene exhibits a normal phenotype, thus suggesting that a BACE-specific inhibitor can be developed as a safer and efficient anti-dementia

drug. Taken altogether, these findings indicate that BACE is a more feasible target for the treatment of Alzheimer's disease, as compared to gamma-secretase.

As discussed above, upon considering that conventional commercially available drugs merely exhibit palliative effects of disease symptoms, thus providing substantially no effects on the progression of the concerned disease, it is certain that development of the BACE-specific inhibitors will lead to the development of a novel and remarkable drug against Alzheimer's disease. In addition, studies of BACE inhibitors have only relatively recently started, so there is no report yet on satisfactory small molecule chemical compounds other than peptide variants. Therefore, it is believed that the identification and development of small molecule chemical compounds as a promising candidate of the BACE inhibitor will provide a good opportunity for development of new worldwide drugs.

In recent years, many pharmaceutical companies have published study results on BACE inhibitors, for example by Merck (WO 2006/078577, WO 2006/060109 and WO 2006/057983), Elan (WO 2004/022523 and WO 2005/095326), Schering-Plough (WO 2006/014762 and WO 2006/014944), BMS (WO 2005/182105 and WO 2005/030758), and Eli-Lilly (WO 2005/108358 and WO 2006/034093). In the beginning, Elan has reported BACE inhibitor compounds, which were compounds having a small molecular weight and low blood-brain barrier (BBB) permeability. However, these compounds exhibited various limitations due to selectivity for cathepsin D (Cat. D) or *in vivo* cytotoxicity. A great deal of attention has been recently focused on Merck compounds. Through early introduction of dicarbonyl compounds and subsequently sulfonamide compounds from fellow pharma giant Sunesis, Merck reported excellent inhibitory effects and selectivity of the drug compounds. Since then, numerous drug companies issued various compounds in the form of Merck compounds.

Due to excellent BACE inhibitory effects and high Cat. D selectivity, these compounds, particularly sulfonamide compounds were expected to provide the possibility that they may be developed as an anti-Alzheimer drug. However, their low oral applicability and poor BBB permeability are obstacles to development of the drug. For these reasons, there are very little reports on compounds that lower the production of Ab42 in practical animal models. Apart from sulfonamide compounds, Lilly reported efficacy of drug compounds (lowering of Ab42 levels) in a practical animal model, through S.C administration of peptidomimetic compounds having a low molecular weight. However, these peptidomimetic compounds still suffer from disadvantages such as low oral applicability and poor BBB permeability. Therefore, there is no report yet on compounds exerting excellent effects. Schering-Plough also reported peptidic compounds, but these compounds failed to show desired efficacy in the brain of a practical animal model as they were known to be a Pgp substrate.

That is, peptidic beta-secretase inhibitors exhibit limited applicability as oral preparations, due to their intrinsic molecular specificities. In particular, sulfonamide type compounds also have poor BBB permeability which makes it difficult to observe desired efficacy of the drug in an animal model.

To this end, there is a strong need for development of a compound having excellent beta-secretase inhibitory activity, distinctively different from sulfonamide compounds.

SUMMARY OF THE INVENTION

Therefore, the present invention has been made to solve the above problems and other technical problems that have yet to be resolved.

More specifically, the present invention is intended to improve blood-brain barrier (BBB) permeability of a drug compound which is a technical problem suffered by conventional
5 sulfonamide compounds.

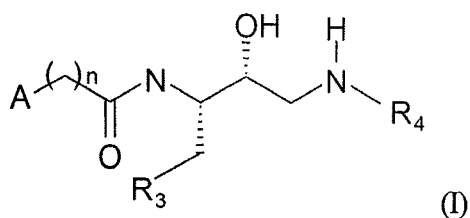
Therefore, an object of the present invention is to provide a novel compound having beta-secretase inhibitory activity through five-membered heterocyclic compounds apart from sulfonamide in terms of a chemical structure, and pharmaceutically acceptable salts and isomers thereof.

10 It is another object of the present invention to provide a method for preparing the said compound.

It is a further object of the present invention to provide a composition for inhibiting beta-secretase activity, comprising a therapeutically effective amount of the said compound as an active ingredient.

15 It is yet another object of the present invention to provide a use of the said novel compound for improvement of cognitive functions or treatment or prevention of neurodegenerative diseases such as Alzheimer's disease.

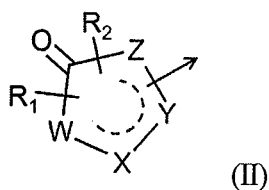
In accordance with an aspect of the present invention, the above and other objects can be accomplished by the provision of a compound represented by Formula I:



wherein:

n is 0 to 2;

5 A is represented by Formula II:



wherein W, X, Y and Z are each independently C or N atom, one or two of which being N;

10 R_1 is $-(CR_6R_7)_m-R_8$ wherein m is 0 to 2, R_6 is selected from the group consisting of hydrogen, alkyl and alkoxy, R_7 is selected from the group consisting of hydrogen, alkyl and $-C(O)NHR_6$, and R_8 is selected from the group consisting of alkyl, alkoxy, arylalkoxy, cycloalkyl, heterocycle, heteroaryl, $-C(O)R_6$ and $-C(O)NR_6R_7$ wherein R_6 and R_7 are as defined above;

R_2 is selected from the group consisting of hydrogen, alkyl and $-(CR_6R_7)_m-R_9$ wherein m is 0 to 2, R_6 and R_7 are as defined above, and R_9 is aryl;

R₃ is selected from the group consisting of hydrogen, alkyl, alkoxy and $-(CR_6R_7)_p-R_{10}$ wherein p is 0 to 2, R₆ and R₇ are as defined above, and R₁₀ is selected from the group consisting of cycloalkyl, aryl, heterocycle and heteroaryl;

R₄ is selected from the group consisting of hydrogen, alkyl, cycloalkyl and $-(CH_2)_p-A'R_{11}$ wherein p is as defined above, A' is aryl or heteroaryl and R₁₁ is selected from the group consisting of hydrogen, halogen, hydroxy, alkyl, alkoxy and $-NR_6R_7$ wherein R₆ and R₇ are as defined above; and

R₆ and R₇ may be taken together to form a cyclic or acyclic structure; or a pharmaceutically acceptable salt or isomer thereof.

The alkyl, alkoxy, aryl, cycloalkyl, heterocycle, and heteroaryl may be substituted or unsubstituted. When substituted, the substituent may be at least one selected from the group consisting of halogen, amino, alkylamino, dialkylamino, alkylacylamino, C₁-C₄ alkyl, hydroxy, C₁-C₄ alkoxy, aryl alkoxy and oxo. Where appropriate, these substituents may also be substituted. When substituted, the substituents are as exemplified above. Further, the substituents may be taken together to form a cyclic structure.

Further, the heteroaryl and heterocycle are each independently a 4 to 8-membered ring containing 1 to 3 hetero atoms selected from the group consisting of O, N and S, and having 0 to 2 double bonds, and preferably a 5 or 6-membered ring having 1 or 2 double bonds.

Compounds of Formula I in accordance with the present invention have a chemical structure that is distinctly different from that of conventional known sulfonamide- or peptide-based beta-secretase inhibitor compounds. As will be illustrated in Experimental Examples which will

follow, the compounds of the present invention exhibit excellent inhibitory effects on human beta-secretase which is correlated with improvement of cognitive functions or prevention and treatment of neurodegenerative diseases such as Alzheimer's disease.

The term "alkyl" means an aliphatic hydrocarbon group. The alkyl moiety may be a
5 "saturated alkyl" group, which means that it does not contain any alkene or alkyne moiety. The alkyl moiety may also be an "unsaturated alkyl" group, which means that it contains at least one alkene or alkyne moiety. An "alkene" moiety refers to a group consisting of at least two carbon atoms and at least one carbon-carbon double bond, and an "alkyne" moiety refers to a group consisting of at least two carbon atoms and at least one carbon-carbon triple bond. The alkyl moiety, whether saturated or
10 unsaturated, may be branched, straight chain, or cyclic.

The alkyl group may have 1 to 20 carbon atoms. The alkyl group may also be C₁-C₈ alkyl having, preferably 1 to 8 carbon atoms. By way of example only, "C₁-C₄ alkyl" indicates that there are one to four carbon atoms in the alkyl chain, *i.e.*, the alkyl chain is selected from the group consisting of methyl, ethyl, propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl, and t-butyl. When the
15 alkyl group is used alone or in combination with alkyloxy, it may be a linear or branched hydrocarbon radical.

Typical examples of the alkyl group may include, but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, hexyl, ethenyl, propenyl, butenyl, cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl.

20 The term "alkoxy" refers to oxo alkyl having 1 to 8 carbon atoms.

The term "cycloalkyl" refers to an unsaturated aliphatic 3 to 10-membered ring, including cyclohexyl.

The term "aryl" refers to an aromatic group which has at least one ring having a conjugated pi (π) electron system and includes both carbocyclic aryl (for example, phenyl) and
5 heterocyclic aryl (for example, pyridine) groups. This term is intended to include monocyclic or fused-ring polycyclic (*i.e.*, rings which share adjacent pairs of carbon atoms) groups. Specifically, aryl means a 4 to 10-membered, preferably 6 to 10-membered aromatic monocyclic or multicyclic group, including phenyl, naphthyl, and the like.

The term "heteroaryl" refers to an aromatic 4 to 8-membered ring, preferably a 5 or 6-
10 membered ring, which contains 1 to 3 hetero atoms selected from the group consisting of N, O and S and may be fused with benzo or C₃-C₈cycloalkyl. Examples of monocyclic heteroaryl may include, but are not limited to, thiazole, oxazole, thiophene, furan, pyrrole, imidazole, isoxazole, pyrazole, triazole, thiadiazole, tetrazole, oxadiazole, pyridine, pyridazine, pyrimidine, pyrazine, and the like. Examples of bicyclic heteroaryl may include, but are not limited to, indole, indoline,
15 benzothiophene, benzofuran, benzimidazole, benzoxazole, benzisoxazole, benzothiazole, benzothiadiazole, benzotriazole, quinoline, isoquinoline, purine, furopyridine and the like.

The term "heterocycle" refers to a 3 to 10-membered ring which has 1 to 2 hetero atoms selected from the group consisting of N, O and S, may be fused with benzo or C₃-C₈cycloalkyl, and contains 1 or 2 double bonds or may be saturated. The heterocycle may preferably be a 4 to 8-
20 membered ring, and more preferably a 5 or 6-membered ring. Examples of the heterocycles may include, but are not limited to, furan, thiophene, pyrrole, pyrroline, pyrrolidine, oxazole, thiazole, imidazole, imidazoline, imidazolidine, pyrazole, pyrazoline, pyrazolidine, isothiazole, triazole,

thiadiazole, pyrane, pyridine, piperidine, morpholine, thiomorpholine, pyridazine, pyrimidine, pyrazine, pyperazine, triazine, and hydrofuran.

Other terms used herein can be interpreted as having their usual meanings in the art to which the present invention pertains.

5 Preferably, in the compound of Formula I in accordance with the present invention,

R_1 is $-(CR_6R_7)_m-R_8$ wherein m is 0 to 2, R_6 is hydrogen or C_1-C_8 alkyl, R_7 is selected from the group consisting of hydrogen, C_1-C_8 alkyl and $-C(O)NHR_6$ wherein R_6 is as defined above, and R_8 is selected from the group consisting of C_1-C_8 alkyl, C_1-C_8 alkoxy, arylalkoxy, cycloalkyl, heteroaryl, $-C(O)R_6$ and $-C(O)NR_6R_7$ wherein R_6 and R_7 are as defined above;

10 R_2 is selected from the group consisting of hydrogen, C_1-C_8 alkyl and $-(CR_6R_7)_m-R_9$ wherein m is 0 to 2, R_6 and R_7 are as defined above and R_9 is phenyl;

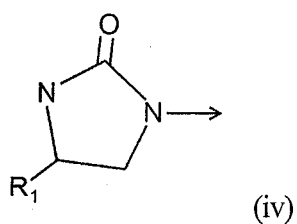
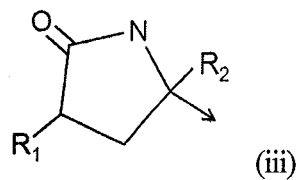
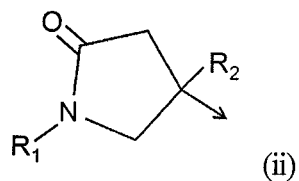
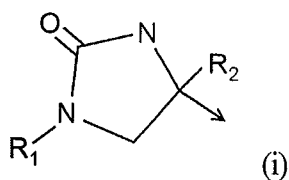
R_3 is selected from the group consisting of hydrogen, C_1-C_8 alkyl, C_1-C_8 alkoxy and $-(CR_6R_7)_p-R_{10}$ wherein p is 0 to 2, R_6 and R_7 are as defined above and R_{10} is selected from the group consisting of cycloalkyl, aryl, heterocycle and heteroaryl; and

15 R_4 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, 5 or 6-membered cycloalkyl and $-(CH_2)_p-A'R_{11}$ wherein p is as defined above, A' is aryl or heteroaryl and R_{11} is selected from the group consisting of hydrogen, halogen, hydroxy, C_1-C_8 alkyl, C_1-C_8 alkoxy and $-NR_6R_7$ wherein R_6 and R_7 may be taken together to form a cyclic structure.

In Formula I, A is a 5-membered ring having a structure of Formula II and containing one or two N atoms. In the substituent A, R_1 and R_2 may be attached to the same or different ring atom, or otherwise may be attached to the ring atom which is attached to the substituent.

For example, the substituent A may be any one of heterocycles of Formulae (i) to (iv):

5



10

Preferably, R_1 is $-(CR_6R_7)_m-R_8$ wherein m is 0 to 2, R_6 is hydrogen or C_1-C_8 alkyl, R_7 is selected from the group consisting of hydrogen, C_1-C_8 alkyl and $-C(O)NHR_6$ wherein R_6 is as defined above, and R_8 is selected from the group consisting of C_1-C_8 alkyl, C_1-C_8 alkoxy, arylalkoxy, cycloalkyl, heteroaryl, $-C(O)R_6$ and $-C(O)NR_6R_7$.

- 5 More preferably, R_1 is $-(CHR_7)_m-R_8$ wherein m is 0 or 1, R_7 is hydrogen, C_1-C_4 alkyl or $-C(O)NHR_6$ wherein R_6 is as defined above, and R_8 is selected from the group consisting of C_1-C_8 alkyl, arylalkoxy, cycloalkyl, $-C(O)R_6$ and $-C(O)NR_6R_7$ wherein R_6 and R_7 are as defined above. Particularly preferably, R_1 is selected from the group consisting of hydrogen, benzyl, dimethylamino-carbonyl, propyl-carbonyl, phenethyl, butyl, pentyl, ethylcarbamoyl, trifluorobenzyl, 4-trifluoromethylbenzyl, 2-trifluoromethylbenzyl, phenoxyethyl, 2,4,5-trifluorobenzyl, 4-
10 fluorobenzyl, 3,4-difluorobenzyl, 2,4-difluorobenzyl, 3,5-difluorobenzyl, 3,5-dimethoxybenzyl, 3-methoxybenzyl, 3,5-dibromophenyl, cyclohexylmethyl and 2-acetylaminophenyl.

Preferably, R_2 is selected from the group consisting of hydrogen, C_1-C_8 alkyl and benzyl.

More preferably, R_2 is selected from the group consisting of hydrogen, methyl and benzyl.

- 15 Preferably, R_3 is selected from the group consisting of hydrogen, C_1-C_8 alkyl, cycloalkyl and aryl, wherein alkyl, cycloalkyl and aryl may be independently unsubstituted or substituted with one or more substituents selected from the group consisting of halogen, hydroxy, C_1-C_4 alkyl, C_1-C_4 alkoxy and arylalkoxy. More preferably, R_3 is phenyl unsubstituted or substituted with one or more substituents selected from the group consisting of fluorine, chlorine and methyl. Particularly
20 preferably, R_3 is phenyl or 3,5-difluorobenzyl.

Preferably, R_4 is selected from the group consisting of hydrogen, C_1 - C_6 alkyl, cycloalkyl and $-(CH_2)-A'R_{11}$ wherein A' is aryl or heteroaryl and R_{11} is selected from the group consisting of hydrogen, halogen, hydroxy, alkyl, alkoxy and $-NR_6R_7$ wherein R_6 is selected from the group consisting of hydrogen, C_1 - C_3 alkyl and C_1 - C_3 alkoxy and R_7 is selected from the group consisting of hydrogen, C_1 - C_4 alkyl and $-C(O)NHR_6$. More preferably, R_4 is selected from the group consisting of 3-dimethylaminobenzyl, 3-isopropylbenzyl, 3-trifluoromethoxybenzyl, 3-trifluoromethylbenzyl, 3-ethylbenzyl, 3-*t*-butylbenzyl, ethyl and cyclohexyl.

The compound in accordance with the present invention may form a pharmaceutically acceptable salt. The term "pharmaceutically acceptable salt" means acid addition salts of the compound with acids capable of forming a non-toxic acid addition salt containing pharmaceutically acceptable anions, for example, inorganic acids such as sulfuric acid, hydrochloric acid, nitric acid, phosphoric acid, hydrobromic acid and hydroiodic acid; organic carboxylic acids such as tartaric acid, formic acid, citric acid, acetic acid, trichloroacetic acid, trifluoroacetic acid, gluconic acid, benzoic acid, lactic acid, fumaric acid, and maleic acid; or sulfonic acids such as methanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid and naphthalenesulfonic acid. These acid addition salts may be prepared by conventional techniques known in the art, based on the chemical structure of Formula I.

The compound of the present invention or a pharmaceutically acceptable salt thereof may be in the form of a hydrate or solvate.

As used herein, the term "isomer" means a compound of the present invention or a salt thereof, that has the same chemical formula or molecular formula but is optically or stereochemically different therefrom. For examples, the compounds of Formula I in accordance with

the present invention may have an asymmetric carbon center, and therefore can be present in the form of optical isomers (R or S isomeric forms), racemates, diastereomeric mixtures, and individual diastereomers. When the compound of the present invention has a double bond, there may be present geometrical isomers (trans and cis isomeric forms). The present invention encompasses all these isomeric forms and mixtures.

Unless otherwise specified, it should be understood that the compound of Formula I in the context of the present invention is intended to encompass pharmaceutically acceptable salts and isomers thereof, all of which are included within the scope of the present invention. For convenience of illustration, the compound of the present invention is simply expressed as a compound of Formula I.

Representative examples of the compound of Formula I in accordance with the present invention include the following compounds:

(3S)-1-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(3R)-1-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(2S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide;

(2R)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide;

2-((3S)-1-benzyl-5-oxopyrrolidin-3-yl)-N-[(2R, 3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]acetamide;

2-((3R)-1-benzyl-5-oxopyrrolidin-3-yl)-N-[(2R, 3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]acetamide;

5 (4S)-1-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

10 (4S)-1-[2-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]amino-2-oxoethyl]-N,N-dimethyl-2-oxoimidazolidine-4-carboxamide;

(4S)-1-butanoyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-phenethylimidazolidine-4-carboxamide;

15 N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-(1-methyl-oxopyrrolidin-3-yl)acetamide;

1-butyl-N-[4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(3S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxo-1-phenethylpyrrolidine-3-carboxamide;

(3R)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxo-1-phenethylpyrrolidine-3-carboxamide;

5 1-(2-amino-2-oxoethyl)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(3S)-1-benzyl-N-{(2S,3R)-3-hydroxy-1-phenyl-4-[(3-trifluoromethoxyphenyl)methylamino]butan-2-yl}-5-oxopyrrolidine-3-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-N-ethyl-2-oxoimidazolidine-1,4-dicarboxamide;

10

(3S)-1-[(2S)-3-(4-chlorophenyl)-1-ethylamino-1-oxopropan-2-yl]-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(3R)-1-[(2S)-3-(4-chlorophenyl)-1-ethylamino-1-oxopropan-2-yl]-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

15

(3S)-1-benzyl-N-{(2S,3R)-3-hydroxy-1-phenyl-4-[(3-trifluoromethoxyphenyl)methylamino]butan-2-yl}-2-oxoimidazolidine-4-carboxamide;

(3S)-2,4-dibenzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide;

(3R)-2,4-dibenzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide;

5 (2S)-4-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide;

(4S)-1,4-dibenzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

10 (4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(4-trifluoromethylphenyl)methyl]imidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(4-trifluoromethoxyphenyl)methyl]imidazolidine-4-carboxamide;

15 (4S)-1-benzyl-N-[(2S,3R)-1-(3,5-difluorophenyl)-4-(3-dimethylaminophenyl)methylamino-3-hydroxybutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(3S)-1-benzyl-N-[(2S,3R)-1-(3,5-difluorophenyl)-4-(3-dimethylaminophenyl)methylamino-3-hydroxybutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-(2-phenoxyethyl)imidazolidine-4-carboxamide;

(4R)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-(2-phenoxyethyl)imidazolidine-4-carboxamide;

5 (4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(2-trifluoromethylphenyl)methyl]imidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-1-(3,5-difluorophenyl)-4-(3-dimethylaminophenyl)methylamino-3-hydroxybutan-2-yl]-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

10 (4S)-1-benzyl-N-[(2S,3R)-4-(cyclohexylamino)-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4R)-1-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-4-methyl-2-oxoimidazolidine-4-carboxamide;

(4S)-1-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-4-methyl-2-oxoimidazolidine-4-carboxamide;

15 (4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-1-[(4-fluorophenyl)methyl]-2-oxoimidazolidine-4-carboxamide;

(4S)-1-[(3,4-difluorophenyl)methyl]-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-1-[(2,4-difluorophenyl)methyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-1-benzyl-N-[(2S,3R)-3-hydroxy-1-phenyl-4-[(3-trifluoromethylphenyl)]methylamino]butan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-1-benzyl-N-{(2S,3R)-3-hydroxy-1-phenyl-4-[(3-propan-2-ylphenyl)methylamino]butan-2-yl}-2-oxoimidazolidine-4-carboxamide;

(4S)-N-{(2S,3R)-1-(3,5-difluorophenyl)-3-hydroxy-4-[(3-propan-2-ylphenyl)methylamino]butan-2-yl}-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

(4S)-1-(3,5-dimethoxyphenyl)methyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-ethylphenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-t-butylphenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

(4S)-1-[(3,5-difluorophenyl)methyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-1-[(3-methoxyphenyl)methyl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-1-[(4-methoxyphenyl)methyl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-t-butylphenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-1-(3,5-difluorophenyl)methyl-2-oxoimidazolidine-4-carboxamide;

(4S)-1-(3,5-difluorophenyl)methyl-N-{(2S,3R)-3-hydroxy-1-phenyl-4-[(3-propan-2-ylphenyl)methylamino]butan-2-yl}-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-t-butylphenyl)methylamino-1-(3,5-difluorophenyl)-3-hydroxybutan-2-yl]-1-(3,5-difluorophenyl)methyl-2-oxoimidazolidine-4-carboxamide;

(4S)-N-((2S,3R)-4-ethylamino-3-hydroxy-1-phenylbutan-2-yl)-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

(4S)-1-(3,5-dibromo)phenyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-phenylimidazolidine-4-carboxamide;

(3S)-1-cyclohexylmethyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(3R)-1-cyclohexylmethyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

5 (4S)-N-[(2S,3R)-4-(3-*t*-butylphenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-[(3-dimethylaminophenyl)methylamino]-3-hydroxy-1-phenylbutan-2-yl]-5-oxo-1-pentylpyrrolidine-4-carboxamide; and

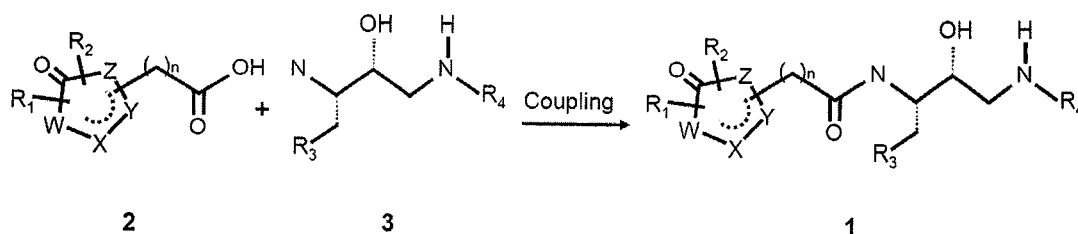
(4R)-N-[(2S,3R)-4-[(3-dimethylaminophenyl)methylamino]-3-hydroxy-1-phenylbutan-2-yl]-5-oxo-1-pentylpyrrolidine-4-carboxamide.

10

Further, the present invention relates to a method for preparing a compound of Formula I. As shown in Reaction Scheme 1 below, the compound of Formula I can be prepared by amide coupling reaction of a compound of Formula 2 with a compound of Formula 3.

15

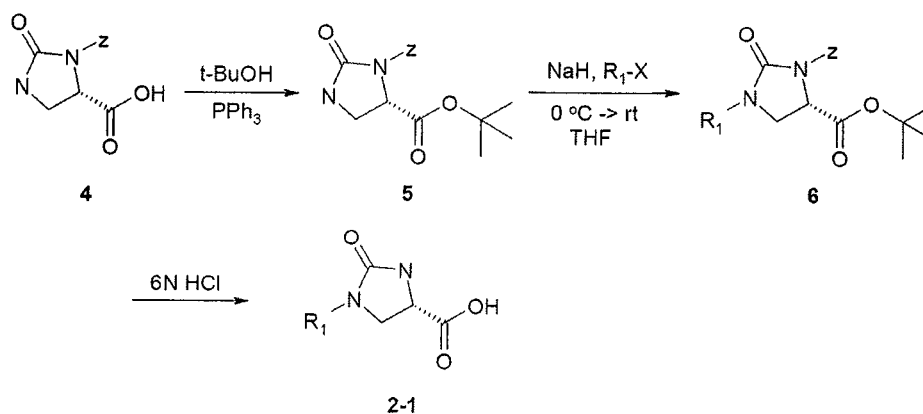
[Reaction Scheme 1]



In Reaction Scheme 1, n, R₁, R₂, R₃, R₄, W, X, Y, and Z are as defined above.

Examples of known coupling agents usable in the amide coupling may include, but are not limited to, carbodiimides such as dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), 1,1'-dicarbonyldiimidazole (CDI), etc. which are used in a mixture with 1-hydroxybenzotriazole (HOBT) or 1-hydroxy-7-azabenzotriazole (HOAT); bis-(2-oxo-3-oxazolidinyl)-phosphinic acid chloride (BOP-Cl), diphenylphosphorylazide (DPPA), N-[dimethylamino-1H-1,2,3-triazole[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium (HATU), etc. Examples of solvents that are conventionally used in amination may include DCM, DMF, and DCE. Examples of bases for activation of the reaction may include triethylamine, diisopropylethylamine, etc.

In the compound of Formula 2, a simple lactam compound is commercially available. Analogously to the procedure described in Reaction Scheme 2 below, cyclic urea may also be prepared from a commercially available compound.

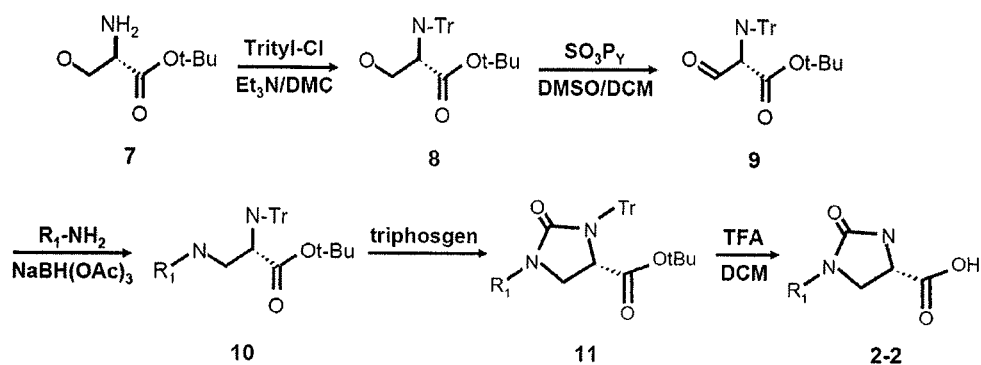


In Reaction Scheme 2, R_1 is as defined above, and Z means benzyloxycarbonyl (cbz). A compound of Formula 4 is commercially available, and a compound of Formula 2-1 can be prepared by preparing ester of Formula 5 using triphenyl phosphine (PPh_3) and t-BuOH, obtaining a substituted compound of Formula 6 via alkylation or acylation of the ester of Formula 5, and preparing a compound of Formula 2-1 via deprotection and hydrolysis of the compound of Formula 6 in the presence of strong acid (HCl).

On the other hand, cyclic urea directly substituted with aryl can be synthesized according to the procedure described in Reaction Scheme 3 below.

10

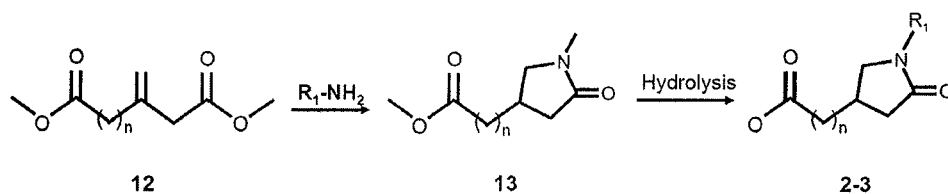
[Reaction Scheme 3]



In Reaction Scheme 3, R_1 is as defined above. Specifically, N of Serine is protected with a trityl group to obtain a compound of Formula 8, a diamino compound of Formula 10 is obtained by oxidation and reductive amination of the compound of Formula 8, and a compound of Formula 2-2 is then obtained by cyclization of compound of Formula 10 using triphosgene, followed by deprotection of Formula 11 in the presence of strong acid.

The lactam compound that is not readily commercially available can be prepared according to the procedure described in Reaction Scheme 4 below.

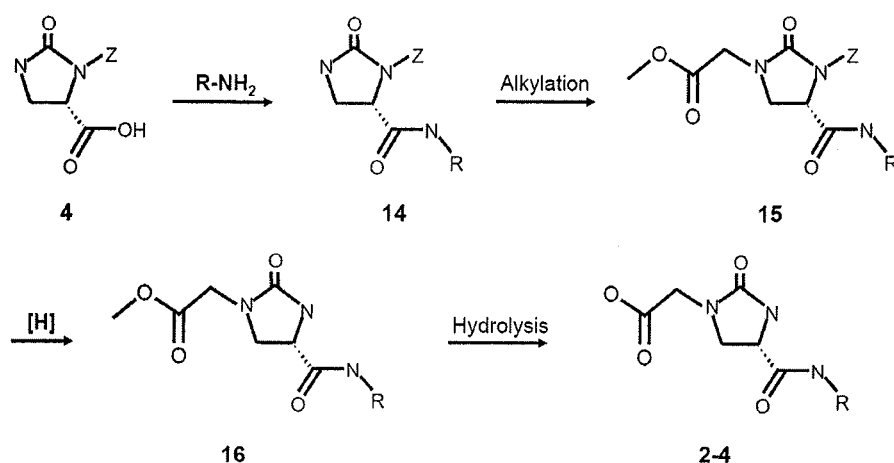
10 [Reaction Scheme 4]



In Reaction Scheme 4, n is 0 or 1, R₁ is as defined above. When a compound of Formula 12 is reacted with amine, cyclization immediately takes place, so a compound of Formula 13 can be obtained. Subsequent hydrolysis is the same as in synthesis of Compound 2-1.

Among cyclic urea compounds, a compound of Formula 2-4 which is directly attached to
 5 N can be synthesized from a compound of Formula 4 that is commercially available, according to the procedure described in Reaction Scheme 5 below.

[Reaction Scheme 5]



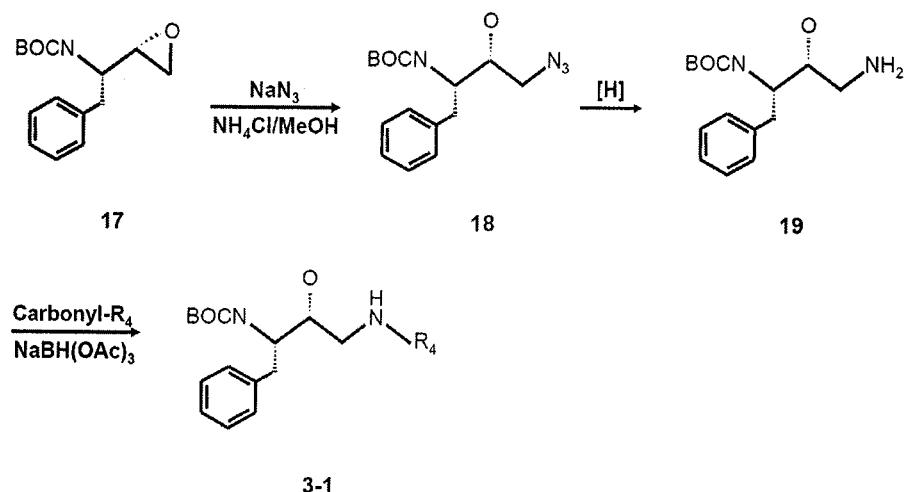
10 In Reaction Scheme 5, R is an alkyl group and Z is cbz. First, a compound of Formula 4 that is commercially available is subjected to amide coupling reaction to obtain a compound of Formula 14. Then, the compound of Formula 14 is alkylated to introduce an acetyl group, followed by deprotection of a Z group under hydrogen conditions to obtain a compound of Formula 16. Hydrogenation can be carried out using a Pd/C catalyst. The reaction is carried out in a solvent such
 15 as methanol, ethanol, and dioxane, at 1 to 3 atm.

A compound of Formula 2-4 can be obtained by hydrolysis of the compound of Formula 16, in the same manner as in synthesis of the compound of Formula 2-1.

In the compound of Formula 3, when R_3 is unsubstituted benzyl, it can be synthesized according to the procedure described in Reaction Scheme 6 below.

5

[Reaction Scheme 6]



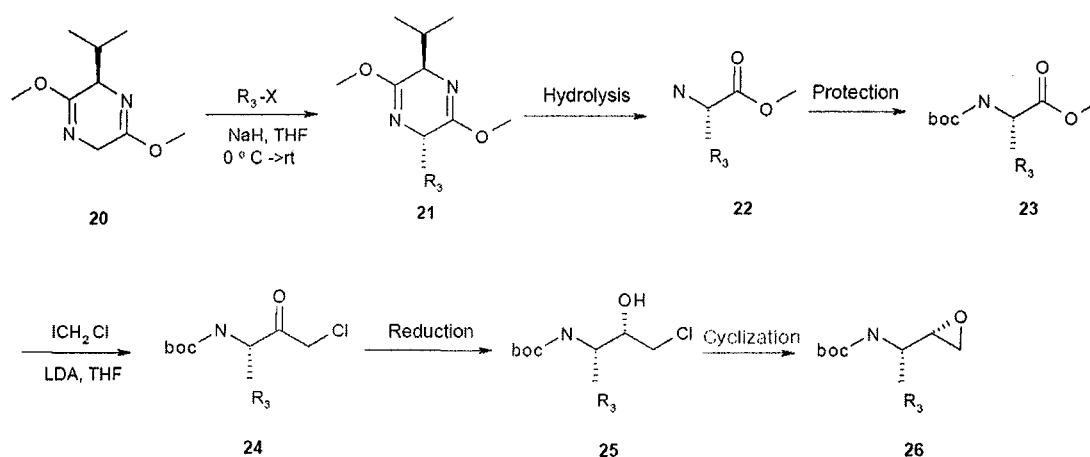
In Reaction Scheme 6, R_4 is as defined above. erythro-N-BOC-L-phenylalanine epoxide is commercially available. Azide is introduced into the epoxide in the presence of saturated ammonium and alcohol, followed by hydrogenation to obtain an amine compound of Formula 19 which is then subjected to reductive amination to give a compound of Formula 3-1.

Reductive amination is carried out using a compound (for example, ketone or aldehyde) containing a carbonyl group. Examples of an available reducing agent may include sodium borohydride, sodium cyanoborohydride, sodium triacetoxyborohydride, etc. In order to facilitate the

reaction, acid may be used as a catalyst. Examples of an available acid catalyst may include inorganic acids such as hydrochloric acid, sulfuric acid, nitric acid, and phosphoric acid; organic carbonic acids such as acetic acid, and trifluoroacetic acid; and amine salts such as ammonium chloride. Particularly preferred is hydrochloric acid or acetic acid.

- 5 When R_3 is a substituted benzyl epoxide, the desired compound can be synthesized according to a conventional amino acid synthesis as disclosed in Reaction Scheme 7 below.

[Reaction Scheme 7]



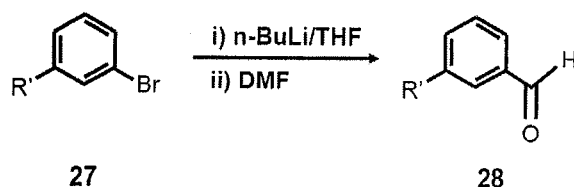
- 10 In Reaction Scheme 7, R_3 is as defined above.

Stereoselective alkylation is carried out using a chiral auxiliary of Formula 20. Then, hydrolysis of the compound of Formula 21 is carried out to prepare an amino acid derivative of Formula 22. The hydrolysis reaction to obtain the compound of Formula 22 may be carried out in a mixed solution of water and an organic solvent, using a base. Examples of the organic solvent may

- include THF, methanol, dioxane, and the like. Examples of the base may include LiOH, KOH, NaOH, and the like. Further, the reaction may be carried out using chloriodomethane in the presence of a base to obtain an chlorocarbonyl compound of Formula 24. Reduction of the compound of Formula 24 is then carried out to obtain an iodohydrin compound of Formula 25.
- 5 Reduction may be carried out using NaBH_4 , NaB(CN)H_3 , or the like. Further, an epoxide compound of Formula 26 can be obtained by cyclization of the chlorohydrin compound in the presence of a base.

- Most of the compounds wherein R_4 is as defined above are commercially available, but some of them can be obtained by conversion of bromide into aldehyde, according to the procedure
- 10 described in Reaction Scheme 8 below.

[Reaction Scheme 8]



- In Reaction Scheme 8, R' is alkyl or aryl. The reaction may be preferably carried out in a conventional solvent that is not detrimental to the reaction. Particularly preferably, examples of the solvent may include, but are not limited to, at least one solvent selected from the group consisting of DMF, dimethylacetamide, tetrahydrofuran, methylene chloride, dichloroethane, methanol, water and any combination thereof.
- 15

Separation of a conventional mixture is carried out by column chromatography, whereas a final compound may be separated by recrystallization or normal-phase or reverse-phase HPLC (Waters, Delta Pack, 300 x 50 mmI.D., C18 5 μ m, 100A). When recrystallization or HPLC is employed, a desired compound can be obtained in the form of a trifluoroacetate. When it is desired
5 to obtain hydrochloride, an ion exchange resin may be employed.

As described above, after the reaction according to the method of the present invention is complete, the reaction product can be separated and purified by conventional post-treatments, for example, chromatography, recrystallization, and the like.

In accordance with another aspect of the present invention, there is provided a
10 pharmaceutical composition for inhibiting beta-secretase, comprising a therapeutically effective amount of a compound of Formula I or a pharmaceutically acceptable salt or isomer thereof as an active ingredient and a pharmaceutically acceptable carrier. If desired, the composition may further comprise one or more diluents or excipients.

The compound of Formula I exhibits excellent inhibitory effects on beta-secretase, so the
15 present invention provides a beta-secretase inhibitor composition comprising a compound of Formula I in conjunction with a pharmaceutically acceptable carrier. In particular, the composition in accordance with the present invention exhibits excellent effects on improvement of cognitive functions or treatment or prevention of neurodegenerative diseases, particularly Alzheimer's disease without being limited thereto.

20 The term "pharmaceutical composition" as used herein means a mixture of a compound of the invention with other chemical components, such as diluents or carriers. The pharmaceutical

composition facilitates administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but are not limited to oral, injection, aerosol, parenteral, and topical administrations. Pharmaceutical compositions can also be obtained by reacting compounds with acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

The term "therapeutically effective amount" means that amount of the compound being administered which will relieve to some extent one or more of the symptoms of the disease being treated. Thus, a therapeutically effective amount refers to that amount which has the effect of (i) reversing the rate of progress of a disease, (ii) inhibiting or slowing to some extent further progress of the disease, and/or, (iii) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the disease.

The term "carrier" means a chemical compound that facilitates the incorporation of a compound into cells or tissues. For example, dimethyl sulfoxide (DMSO) is a commonly utilized carrier as it facilitates the uptake of many organic compounds into the cells or tissues of an organism.

The term "diluent" defines chemical compounds diluted in water that will dissolve the compound of interest as well as stabilize the biologically active form of the compound. Salts dissolved in buffered solutions are utilized as diluents in the art. One commonly used buffered solution is phosphate buffered saline because it mimics the salt conditions of human body fluid. Since buffer salts can control the pH of a solution at low concentrations, a buffered diluent rarely modifies the biological activity of a compound.

The term “physiologically acceptable” defines a carrier or diluent that does not abrogate the biological activity and properties of the compound.

The compounds described herein can be administered to a human patient *per se*, or in pharmaceutical compositions in which they are mixed with other active ingredients, as in combination therapy, or suitable carriers or excipients. Techniques for formulation and administration of the compounds may be found in “Remington’s Pharmaceutical Sciences,” Mack Publishing Co., Easton, PA, 18th edition, 1990.

a) Routes of Administration

Compounds of the present invention may be administered via any conventional routes, depending upon desired applications. Preferred routes of administration may, for example, include injection, oral and intranasal administrations. Alternatively, the active compounds may be administered by dermal, intraperitoneal, retroperitoneal and rectal routes.

b) Composition/Formulation

The pharmaceutical composition of the present invention may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions for use in accordance with the present invention thus may be

formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Any of the well-known techniques, carriers, and excipients may be used as
5 suitable and as understood in the art; *e.g.*, in Remington's Pharmaceutical Sciences, *supra*.

Injectable preparations, for example aqueous or oily suspensions for sterile injection may be prepared by a conventional method known in the art, using suitable dispersants, wetting agents and/or suspending agents. Examples of solvents that can be used in the formulation of injectable preparations may include water, Ringer's solution, and isotonic NaCl solution. In addition, sterile
10 fixing oil is also conventionally used as a solvent or suspending medium. Any non-irritable fixing oil including monoglyceride and diglyceride may be used for this purpose. In addition, fatty acids such as oleic acid may also be used for injectable preparations.

Examples of solid dosage forms for oral administration may include capsules, tablets, pills, powders and granules. Particularly preferred are capsules and tablets. Tablets and pills may be
15 preferably provided with enteric coatings. The solid dosage form may be prepared by mixing an active compound of Formula I in accordance with the present invention with one or more inert diluents (such as sucrose, lactose, and starch) and carriers such as lubricants (such as magnesium stearate), disintegrants, binders, and the like.

20 c) Effective Dosage

Pharmaceutical compositions suitable for use in the present invention include compositions in which the active ingredient is contained in an amount effective to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount of compound effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival
5 of the subject being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

When the compound of the present invention is administered for clinical purposes, a daily dosage of the compound, which may be administered to a subject in a single or divided doses, is preferably in a range of 10 to 100 mg/kg. As will be apparent to those skilled in the art, a specific
10 dose of the active ingredient for individual patients may vary depending on various factors such as kinds of compounds to be used, weight, sex, health conditions, and dietary habits of patients, treatment duration, administration manners, excretion rates, drug mixing and severity of disease.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

15 Now, the present invention will be described in more detail with reference to the following Examples. These examples are provided only for illustrating the present invention and should not be construed as limiting the scope and spirit of the present invention.

Abbreviations used in the preceding Reaction Schemes and the following Preparations
20 and Examples are as follows in Table 1:

[Table 1]

Ac: acetyl

Bu: butyl

5 CBZ (Cbz): benzyloxycarbonyl

BOC (BOC): t-butoxycarbonyl

c-Hex: cyclohexyl

DCE: dichloroethane

DCM: dichloromethane

10 DIPEA: diisopropylethylamine

DMAP: 4-dimethylaminopyridine

DMF: N,N-dimethylformamide

EDC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, hydrochloride

Hex: n-hexane

HOBt: hydroxybenzotriazole

HBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluoro phosphate

i-Pr: isopropyl

Ph: phenyl

5 TEA: triethylamine

TFA: trifluoroacetic acid

Hereinafter, preparation of reactants (intermediates) necessary for synthesis of compounds of Inventive Examples will be described in more detail with reference to the following Preparations.

10

Preparation 1: (S)-1-benzyl-2-oxo-imidazolidine-4-carboxylic acid

Step A: (S)-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester 5-t-butyl ester

Commercially available (S)-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester (2.64 g, 10 mmol) was dissolved in THF (20 mL), to which PPh₃ (2.62 g, 10 mmol) was then added. 15 t-BuOH (81 mg, 11 mmol) was added dropwise thereto, followed by stirring at room temperature for 3 hours. After the reaction was complete, 1N HCl was added dropwise to the reaction mixture,

followed by extraction with EtOAc. The organic layer was washed with water and brine, dried over MgSO_4 , and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/4) to afford the title compound (2.94 g, 92%).

$\text{MS}[\text{M}+1] = 321 (\text{M}+1)$

5

Step B: (S)-3-benzyl-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester, 5-t-butyl ester

(S)-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester 5-t-butyl ester (2.24 g, 7 mmol) obtained in Step A was dissolved in THF, to which NaH (392 mg, 60% mineral oil, 8.4 mmol) was then added dropwise at 0°C. 30 min later, benzyl chloride (970 mg, 7.7 mmol) was added dropwise thereto. The reaction solution was stirred at room temperature for 4 hours, followed by addition of a saturated aqueous solution of NH_4Cl and extraction with EtOAc. The organic extract was dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/4) to afford the title compound (2.43 g, 85%).

10

$\text{MS}[\text{M}+1] = 411 (\text{M}+1)$

15

Step C: (S)-1-benzyl-2-oxo-imidazolidine-4-carboxylic acid

(S)-3-benzyl-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester, 5-t-butyl ester (2.43 g, 5.93 mmol) obtained in Step B was dissolved in a 4N HCl aqueous solution, followed by stirring at room temperature for 3 hours. After the reaction was complete, the reaction mixture was

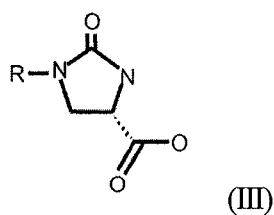
neutralized with a saturated aqueous solution of NaHCO_3 , followed by extraction with EtOAc. The residue was recrystallized to afford the title compound (1.14 g, 87%).

$^1\text{H NMR}$ (CDCl_3) δ 7.37-7.27 (m, 5H), 4.43-4.30 (m, 2H), 4.23-4.21 (m, 1H), 3.62 (t, 1H, $J=8.0\text{Hz}$),
5 3.46-3.37 (m, 1H),

$\text{MS}[\text{M}+1] = 221 (\text{M}+1)$

Preparations 2 to 13

Analogously to the procedure described in Preparation 1, compounds of Preparations 2 to
10 13 as listed in Table 2 below and represented by the following general formula were synthesized using a commercially available halide derivative and (S)-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester.



15 [Table 2]

Preparation No.	R	MS(M+H)
2	4-F-benzyl	239
3	4-Cl-benzyl	255
4	4-CF ₃ -benzyl	289
5	4-MeO-benzyl	251
6	3,4-DiF-benzyl	257
7	3,5-DiF-benzyl	257
8	2,4,5-triF-benzyl	275
9	3,5-diOMe-benzyl	281
10	4-OCF ₃ -benzyl	305
11	2-CF ₃ -benzyl	289
12	2-PhO-ethyl	313
13	n-pentyl	201

Preparation 14: (S)-1-butyryl-2-oxo-imidazolidine-4-carboxylic acid

Step A: (S)-3-butyryl-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester 5-t-butyl ester

(S)-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester 5-t-butyl ester (620 mg, 2 mmol) obtained in Step A of Preparation 1 was dissolved in DCM, to which Et₃N (560 μ l, 4 mmol) was then added dropwise, followed by addition of propyl acyl chloride (297 mg, 2.8 mmol) and stirring at room temperature for 2 hours. After the reaction was complete, a saturated aqueous solution of NH₄Cl was added thereto, followed by extraction with EtOAc. The organic extract was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/5) to afford the title compound (725 mg, 93%).

MS[M+1] = 391 (M+1)

10 Step B: (S)-1-butyryl-2-oxo-imidazolidine-4-carboxylic acid

Analogously to the procedure described in Step C of Preparation 1, the title compound was obtained using (S)-3-butyryl-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester 5-t-butyl ester obtained in Step A.

MS[M+1] = 201 (M+1)

15

Preparation 15: (S)-1-ethylcarbamoyl-2-oxo-imidazolidine-4-carboxylic acid

Analogously to the procedure described in Preparation 14, the title compound was obtained using (S)-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester 5-t-butyl ester obtained in Step A of Preparation 1 and ethyl isocyanate.

MS[M+1] = 202 (M+1)

Preparation 16; (S)-2-oxo-1-phenyl-imidazolidine-4-carboxylic acid

Step A: (S)-3-oxo-2-(tritylamino)-propionic acid t-butyl ester

5 Commercially available Tr-N-Ser-O(t-Bu) (2.02 g, 5 mmol) was dissolved in DCM and DMSO solution, and Et₃N (2.8 mL, 20 mmol) and SO₃Py (1.59 g, 10 mmol) were added thereto. The reaction solution was stirred at room temperature for 2 hours. After the reaction was complete, DCM was distilled under reduced pressure, followed by addition of a saturated NH₄Cl aqueous solution and extraction with ethyl ester. The organic extract was washed with water, dried over
10 MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/2) to afford the title compound (1.9 g, 95%).

MS[M+1] = 402 (M+1)

Step B: (S)-3-phenylamino-2-tritylamino-propionic acid t-butyl ester

15 (S)-3-oxo-2-(tritylamino)-propionic acid t-butyl ester (1.5 g, 3.74 mmol) obtained in Step A and aniline (465 mg, 5 mmol) were dissolved in dichloroethane, to which NaBH(OAc)₃ (1.72 g, 7.48 mmol) was then added. The reaction solution was stirred at room temperature for 4 hours. After the reaction was complete, the reaction solution was diluted with a saturated aqueous solution of NaHCO₃, followed by extraction with DCM and EtOAc. The organic extract was washed with

brine, dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/2) to afford the title compound (2.07 g, 87%).

$\text{MS}[\text{M}+1] = 479 (\text{M}+1)$

5 Step C: (S)-2-oxo-1-phenyl-3-tri-imidazolidine-4-carboxylic acid t-butyl ester

(S)-3-phenylamino-2-tritylamino-propionic acid t-butyl ester (2.0 g, 4.18 mmol) obtained in Step B was dissolved in DCM (10 mL), to which phosgene (in toluene, 2M, 4 mL) was then added, followed by stirring at room temperature for 4 hours. After the reaction was complete, the solvent was distilled under reduced pressure. The residue was purified by column chromatography
10 (eluent: EtOAc: n-Hex = 1/3) to afford the title compound (1.09 g, 54%).

$\text{MS}[\text{M}+1] = 521 (\text{M}+1)$

Step D: (S)-2-oxo-1-phenyl-imidazolidine-4-carboxylic acid

Analogously to the procedure described in Step C of Preparation 1, the title compound
15 was obtained using (S)-2-oxo-1-phenyl-3-tri-imidazolidine-4-carboxylic acid t-butyl ester obtained in Step C.

$\text{MS}[\text{M}+1] = 223 (\text{M}+1)$

Preparation 17: (S)-1-(3,5-dibromophenyl)-2-oxo-imidazolidine-4-carboxylic acid

Analogously to the procedure described in Preparation 16, the title compound was obtained using (S)-3-oxo-2-(tritylamino)-propionic acid t-butyl ester obtained in Step A of
5 Preparation 16 and 3,5-dibromo aniline.

MS[M+1] = 381 (M+1)

Preparation 18: ((S)-4-dimethylcarbamoyl-2-oxo-imidazolidine-1-yl)acetic acid

Step A: (S)-5-dimethylcarbamoyl-2-oxo-imidazolidine-1-carboxylic acid benzyl ester

10 Commercially available (S)-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester (2.64 g, 10 mmol) was dissolved in DHF (20 mL), to which EDC (2.33 g, 13 mmol) and HOBT (2.30 g, 15 mmol) were then added, followed by addition of Et₃N (2.8 mL, 20 mmol). After confirming that a pH of the solution was basic, dimethylamine hydrochloride (1.2 g, 15 mmol) was added thereto and a pH of the solution was measured again to confirm that it was basic, followed by stirring
15 at room temperature for 12 hours. After the reaction was complete, DMF was distilled under reduced pressure. The residue was diluted with EtOAc, washed with a saturated aqueous solution of NaHCO₃ and 1N HCl, and water. The filtrate was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/1) to afford the title compound (2.50 g, 87%).

MS[M+1] = 392 (M+1)

Step B: (S)-3-t-butoxycarbonylmethyl-5-dimethylcarbamoyl-2-oxo-imidazolidine-1-carboxylic acid benzyl ester

5 (S)-5-dimethylcarbamoyl-2-oxo-imidazolidine-1-carboxylic acid benzyl ester (2.0 g, 6.87 mmol) obtained in Step A was dissolved in THF (20 mL), and the reaction solution was cooled to 0 °C, followed by addition of NaH and stirring for 30 min. A solution of t-butylbromoacetate in THF was gradually added and the reaction solution was warmed to room temperature, followed by stirring for 4 hours. After the reaction was complete, a saturated aqueous solution of NH₄Cl was
10 added thereto, followed by extraction with EtOAc. The organic extract was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/3) to afford the title compound (2.50 g, 87%).

MS[M+1] = 406 (M+1)

15 Step C: ((S)-4-dimethylcarbamoyl-2-oxo-imidazolidine-1-yl)acetic acid

Analogously to the procedure described in Step C of Preparation 1, the title compound was obtained using (S)-3-t-butoxycarbonylmethyl-5-dimethylcarbamoyl-2-oxo-imidazolidine-1-carboxylic acid benzyl ester obtained in Step B.

MS[M+1] = 216 (M+1)

Preparation 19: 1-butyl-5-oxo-pyrrolidine-3-carboxylic acidStep A: 1-butyl-5-oxo-pyrrolidine-3-carboxylic acid methyl ester

Dimethyl itaconate (1.58 g, 10 mmol) was dissolved in THF (30 mL) and n-butylamine
5 (870 mg, 10 mmol) was then added thereto. The reaction solution was heated with stirring to 50 °C
for 12 hours. After the reaction was complete, a saturated aqueous solution of NH₄Cl was added to
the reaction solution, followed by extraction with EtOAc. The organic extract was dried over
MgSO₄ and concentrated under reduced pressure. The residue was purified by column
chromatography (eluent: EtOAc: n-Hex = 1/1) to afford the title compound (1.84 g, 80%).

10 MS[M+1] = 246 (M+1)

Step B: 1-butyl-5-oxo-pyrrolidine-3-carboxylic acid

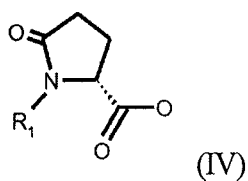
1-butyl-5-oxo-pyrrolidine-3-carboxylic acid methyl ester (1 g, 4.6 mmol) obtained in Step
A was dissolved in MeOH (1 mL), and water (5 mL) was added thereto, followed by addition of
15 LiOH (529 mg, 23 mmol). The reaction solution was stirred at room temperature for 4 hours. After
the reaction was complete, MeOH was removed by distillation under reduced pressure and the
reaction solution was adjusted to a pH of 2 to 3 with addition of 1N HCl, followed by extraction
with EtOAc to afford the title compound (851 mg, 93%).

MS[M+1] = 200 (M+1)

Preparations 20 to 26

Analogously to the procedure described in Preparation 19, compounds of Preparations 20 to 26 as listed in Table 3 below and represented by the following general formula were synthesized

5 using commercially available amine compound and dimethyl itaconate.



[Table 3]

Preparation No.	R ₁	MS(M+H)
20	c-Hex-CH ₂	226
21	Ph-CH ₂ -CH ₂	233
22	(3-AcNH)phenyl	263
24	[2-(Cl-phenyl)-1-ethylcarbamoyl]ethyl	339
25	[2-(Cl-phenyl)-1-carbamoyl]ethyl	310
26	(2-carbamoyl)ethyl	189

Preparation 27: 1-benzyl-4-methyl-2-oxo-imidazolidine-4-carboxylic acid

Step A: 3-benzyl-5-methyl-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester 5-t-butyl ester

(S)-3-benzyl-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester, 5-t-butyl ester

- 5 (410 mg, 1 mmol) obtained in Step B of Preparation 1 was dissolved THF (5 mL), and the reaction solution was cooled to -78 °C, to which a solution of LiHMDS (1N THF, 1.4 mL, 1.4 mmol) was then added, followed by addition of methyl iodide (135 mg, 1 mmol) 10 min later. The reaction solution was stirred at -78 °C for 1 hour, and the reaction was terminated with addition of a saturated NH₄Cl aqueous solution, followed by extraction with EtOAc. The organic extract was dried over
- 10 MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/2) to afford the title compound (339 mg, 80%).

MS[M+1] = 425 (M+1)

Step B: 1-benzyl-4-methyl-2-oxo-imidazolidine-4-carboxylic acid

- 15 Analogously to the procedure described in Step C of Preparation 1, the title compound was obtained using 3-benzyl-5-methyl-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester 5-t-butyl ester prepared in Step A.

MS[M+1] = 235 (M+1)

Preparation 28: 1,4-dibenzyl-2-oxo-imidazolidine-4-carboxylic acid

Analogously to the procedure described in Preparation 27, the title compound was obtained using (S)-3-benzyl-2-oxo-imidazolidine-1,5-dicarboxylic acid 1-benzyl ester, 5-*t*-butyl ester obtained in Step B of Preparation 1 and benzyl chloride.

MS[M+1] = 311 (M+1)

Preparation 29: (1-methyl-5-oxo-pyrrolidin-3-yl)-acetic acid

Step A: 3-methanesulfonyloxy-pentanedioic acid dimethyl ester

Commercially available methyl 3-hydroxy-pentanedioic acid dimethyl ester (3.5 g, 20 mmol) was dissolved in DCM (50 mL) and Et₃N (5.6 mL, 40 mmol) was added thereto. The reaction solution was cooled to 0 °C, and MsCl (2.75 g, 24 mmol) was added dropwise thereto, followed by stirring at room temperature for 2 hours. After the reaction was complete, NH₄Cl was added to the reaction solution, followed by extraction with EtOAc. The organic extract was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/5) to afford the title compound (4.77 g, 94%).

MS[M+1] = 241 (M+1)

Step B: 3-nitromethyl-pentanedioic acid dimethyl ester

3-methanesulfonyloxy-pentanedioic acid dimethyl ester (4.0 g, 15.7 mmol) obtained in Step A was dissolved DMF (950 mL), to which DMAP (9200 mg, 1.57 mmol) was then added, followed by addition of nitro methane (4.71 g, 78.5 mmol) and stirring at 80°C for 12 hours. After
5 the reaction was complete, NH_4Cl was added thereto, followed by extraction with EtOAc. The organic extract was dried over MgSO_4 and concentrated under reduced pressure. The residue was recrystallized from EtOAc/n-Hex to afford the title compound (2.78 g, 81%).

$$\text{MS}[\text{M}+1] = (\text{M}+1)$$

10 Step C: 3-aminomethyl-pentanedioic acid dimethyl ester

3-nitromethyl-pentanedioic acid dimethyl ester (2.19 g, 10 mmol) obtained in Step B was dissolved in MeOH (20 mL), and Pd/C (200 mg) was added thereto, followed by reaction in a hydrogen reaction vessel for 12 hours. After the reaction was complete, the reaction mixture was filtered through celite. The residue was directly used in subsequent reactions without further
15 purification.

$$\text{MS}[\text{M}+1] = 176 (\text{M})$$

Step D: (5-oxo-pyrrolidin-3-yl)-acetic acid methyl ester

Analogously to the procedure described in Step A of Preparation 19, the title compound was obtained using 3-aminomethyl-pentanedioic acid dimethyl ester obtained in Step C.

$$MS[M+1] = 144 (M+1)$$

5 Step E: (1-methyl-5-oxo-pyrrolidin-3-yl)acetic acid methyl ester

Analogously to the procedure described in Step B of Preparation 18, the title compound was obtained using (5-oxo-pyrrolidin-3-yl)-acetic acid methyl ester obtained in Step D and methyl iodide.

$$MS[M+1] = 158 (M+1)$$

10

Step F: (1-methyl-5-oxo-pyrrolidin-3-yl)-acetic acid

Analogously to the procedure described in Step C of Preparation 19, the title compound was obtained using (1-methyl-5-oxo-pyrrolidin-3-yl)acetic acid methyl ester obtained in Step E.

$$MS[M+1] = 144 (M+1)$$

15

Preparation 30: (1-benzyl-5-oxo-pyrrolidin-3-yl)-acetic acid

Analogously to the procedure described in Steps E and F of Preparation 22, the title compound was obtained using (5-oxo-pyrrolidin-3-yl)-acetic acid methyl ester obtained in Step D of Preparation 29 and benzyl chloride.

MS[M+1] = 220 (M+1)

5

Preparation 31: (S)-4-benzyl-5-oxo-pyrrolidine-2-carboxylic acid

Step A: (S)-2-benzyl-4-t-butoxycarbonylamino-pentanedioic acid dimethyl ester

Analogously to the procedure described in Step A of Preparation 27, the title compound was obtained using commercially available NBOC-ASP(OMe)-OMe and benzyl bromide.

10 MS[M+1] = 366 (M+1)

Step B: (S)-2-amino-4-benzyl-pentanedioic acid methyl ester

(S)-2-benzyl-4-t-butoxycarbonylamino-pentanedioic acid dimethyl ester obtained in Step A was dissolved in DCM and TFA was added thereto, followed by stirring at room temperature for 15 2 hours. After the reaction was complete, the solvent was removed by distillation under reduced pressure and the reaction mixture was diluted with DCM, followed by removal of the solvent under reduced pressure. The residue was dissolved again in DCM and recrystallized from diethyl ester to afford the title compound.

$$MS[M+1] = 266 (M+1)$$

Step C: (S)-4-benzyl-5-oxo-pyrrolidine-2-carboxylic acid methyl ester

Analogously to the procedure described in Step B of Preparation 19, the title compound
5 was obtained using (S)-2-amino-4-benzyl-pentanedioic acid methyl ester obtained in Step B.

$$MS[M+1] = 235 (M+1)$$

Step D: (S)-4-benzyl-5-oxo-pyrrolidine-2-carboxylic acid

Analogously to the procedure described in Step C of Preparation 19, the title compound
10 was obtained using (S)-4-benzyl-5-oxo-pyrrolidine-2-carboxylic acid methyl ester obtained in Step C.

$$MS[M+1] = 221 (M+1)$$

Preparation 32: (R)-2,4-dibenzyl-5-oxo-pyrrolidine-2-carboxylic acid

A dialkylation compound was obtained during purification of a compound in Step A of Preparation 31. Analogously to the procedure described in Steps B, C, and D of Preparation 31, the title compound was obtained using the dialkylation compound.

MS[M+1] = 310 (M+1)

5

Preparation 33: ((1S,2R)-3-amino-1-benzyl-2-hydroxy-propyl)carbamic acid t-butyl ester

Step A: ((1S,2R)-3-azido-1-benzyl-2-hydroxy-propyl)carbamic acid t-butyl ester

(2S,3S)-erythro-N-BOC-L-phenylalanine epoxide (10.4 g, 50 mmol) was dissolved in ethanol (150 mL) and a saturated aqueous solution of NH₄Cl (40 mL) was added thereto. When a
10 homogeneous solution was obtained, NaN₃ (9.83 g, 150 mmol) was added thereto, followed by stirring at 80 °C for 12 hours. After the reaction was complete, extraction with EtOAc was carried out. The organic extract was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/4) to afford the title compound (13.2 g, 86%).

15 MS[M+1] = 207 (M+1)

Step B: ((1S,2R)-3-amino-1-benzyl-2-hydroxy-propyl)carbamic acid t-butyl ester

Analogously to the procedure described in Step C of Preparation 29, the title compound was obtained using ((1S,2R)-3-azido-1-benzyl-2-hydroxy-propyl)carbamic acid t-butyl ester obtained in Step A.

MS[M+1] = 181 (M+1)

5

Preparation 34: ((1S,2R)-3-amino-1-(3,5-difluorobenzyl)-2-hydroxy-propyl)carbamic acid t-butyl ester

Step A: (2S,5R)-2-(3,5-difluorobenzyl)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine

(R)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine was dissolved THF and n-BuLi was added thereto at -78°C, followed by addition of difluorobenzyl chloride. After the reaction was complete, NH₄Cl was added to the reaction mixture, followed by extraction with EtOAc. The organic extract was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/1) to afford the title compound (339 mg, 80%).

15 MS[M+1] = 313 (M+1)

Step B: (S)-2-amino-3-(3,5-difluorophenyl)propionic acid methyl ester

(2S,5R)-2-(3,5-difluorobenzyl)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine obtained in Step A was dissolved in acetonitrile, to which 2N HCl was then added, followed by stirring at room temperature for 4 hours. After the reaction was complete, NaHCO₃ was added thereto, followed by extraction with EtOAc. The organic extract was dried over MgSO₄ and concentrated under reduced pressure to afford the title compound (339 mg, 80%).

MS[M+1] = 215 (M+1)

Step C: (S)-2-t-butoxycarbonylamino-3-(3,5-difluorophenyl)propionic acid methyl ester

(S)-2-amino-3-(3,5-difluorophenyl)propionic acid methyl ester obtained in Step B was dissolved in water and NaOH was added thereto, followed by addition of (BOC)₂O. 12 hours later, the reaction was terminated with addition of 1N HCl, followed by extraction with EtOAc. The organic extract was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/8) to afford the title compound (339 mg, 80%).

MS[M+1] = 315 (M+1)

Step D: [(3S)-1-(3,5-difluorobenzyl)-3-chloro-2-oxo-propyl]-carbamic acid t-butyl ester

Chloriodomethane was dissolved in THF and LDA was added thereto, followed by addition of (S)-2-t-butoxycarbonylamino-3-(3,5-difluorophenyl)propionic acid methyl ester

obtained in Step C and stirring at room temperature for 12 hours. After the reaction was complete, NH_4Cl was added thereto, followed by extraction with EtOAc. The organic extract was dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/5) to afford the title compound (339 mg, 80%).

5 MS[M+1] = 426 (M+1)

Step E: [(1S,2S)-1-(3,5-difluorobenzyl)-2-hydroxy-3-chloro-propyl]carbamic acid t-butyl ester

[(3S)-1-(3,5-difluorobenzyl)-3-chloro-2-oxo-propyl]-carbamic acid t-butyl ester obtained in Step D was dissolved in MeOH and NaBH_4 was added thereto, followed by stirring at room temperature for 12 hours. After the reaction was complete, water was added to the reaction mixture, followed by addition of 1N HCl, stirring for 30 min and then extraction with EtOAc. The organic extract was dried over MgSO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/3) to afford the title compound (339 mg, 80%).

15 MS[M+1] = 428 (M+1)

Step F: [(S)-2-(3,5-difluoro-phenyl)-1-(S)-oxiranyl-ethyl]-carbamic acid t-butyl ester

[(1S,2S)-1-(3,5-difluorobenzyl)-2-hydroxy-3-chloro-propyl]carbamic acid t-butyl ester obtained in Step E was dissolved in THF and NaOH was added thereto, followed by stirring at room

temperature for 12 hours. After the reaction was complete, 1N HCl was added to the reaction mixture, followed by extraction with EtOAc. The organic extract was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/1) to afford the title compound (339 mg, 80%).

5 MS[M+1] = 300 (M+1)

Step G: ((1S,2R)-3-amino-1-(3,5-difluorobenzyl)-2-hydroxy-propyl)carbamic acid t-butyl ester

Analogously to the procedure described in Steps A and B of Preparation 33, the title compound was obtained using [(S)-2-(3,5-difluoro-phenyl)-1-(S)-oxiranyl-ethyl]-carbamic acid t-

10 butyl ester obtained in Step F.

MS[M+1] = 217 (M+1)

Preparation 35: 3-dimethylamino benzaldehyde

Analogously to the procedure described in Step A of Preparation 16, the title compound

15 was obtained using 3-dimethylamino benzyl alcohol.

MS[M+1] = 150 (M+1)

Preparation 36: 3-t-butyl benzaldehyde

3-t-butyl phenylbromide was dissolved in THF and n-BuLi was added thereto, followed by addition of DMF and stirring at 0°C for 3 hours. After the reaction was complete, NH₄Cl was added to the reaction mixture, followed by extraction with EtOAc. The organic extract was dried
5 over MgSO₄ and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: EtOAc: n-Hex = 1/3) to afford the title compound (339 mg, 80%).

MS[M+1] = 163 (M+1)

10 Preparation 37: [(1S,2R)-1-benzyl-3-(3-dimethylamino-benzylamino)-2-hydroxy-propyl]-carbamic acid t-butyl ester

Analogously to the procedure described in Step B of Preparation 16, the title compound was obtained using ((1S,2R)-3-amino-1-benzyl-2-hydroxy-propyl)carbamic acid t-butyl ester and 3-dimethylaminobenzaldehyde.

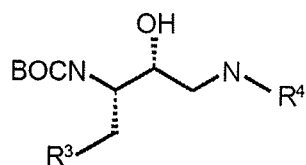
MS[M+1] = 414 (M+1)

15

Preparations 38 to 50

Analogously to the procedure described in Step B of Preparation 16, compounds of Preparations 38 to 50 as listed in Table 4 below and represented by the following general formula

were synthesized using amine compounds synthesized in Preparations 33 and 34 and aldehydes synthesized in Preparations 35 and 36 or commercially available aldehydes.



5

[Table 4]

Preparation No.	R ₃	R ⁴	MS[M+1]
38	phenyl	ethyl	309
39	phenyl	cyclohexyl	363
40	phenyl	3-(t-butyl)benzyl	427
41	phenyl	3-(ethyl)benzyl	399
42	phenyl	3-(isopropyl)benzyl	413
43	phenyl	3-CF ₃ O-benzyl	455
44	phenyl	3-CF ₃ benzyl	439

45	phenyl	3-(amino)benzyl	386
46	phenyl	3-methylaminobenzyl	400
47	phenyl	3-nitrobenzyl	416
48	3,5-difluorophenyl	3-(t-butyl)benzyl	463
49	3,5-difluorophenyl	3-(isopropyl)benzyl	449
50	3,5-difluorophenyl	3-dimethylaminobenzyl	450

Example 1: (2S)-N-[(2S,3R)-4-[(3-dimethylaminophenyl)methylamino]-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide

5 Step A: (2R,3S)-3-amino-1-(3-dimethylamino-benzylamino)-4-phenyl-butan-2-ol

[(1S,2R)-1-benzyl-3-(3-dimethylamino-benzylamino)-2-hydroxy-propyl]-carbamic acid t-butyl ester (414 mg, 1 mmol) obtained in Preparation 37 was dissolved in dichloromethane (5 mL), to which TFA (2 mL) was then added, followed by stirring at room temperature for 2 hours. After the reaction was complete, the solvent was removed under reduced pressure, followed by drying.

10 The residue was directly used in subsequent reactions without further purification.

Mass [M+1]= 314(M+H)

Step B: (2S)-N-[(2S,3R)-4-[(3-dimethylaminophenyl)methylamino]-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide

Commercially available (S)-5-oxo-proline (129 mg, 1 mmol) was dissolved in DMF (5 mL) and Et₃N (280 μM, 2 mmol) was added thereto. HOBT (229.5 mg, 1.5 mmol) and EDC (232 mg, 1.3 mmol) were added to the mixture which was then stirred at room temperature for 30 min, followed by addition of (2R,3S)-3-amino-1-(3-dimethylamino-benzylamino)-4-phenyl-butan-2-ol (313 mg, 1 mmol) obtained in Step A. After the reaction was complete, the solvent was removed under reduced pressure, and the reaction mixture was diluted with a saturated aqueous solution of NaHCO₃, followed by extraction with EtOAc. The organic layer was washed with brine, dried over MgSO₄, and distilled under reduced pressure. The residue was purified by Prep-TLC (10% MeOH/90% DCM) to afford the title compound (305 mg, 72%).

¹H NMR (400MHz, CDCl₃) δ 7.26-7.14 (m, 6H), 6.72-6.64 (m, 3H), 4.23-4.19 (m, 1H), 3.96-3.92 (m, 1H), 3.87-3.76 (m, 3H), 3.20-3.10 (m, 1H), 2.94 (s, 6H), 2.87-2.77 (m, 3H), 2.23-1.85 (m, 3H), 1.51-1.46 (m, 1H)

Mass [M+1]= 425(M+H)

Example 2: (2R)-N-[(2R,3S)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-carboxamide

Analogously to the procedure described in Example 1, the title compound was obtained using commercially available (R)-5-oxo-proline and (2R,3S)-3-amino-1-(3-dimethylamino-benzylamino)-4-phenyl-butan-2-ol obtained in Step A of Example 1.

^1H NMR (400MHz, CDCl_3) δ 7.26-7.14 (m, 6H), 6.72-6.64 (m, 3H), 4.23-4.19 (m, 1H), 3.96-3.92 (m, 1H), 3.87-3.76 (m, 3H), 3.20-3.10 (m, 1H), 2.94 (s, 6H), 2.87-2.77 (m, 3H), 2.23-1.85 (m, 3H), 1.51-1.46 (m, 1H)

Mass $[M+1]=425(M+H)$

Example 3: (4S)-1-benzyl-N-[1-(3,5-difluorophenyl)-4-(3-dimethylaminophenyl)methylamino-3-hydroxybutan-2-yl]-2-oxoimidazolidine-4-carboxamide

Analogously to the procedure described in Example 1, the title compound was obtained using [(1S,2R)-1-(3,5-difluoro-benzyl)-3-(3-dimethylamino-benzylamino)-2-hydroxy-propyl]-carbamic acid t-butyl ester obtained in Preparation 50.

^1H NMR (CDCl_3) δ 8.13 (brs, 1H), 7.28-7.14 (m, 6H), 6.79-6.72 (m, 4H), 6.67-6.65 (m, 1H), 6.58 (t, 1H, $J=8.0\text{Hz}$), 4.58 (d, 1H, $J=16\text{Hz}$), 4.27-3.90 (m, 3H), 3.77 (brs, 2H), 3.47-3.41 (m, 1H), 2.89 (s, 6H), 2.85-2.67 (m, 2H), 2.53 (brs, 1H), 2.30 (brs, 1H)

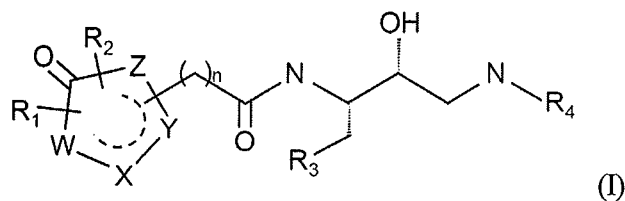
Mass $[M+1]=516$ ($M+H$)

5

Examples 4 to 60

Analogously to the procedure described in Example 3, compounds of Examples 4 to 60 as listed in Table 5 below and represented by Formula I were synthesized using acids synthesized in Preparations 1 to 30 and amine compounds synthesized in Preparations 33 and 34.

10



[Table 5]

Ex. No.	R_1	R_2	Ar	n	*	R_3	R_4

H ¹ NMR data							
4	benzyl	hydrogen	(iii)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, MeOD); δ 7.24-7.05(m, 11H), 6.78(s, 1H), 6.69(m, 2H), 4.03(m, 1H), 3.96(q, 2H), 3.87(t, 1H), 3.78(td, 1H), 3.15(dd, 1H), 3.08(dd, 1H), 2.99(dd, 1H), 2.89(s, 6H), 2.87(m, 1H), 2.81(dd, 3H), 2.68-2.54(m, 2H), 2.28(dd, 1H), 2.15(m, 1H)						
5	benzyl	benzyl	(iii)	0	R	phenyl	(3-dimethylamino)benzyl
	(500MHz, MeOD); δ 7.29-7.07(m, 16H), 6.79-6.68(m, 3H), 3.97-3.84(m, 3H), 3.74(td, 1H), 3.07(d, 1H), 2.93-2.86(m, 2H), 2.91(s, 6H), 2.84-2.74(m, 3H), 2.70-2.48(m, 3H), 2.17(dd, 1H), 1.72(dd, 1H)						
6	benzyl	benzyl	(iii)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, MeOD); δ 7.32-7.05(m, 15H), 6.96(s, 1H), 6.80-6.69(m, 3H), 3.98(m, 2H), 3.89(m, 1H), 3.72(m, 1H), 3.08(d, 1H), 2.93(s, 6H), 2.92(m, 1H), 2.86-2.69(m, 3H), 2.63-2.48(m, 3H), 2.00(dd, 1H), 1.33(dd, 1H)						
7	dimethylcarbamoyl	hydrogen	(iv)	1	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.32-7.15(m, 6H), 6.83 (m, 1H), 6.82-6.75 (m, 2H), 4.34 (t, 1H), 4. 10 (m, 1H), 3.92 (m, 1H), 3.84 (s, 2H), 3. 30 – 3.21 (m, 2H), 3. 10 – 3.06 (m, 2H), 2.94 (s, 6H), 2.88 (s, 6H), 2.76 – 2.73 (m, 2H), 4.01 (s, 2H), 3.92-3.89 (m, 2H), 3.33-3.13 (m, 4H), 2.97-2.85 (m, 9H), 2.70 (m, 1H), 1.68 (q, J=12Hz, 2H), 0.98 (t, J=12Hz, 3H)						
8	benzyl	methyl	(i)	0	R	phenyl	(3-dimethylamino)benzyl

	(400MHz, CDCl ₃); δ 7.53(d, 1H), 7.32(t, 2H), 7.26-7.06(m, 8H), 6.75(m, 1H), 6.65(m, 2H), 4.46(d, 1H), 4.15(d, 1H), 4.12(m, 1H), 3.93(m, 1H), 3.85(s, 2H), 3.51(d, 1H), 2.96(m, 2H), 2.93(s, 6H), 2.78(m, 3H)						
9	2,4,5-tri-F-benzyl	hydrogen	(i)	0	S	phenyl	ethyl
	(400MHz, CDCl ₃); δ 7.27-7.16 (7H, m), 4.38-4.22 (2H, m), 4.18-4.08 (2H, m), 3.95-3.90 (1H, m), 3.59-3.50 (1H, m), 3.29-3.21 (2H, m), 3.18-3.03 (2H, m), 3.01-2.96 (1H, m), 2.78-2.72 (2H, m), 1.37-1.32 (3H, m)						
10	2,4,5-tri-F-benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃); δ 7.84 (1H, brs), 7.53 (1H, brs), 7.30-7.02 (7H, m), 6.90-6.71 (3H, m), 6.67-6.65 (1H, m), 5.22 (2H, brs), 4.30-4.26 (1H, m), 4.12-4.00 (3H, m), 3.79 (2H, brs), 3.43-3.36 (1H, m), 2.91 (6H, s), 2.76-2.73 (2H, m), 2.64-2.61 (2H, m)						
11	2,4,5-tri-F-benzyl	hydrogen	(i)	0	S	phenyl	(3-t-butyl)benzyl
	(400MHz, CDCl ₃); δ 7.38 (1H, s), 7.40-7.11 (10H, m), 4.34-4.31 (2H, m), 4.11-4.07 (1H, m), 4.03-4.00 (1H, m), 3.83-3.69 (3H, m), 3.49-3.45 (1H, m), 3.09-3.06 (1H, m), 2.79-2.71 (2H, m), 2.67-2.61 (2H, m), 1.30 (9H, s)						
12	2,4,5-tri-F-benzyl	hydrogen	(i)	0	S	phenyl	(3-ethyl)benzyl

	(400MHz, CDCl ₃); δ 7.32-7.18 (14H, m), 7.11-7.09 (4H, m), 6.20 (1H, brs), 4.42-4.39 (1H, m), 4.22-4.18 (1H, m), 4.11-4.01 (4H, m), 3.42-3.37 (1H, m), 3.01-2.98 (2H, m), 2.91-2.75 (3H, m), 2.63-2.59 (1H, m), 1.22 (6H, d, J=7.2Hz)						
13	2,4,5-tri-F-benzyl	hydrogen	(i)	0	S	3,5-di-F-phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃); δ 7.27 (1H, brs), 7.21-7.17 (2H, m), 7.08 (1H, brs), 6.91-6.86 (2H, m), 6.71-6.58 (8H, m), 4.46-4.24 (1H, m), 4.06 (3H, brs), 3.52-3.48 (1H, m), 2.94-2.91 (8H, m), 2.84-2.77 (3H, m)						
14	2,4,5-tri-F-benzyl	hydrogen	(i)	0	S	3,5-di-F-phenyl	(3-isopropyl)benzyl
	(400MHz, CDCl ₃); δ 7.65 (1H, brs), 7.26-7.15 (5H, m), 6.92-6.89 (1H, m), 6.69-6.61 (3H, m), 6.25 (1H, brs), 4.48-4.46 (1H, m), 4.12-4.08 (3H, m), 3.80 (2H, brs), 2.87-2.78 (6H, m), 1.22 (6H, d, J=6.8Hz)						
15	benzyl	hydrogen	(i)	0	S	phenyl	cyclohexyl
	(400MHz, CDCl ₃); δ 7.44-7.30 (4H, m), 7.26-7.23 (2H, m), 7.18-7.15 (4H, m), 4.36-4.20 (2H, m), 4.12-4.08 (1H, m), 3.95-3.90 (1H, m), 3.48-3.43 (1H, m), 3.29-3.20 (2H, m), 3.14-3.07 (1H, m), 3.02-2.97 (1H, m), 2.76-2.69 (1H, m), 2.11 (2H, brs), 1.89-1.88 (2H, m), 1.74-1.71 (1H, m), 1.46-1.25 (5H, m)						
16	hydrogen	hydrogen	(i)	0	S	phenyl	(3-t-butyl)benzyl

	(400MHz, CDCl ₃); δ 7.52 (1H, brs), 7.44-7.43 (1H, m), 7.35-7.32 (1H, m), 7.25-7.22 (3H, m), 7.19-7.14 (3H, m), 4.12-4.02 (4H, m), 3.87-3.84 (1H, m), 3.56-3.52 (1H, m), 3.20-3.16 (1H, m), 3.09-3.06 (1H, m), 2.91-2.87 (1H, m), 2.72-2.68 (2H, m), 1.32 (9H, s)						
17	hydrogen	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.30-7.18(m, 6H), 6.87 (s, 1H), 6.83 (d, J=8Hz, 1H), 6.77 (d, J=8Hz, 1H), 4.14-4.11 (m, 3H), 4.09 (m, 1H), 3.88 (m, 1H), 3.57 (t, J=8Hz, 1H), 3.25 (m, 1H), 3.16 (m, 1H), 3.00-2.98 (m, 7H), 2.71 (m, 2H)						
18	2-CF ₃ -benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.61 (2H, d, J=8.0Hz), 7.43-7.39 (1H, m), 7.35-7.31 (3H, m), 7.21-7.16 (1H, m), 7.11 (5H, brs), 6.75-6.73 (2H, m), 6.65-6.64 (1H, m), 4.58-4.55 (1H, m), 4.30-4.23 (1H, m), 4.10-4.05 (2H, m), 3.91 (1H, brs), 3.78 (2H, brs), 3.38-3.34 (1H, m), 2.94-2.88 (8H, m), 2.77-2.69 (2H, m), 2.58-2.55 (1H, m)						
19	benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.36-7.10(m, 12H), 6.73 (m, 2H), 4.43-3.98 (m, 4H), 3.85-3.43 (m, 7H), 3.37 (m, 2H), 2.98 (m, 6H)						
20	benzyl	hydrogen	(i)	0	S	phenyl	(3-CF ₃ O)benzyl
	(400MHz, MeOD) . 7.43-7.03 (m, 14H), 4.29 (s, 2H), 4.03 (m, 1H), 3.90 (m, 2H), 3.65 (m, 1H), 3.12 (m, 2H), 2.94 (m, 1H), 2.73 (m, 2H), 2.58-2.39 (m, 4H)						

21	benzyl	hydrogen	(i)	0	S	phenyl	(3-isopropyl)benzyl
	(400MHz, MeOD) . 7.32-7.18 (14H, m), 7.11-7.09 (4H, m), 6.20 (1H, brs), 4.42-4.39 (1H, m), 4.22-4.18 (1H, m), 4.11-4.01 (4H, m), 3.42-3.37 (1H, m), 3.01-2.98 (2H, m), 2.91-2.75 (3H, m), 2.63-2.59 (1H, m), 1.22 (6H, d, J=7.2Hz)						
22	benzyl	hydrogen	(i)	0	S	phenyl	(3-CF ₃)benzyl
	(400MHz, MeOD) . 7.57 (1H, s), 7.53-7.50 (2H, m), 7.47-7.43 (1H, m), 7.35-7.29 (4H, m), 7.20-7.17 (6H, m), 7.11-7.09 (2H, m), 7.00-6.98 (1H, m), 5.08 (1H, s), 4.47-4.43 (1H, m), 4.18-4.16 (1H, m), 4.09-4.05 (1H, m), 4.01-3.97 (1H, m), 3.89-3.80 (2H, m), 3.64-3.63 (1H, m), 3.49-3.44 (1H, m), 3.02-2.98 (1H, m), 2.73-2.63 (5H, m)						
23	NH ₂ C(O)CH ₂ -	hydrogen	(ii)	0	-	phenyl	(3-dimethylamino)benzyl
	(400MHz, MeOD); δ 7.32-7.18(m, 6H), 6.91(m, 1H), 6.82(dd, 1H), 6.79(d, 1H), 4.19(s, 2H), 4.05(m, 1H), 3.99(d, 0.5H), 3.89(m, 1H), 3.87(d, 0.5H), 3.78(m, 1H), 3.54(m, 1H), 3.42(m, 1H), 3.29(d, 0.5H), 3.14(m, 1H), 3.06(m, 1H), 2.99(s, 6H), 2.96(m, 1H), 2.77(dd, 0.5H), 2.65(m, 1H), 2.51(m, 1H), 2.34(dd, 0.5H) 1.90(dd, 0.5H)						
24	benzyl	methyl	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.30-7.06(m, 11H), 6.71(s, 1H), 6.63(m, 2H), 4.42(d, 1H), 4.11(m, 1H), 4.05(d, 1H), 4.02(m, 1H), 3.96(s, 2H), 3.29(m, 1H), 3.08(m, 1H), 2.96(m, 2H), 2.90(s, 6H), 2.70(m, 2H)						
25	4-CF ₃ O-benzyl	benzyl	(i)	0	S	phenyl	(3-dimethylamino)benzyl

	(500MHz, CDCl ₃); δ 7.19-7.16 (4H, m), 7.13-7.10 (7H, m), 6.80 (1H, brs), 6.71-6.66 (2H, m), 4.37-4.34 (1H, m), 4.19-4.17 (1H, m), 4.07-3.96 (2H, m), 3.38-3.34 (1H, m), 2.98-2.94 (2H, m), 2.91 (6H, s), 2.88-2.74 (2H, m), 2.67-2.51 (3H, m), 2.03-1.98 (1H, m)						
26	benzyl	benzyl	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.26-7.07(m, 14H), 6.96(m, 2H), 6.73(s, 1H), 6.64(m, 1H), 6.68(d, 1H), 4.33(d, 1H), 4.10(m, 1H), 3.86(t, 2H), 3.80(m, 1H), 3.61(m, 1H), 3.27(m, 1H), 3.15(d, 1H), 3.06(m, 2H), 2.89(s, 6H), 2.75(m, 2H), 2.63(m, 2H)						
27	3,5-di-F-benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.26-7.16 (5H, m), 6.92-6.88 (3H, m), 6.79 (1H, brs), 6.72-6.68 (2H, m), 4.35-4.24 (2H, m), 4.14-4.07 (1H, m), 4.05-4.02 (1H, m), 3.82-3.68 (3H, m), 3.51-3.46 (1H, m), 3.14-3.09 (1H, m), 2.95-2.91 (7H, m), 2.79-2.75 (2H, m), 2.68-2.60 (2H, m)						
28	3,5-di-F-benzyl	hydrogen	(i)	0	S	phenyl	(3-t-butyl)benzyl
	(500MHz, CDCl ₃); δ 7.41 (1H, s), 7.34-7.22 (3H, m), 7.15 (6H, brs), 6.92-6.88 (3H, m), 4.35-4.25 (2H, m), 4.16-4.11 (1H, m), 4.08-4.04 (1H, m), 3.86-3.68 (3H, m), 3.52-3.48 (1H, m), 3.12-3.07 (1H, m), 2.82-2.75 (2H, m), 2.70-2.60 (2H, m), 1.34 (9H, m)						
29	3,5-di-F-benzyl	hydrogen	(i)	0	S	phenyl	(3-isopropyl)benzyl
	(500MHz, CDCl ₃); δ 7.29-7.14 (12H, m), 6.92-6.88 (2H, m), 4.35-4.25 (2H, m), 4.16-4.11 (1H, m), 4.08-4.04 (1H, m), 3.85-3.70 (3H, m), 3.52-3.47 (1H, m), 3.12-3.07 (1H, m), 2.94-2.88 (1H, m), 2.81-2.75 (2H, m), 2.69-2.60 (2H, m), 1.28-1.25 (6H, m)						

30	3,5-di-F-benzyl	hydrogen	(i)	0	S	3,5-di-F-phenyl	(3-t-butyl)benzyl
	(500MHz, CDCl ₃); δ 7.45 (1H, s), 7.38-7.36 (1H, m), 7.32-7.28 (1H, m) 7.20-7.18 (1H, m), 6.89-6.86 (3H, m), 6.81-6.74 (3H, m), 4.43-4.39 (1H, m), 4.24-4.21 (1H, m), 4.15-4.09 (2H, m), 3.93-3.83 (2H, m), 3.79-3.74 (1H, m), 3.60-3.56 (1H, m), 3.17-3.12 (1H, m), 2.95-2.85 (2H, m), 2.76-2.68 (2H, m), 1.34 (9H, s)						
31	3,4-di-F-benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.54 (1H, brs), 7.20-6.98 (11H, m), 6.86-6.65 (5H, m), 4.25 (2H, brs), 4.03-3.98 (3H, m), 3.81 (2H, m), 3.34 (1H, brs), 2.95-2.61 (11H, m)						
32	2,4-di-F-benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.62 (1H, brs), 7.26-7.11 (9H, m), 6.79-6.65 (5H, m), 4.50 (1H, brs), 4.37-4.34 (1H, m), 4.10-4.02 (3H, m), 3.93 (1H, brs), 3.79 (2H, brs), 3.40-3.35 (1H, m), 2.95-2.91 (7H, m), 2.76-2.64 (5H, m)						
33	3,5-di-MeO-benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.26-7.12(m, 6H), 6.77-6.69 (m, 3H), 6.38 (m, 1H), 6.35 (m, 2H), 4.07-3.96 (m, 4H), 3.85 (m, 2H), 3.77(s, 6H), 3.69 (m, 2H), 3.43 (m, 1H), 3.20 (m, 1H), 3.12 (m, 1H), 2.96 (s, 6H), 2.67(m, 1H), 2.55 (m, 1H)						
34	4-fluoro-benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl

	(400MHz, CDCl ₃) . 7.47 (1H, brs), 7.12-7.10 (9H, m), 6.98-6.94 (2H, m), 6.75-6.62 (4H, m), 4.35-4.31 (1H, m), 4.11-3.85 (3H, m), 3.33-3.30 (1H, m), 2.95-2.89 (8H, m), 2.75-2.58 (4H, m)						
35	4-CF ₃ -benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.57 (1H, brs), 7.50 (2H, d, J=8.0Hz), 7.22 (2H, d, J=8.0Hz), 7.20-7.17 (1H, m), 7.11-7.08 (6H, m), 6.75 (2H, brs), 6.65-6.64 (1H, m), 4.38-4.35 (1H, m), 4.13-4.05 (2H, m), 3.89 (1H, brs), 3.80-3.77 (2H, m), 3.38-3.33 (1H, m), 2.94-2.93 (1H, m), 2.98 (6H, s), 2.88-2.80 (1H, m), 2.77-2.71 (2H, m), 2.64-2.61 (2H, m)						
36	3-methoxy-benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.30-7.20(m, 4H), 6.88-6.73 (m, 5H), 4.25 (m, 2H), 4.06-4.01 (m, 3H), 3.85 (m, 1H), 3.79 (m, 3H), 3.45(m, 1H), 3.21 (m, 1H), 3.06 (m, 1H), 2.98 (s, 6H), 2.94-2.83 (m, 2H), 2.68 (m, 2H)						
37	4-methoxy-benzyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.30-7.19(m, 4H), 6.88-6.70 (m, 5H), 4.25 (m, 2H), 4.05 (m, 2H), 3.91 (m, 2H), 3.79 (m, 3H), 3.44(m, 1H), 3.15 (m, 1H), 2.97 (s, 6H), 2.91-2.88 (m, 2H), 2.80-2.59 (m, 3H)						
38	2-phenoxy-ethyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.33(d, 1H), 7.26(t, 1H), 7.18(t, 1H), 7.12(m, 3H), 7.04(m, 1H), 6.94(t, 1H), 6.82(d, 2H), 6.74(s, 1H), 6.65(t, 2H), 4.17(m, 1H), 3.96(m, 4H), 3.88(q, 2H), 3.73(t, 1H), 3.58(dt, 1H), 3.28(m, 1H), 2.97-2.88(m, 2H), 2.91(s, 6H), 2.80(m, 2H), 2.70(dd, 1H)						

39	2-phenoxy-ethyl	hydrogen	(i)	0	R	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.27(t, 1H), 7.20-7.09(m, 6H), 7.07(t, 1H), 6.95(t, 1H), 6.84(d, 2H), 6.70-6.61(m, 3H), 4.16(m, 1H), 3.98(m, 3H), 3.80-3.68(m, 4H), 3.61(td, 1H), 3.31(m, 1H), 2.98-2.83(m, 3H), 2.92(s, 6H), 2.70(m, 2H)						
40	n-butyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.23 – 7.14 (m, 6H), 6.73 (s, 1H), 6.64 (m, 2H), 4.08 (t, 1H), 3.76 (m, 1H), 3.66 (m, 1H), 2.91 (s, 6H), 2.90 (m, 2H), 2.83(m, 4H), 2.67(m, 2H), 1.42 – 1.26 (m, 4H), 0.90 (t, 3H)						
41	phenethyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.42 (m, 2H), 7.28 – 7.10 (m, 10H), 6.81 (s, 1H), 6.74 (m, 2H), 4.07 (m, 2H), 3.82 (m, 1H), 3.64 (m, 2H), 2.98-2.77 (m, 6H), 2.90 (s, 6H), 2.57 (m, 2H)						
42	1-oxo-butyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.33-7.15(m, 6H), 6.86 (m, 1H), 6.83-6.76 (m, 2H), 4.10-4.05 (m, 4H), 3.92-3.89 (m, 2H), 3.33-3.13 (m, 4H), 2.97-2.85 (m, 9H), 2.70 (m, 1H), 1.68 (q, J=12Hz, 2H), 0.98 (t, J=12Hz, 3H)						
43	ethylcarbamoyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl

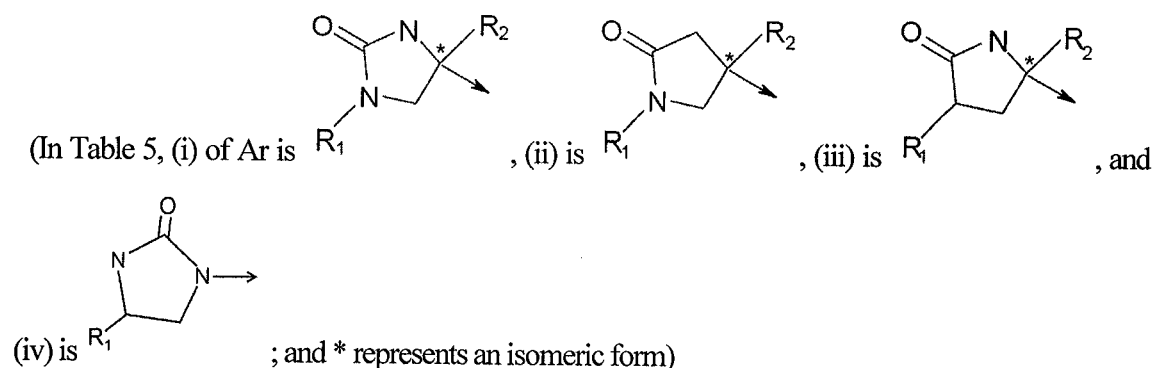
	(400MHz, CDCl ₃) . 7.30-7.14(m, 6H), 6.88 (m, 1H), 6.82-6.75 (m, 2H), 4.13 (m, 1H), 4.10 – 4.03 (m, 1H), 3.98 (m, 1H), 2.96(s, 6H), 2.95 – 2.87 (m, 4H), 2.80 (q, 2H), 1.20 (t, 3H)						
44	phenyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.45(m, 2H), 7.39 (m, 2H), 7.32 (m, 3H), 7.22 (m, 3H), 6.99 (m, 2H), 6.78 (m, 2H), 4.18-4.01(m, 4H), 3.88 (m, 1H), 3.23 (m, 1H), 3.13 (m, 1H), 3.06(m, 1H), 2.98 (s, 6H), 2.97-2.91 (m, 2H), 2.68 (m, 1H)						
45	3,5-di-Br-phenyl	hydrogen	(i)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, CDCl ₃) . 7.75(s, 1H), 7.45 (m, 1H), 7.25-7.11 (m, 6H), 7.02 (m, 1H), 6.77 (s, 1H), 6.71 (m, 2H), 4.30(m, 1H), 4.16-3.90 (m, 5H), 3.20-3.09 (m, 5H), 2.95 (s, 6H), 2.77(m, 1H)						
46	benzyl	hydrogen	(ii)	0	R	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.30-7.22(m, 2H), 7.20-7.11(m, 4H), 6.70-6.56(m, 6H), 4.46(d, 1H), 4.23(d, 1H), 3.96(m, 1H), 3.93(d, 1H), 3.84(m, 1H), 3.83(d, 1H), 3.24(m, 2H), 3.91(m, 2H), 2.88(s, 6H), 2.76(dd, 1H), 2.60(dd, 1H), 2.48(dd, 1H), 2.30(dd, 1H)						
47	benzyl	hydrogen	(ii)	0	S	phenyl	(3-CF ₃ O)benzyl
	(400MHz, MeOD) . 7.34-7.14 (m, 14H), 4.41 (s, 2H), 4.01 (m, 1H), 3.78 (s, 2H), 3.61 (m, 1H), 3.26 (m, 1H), 3.11 (m, 1H), 2.94 (m, 1H), 2.70-2.51 (m, 4H), 2.38 (m, 1H), 2.05 (m, 1H)						

48	benzyl	hydrogen	(ii)	0	R	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.32-7.22(m, 5H), 7.21-7.13(m, 4H), 7.11(d, 2H), 6.63(s, 1H), 6.63-9.59(m, 2H), 6.12(d, 1H), 4.46(d, 1H), 4.27(d, 1H), 4.10(m, 1H), 3.81(d, 1H), 3.70(d, 1H), 3.67(m, 1H), 3.29(m, 1H), 3.24(t, 1H), 2.96(m, 1H), 2.91(s, 6H), 2.85(m, 2H), 2.74(m, 2H) 2.42(dd, 1H), 2.30(dd, 1H)						
49	benzyl	hydrogen	(ii)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, CDCl ₃); δ 7.34-7.26(m, 3H), 7.20-7.13(m, 5H), 7.13-7.08(m, 3H), 6.68-6.61(m, 3H), 6.15(d, 1H), 4.42(d, 1H), 4.22(d, 1H), 4.17(m, 1H), 3.83(d, 1H), 3.74(d, 1H), 3.67(m, 1H), 3.10(t, 1H), 2.91(m, 1H), 2.90(s, 6H), 2.85(m, 2H), 2.79-2.69(m, 3H), 2.62(dd, 1H) 2.52(dd, 1H)						
50	cyclohexyl	hydrogen	(ii)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, MeOD); δ 7.27-7.16(m, 6H), 6.82(s, 1H), 6.73(m, 2H), 3.99(m, 3H), 3.75(td, 1H), 3.23(dd, 1H), 3.01-2.92(m, 4H), 2.93(s, 6H), 2.86(m, 2H), 2.63(dd, 1H), 2.56(dd, 1H), 2.45(dd, 1H), 2.37(dd, 1H), 1.68(m, 3H), 1.53(m, 2H), 1.41(m, 1H), 1.20(m, 3H), 0.84(m, 2H)						
51	cyclohexyl	hydrogen	(ii)	0	R	phenyl	(3-dimethylamino)benzyl
	(500MHz, MeOD); δ 7.24-7.12(m, 6H), 6.76(s, 1H), 6.66(m, 2H), 4.05(m, 1H), 3.73(q, 2H), 3.95(dt, 1H), 3.36(q, 1H), 3.32(t, 1H), 3.13(dd, 1H), 3.03-2.90(m, 3H), 2.91(s, 6H), 2.72(dd, 1H), 2.62(dd, 1H), 2.56(dd, 1H), 2.31(dd, 1H), 2.01(dd, 1H), 1.70(m, 2H), 1.58(m, 4H), 1.19(m, 3H), 0.89(m, 2H)						

52	n-pentyl	hydrogen	(ii)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, MeOD); δ 7.22(m, 2H), 7.15(m, 4H), 6.75(s, 1H), 6.66(m, 2H), 4.04(m, 1H), 3.72(q, 2H), 3.64(dt, 1H), 3.38(m, 1H), 3.32(t, 1H), 3.18(t, 2H), 3.13(dd, 1H), 2.94(m, 1H), 2.91(s, 6H), 2.71(dd, 1H), 2.59(m, 2H), 2.30(dd, 1H), 1.99(dd, 1H), 1.47(m, 2H), 1.32(m, 2H), 1.23(m, 2H), 0.89(t, 3H)						
53	n-pentyl	hydrogen	(ii)	0	R	phenyl	(3-dimethylamino)benzyl
	(500MHz, MeOD); δ 7.25-7.12(m, 6H), 6.75(s, 1H), 6.66(m, 2H), 4.06(m, 1H), 3.72(q, 2H), 3.63(dt, 1H), 3.27(m, 1H), 3.15(m, 2H), 2.94(m, 1H), 2.91(s, 6H), 2.69(m, 2H), 2.62(dd, 1H), 2.54(dd, 1H), 2.46(dd, 1H), 2.36(dd, 1H), 1.38(m, 2H), 1.32(m, 2H), 1.19(m, 2H), 0.90(t, 3H)						
54	phenethyl	hydrogen	(ii)	0	S	phenyl	(3-dimethylamino)benzyl
	(500MHz, MeOD); δ 7.27-7.12(m, 11H), 6.77(s, 1H), 6.68(m, 2H), 4.01(m, 1H), 3.84(q, 2H), 3.69(m, 1H), 3.48(m, 1H), 3.38-3.30(m, 2H), 3.15(dd, 1H), 2.90(s, 6H), 2.87(m, 2H), 2.85-2.68(m, 4H), 2.56(dd, 1H), 2.25(dd, 1H), 1.95(dd, 1H)						
55	phenethyl	hydrogen	(ii)	0	R	phenyl	(3-dimethylamino)benzyl
	(400MHz, MeOD); δ 7.28-7.12(m, 11H), 6.75(s, 1H), 6.66(m, 2H), 4.05(m, 1H), 3.75(q, 2H), 3.65(td, 1H), 3.38(m, 1H), 3.29(m, 1H), 3.16(m, 2H), 2.90(s, 6H), 2.86(m, 1H), 2.76-2.60(m, 5H), 2.55(dd, 1H), 2.43(dd, 1H), 2.30(dd, 1H)						

56	[(S)-2-(Cl-phenyl)-1-ethylcarbamoyl]ethyl	hydrogen	(ii)	0	R	phenyl	(3-dimethylamino)benzyl
	(400MHz, MeOD); δ 7.26-7.12(m, 8H), 6.98(d, 2H), 6.78(m, 1H), 6.73(dd, 1H), 6.68(d, 1H), 4.51(dd, 1H), 4.01(d, 2H), 3.93(m, 1H), 3.71(td, 1H), 3.41(t, 1H), 3.22(dd, 1H), 3.14-3.07(m, 2H), 3.07-2.93(m, 3H), 2.89(m, 1H), 2.88(s, 6H), 2.64(m, 2H), 2.50(dd, 1H), 2.30(m, 2H), 0.99(t, 3H)						
57	[(S)-2-(Cl-phenyl)-1-ethylcarbamoyl]ethyl	hydrogen	(ii)	0	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, MeOD); δ 7.24-7.10(m, 10H), 6.83(s, 1H), 6.76(d, 1H), 6.72(d, 1H), 4.09(d, 2H), 3.97(m, 1H), 3.75(td, 1H), 3.53(dd, 1H), 3.42(dd, 1H), 3.30(dd, 1H), 3.21(dd, 1H), 3.17-3.06(m, 4H), 2.92(s, 6H), 2.90-2.80(m, 3H), 2.52(dd, 1H), 2.16(dd, 1H), 1.63(dd, 1H), 1.00(t, 3H)						
58	methyl	hydrogen	(ii)	1	-	phenyl	(3-dimethylamino)benzyl
	(400MHz, MeOD); δ 7.26-7.14 (m, 6H), 6.77 (s, 1H), 6.66 (m, 2H), 4.05 (m, 1H), 3.79 (m, 2H), 3.65 (m, 1H), 3.19-3.07 (m, 2H), 2.91 (s, 6H), 2.86 (m, 1H), 2.76 (m, 1H), 2.70 (d, 3H, J=10Hz), 2.68-2.62 (m, 2H), 2.53 (m, 1H), 2.41 (m, 1H), 2.24 (m, 1H), 2.16-2.02 (m, 2H), 1.85 (m, 1H)						
59	benzyl	hydrogen	(ii)	1	S	phenyl	(3-dimethylamino)benzyl
	(400MHz, MeOD); δ 7.29-7.04 (m, 11H), 6.69-6.63 (m, 3H), 4.35 (m, 1H), 4.18 (m, 2H), 4.02-3.87 (m, 3H), 3.76 (m, 1H), 3.51 (m, 1H), 2.13-2.87 (m, 7H), 2.60-2.35 (m, 4H), 2.29 (m, 1H), 2.15 (m, 1H), 2.08 (m, 1H), 1.99 (m, 1H)						
60	benzyl	hydrogen	(ii)	1	R	phenyl	(3-dimethylamino)benzyl

(400MHz, MeOD); δ 7.25-7.01 (m, 1H), 6.69-6.56 (m, 3H), 4.36 (d, 1H, $J=15\text{Hz}$), 4.16 (d, 1H, $J=15\text{Hz}$), 3.97-3.79 (m, 3H), 3.73 (m, 1H), 3.13-2.98 (m, 5H), 2.86-2.83 (m, 6H), 2.71 (m, 1H), 2.50 (m, 1H), 2.30 (m, 2H), 2.08 (m, 1H), 1.82 (m, 1H)



5

Experimental Example 1: Enzymatic activity of recombinant beta-secretase 2

Step A: Construction of vector expressing recombinant beta-secretase 2

BACE cDNA (ATCC, Cat. No. 6896840) was purchased which was synthesized based on a human BACE2 gene sequence (Accession No. BC014453) disclosed in the public Genbank data base. Only the ectodomain which corresponds to the region of from the amino acid residues 1 to 466 with exclusion of a transmembrane domain and a cytoplasmic domain in the entire BACE gene was re-cloned, and then the base sequence of Fc region which corresponds to 230 amino acids (from the amino acid residues 1 to 466) of human Immunoglobulin G (hIgG) was linked to the 3' end

thereof. The BACE (ectodomain)-IgG Fc (hereinafter, referred to as "BACE-Fc") was ligated between *Bam*HI and *Xho*I sites of pCDNA3 (Invitrogen) as a mammalian expression vector to construct a BACE2-Fc expression vector, designated as pCDNA3 BACE2-Fc.

5 Step B: Construction of mammalian cell line expressing BACE2-Fc fusion protein

Chinese hamster ovary (CHO) DHFR- cells (ATCC Accession No. CRL9096) were cultured in an alpha-minimum essential medium (α -MEM, GIBCO-BRL) supplemented with 10% fetal bovine serum (FBS, GIBCO-BRL) and then transferred to a 100 mm culture plate. When the cells reached confluence, they were transfected with the BACE2-Fc-expressing vector pCDNA3 BACE2-Fc using Lipofectamine Plus (Life Technologies). Selection of transfectant cells was carried out in a medium containing 10% dialyzed fetal bovine serum (dFBS, JRH) supplemented with 1 mg/mL of Geneticin (G418 sulfate, GIBCO-BRL). The culture medium was replaced with a fresh one every 4 days. Then, 100 clones were isolated and cultured on a 24-well culture plate. Among these isolated clones, 20 clones showing acceptable growth rates were sub-cultured on a 24-well culture plates for 3 days at an equal cell density (2×10^5 cells/mL/24-well). An amount of BACE2-Fc protein secreted into the medium was quantified by ELISA method using goat anti-human IgG (Pierce). As a result, clone #66 showing the highest growth rate and BACE2-Fc expression (3 mg/1 L of culture) was selected.

20 Step C: Production and purification of BACE2-Fc fusion protein

2 x 10⁵ cells/mL of the CHO DHFR- BACE2-Fc #66 cell line were inoculated into a roller bottle containing 250 mL of α -MEM supplemented with 10% dFBS, and cultured in a Roll-In cell incubator (Belco) at 37°C and 40 rpm for 4 days. When cells reached confluence, they were washed once with 250 mL of a serum-free medium (SFII, GIBCO-BRL), and 500 mL of a serum-free medium containing insulin (0.5 μ g/mL, SIGMA) was added, followed by culture for 3 days. After the culture medium was harvested, 500 mL of a serum-free medium was again added thereto and then the cells were cultured for another 3 days. This procedure was repeated two times. All conditioned media harvested were centrifuged at 7000 rpm for 20 min (Beckman, JA 10 rotor) to isolate only the wash solution. The wash solution was filtered through a 0.45 μ m filter and was then passed through a protein A sepharose chromatography column (Pharmacia) equilibrated with 20 mM sodium phosphate buffer (pH 7.0), followed by washing with 20 mM sodium phosphate buffer (pH 7.0) to completely remove the non-adsorbed proteins. 100 mM sodium acetate buffer (pH 3.5) was added to elute the adsorbed proteins, so the BACE2-Fc protein (MW 75 kDa) of over 95% purity was obtained.

Step D: Beta-secretase 2 activity assay using fluorescence-labeled specific substrate

To determine enzymatic activity of beta-secretase 2 and inhibition efficiency of synthetic compounds, a Fluorescence Resonance Energy Transfer (FRET) enzyme activity assay was carried out using the purified BACE2-Fc fusion protein and a fluorescence-labeled beta secretase 2-specific substrate. This will be briefly illustrated in the following.

From the entire amino acid sequence of an amyloid precursor protein (APP) known as an intracellular beta secretase 2-specific substrate, a peptide corresponding to a 10-amino acid region containing a beta-secretase cleavage site was synthesized with inclusion of EDANS as a fluorophore and DABCYL as a quenching group linked thereto. When this fluorescence-labeled substrate is
5 reacted with BACE2-Fc, the BACE2 functional region is cleaved and the quenching group is detached, and EDANS emits fluorescence at 510 nm upon excitation with 350 nm light, whereby the level of fluorescence is measured to accurately and conveniently determine the extent of the cleavage reaction.

Each of synthetic compounds at a concentration of 10 mM was dissolved in DMSO and
10 then stored at 20°C. For determination of activity, a 10 mM DMSO solution was first added to the right row of a 96-well plate, and then nine successive doubling dilutions were carried out with an equal volume of DMSO. 10 μl of the diluted compound solution was added to a 96-well assay plate containing 600 μM of a fluorescence-labeled BACE substrate dissolved in 15 μl of reaction buffer (50 mM sodium acetate, pH 4.5, 0.05% CHAPS) and 10 μl of 50% DMSO, so that DMSO
15 was adjusted to a final concentration of 10%, and the inhibitor was treated through nine successive doubling dilutions from 500 μM . 65 μl of the purified BACE2-Fc fusion protein solution was added to a final concentration of 0.4 $\mu\text{g/mL}$ and reacted at room temperature for 1 hour. An amount of the reaction product was measured by the magnitude of fluorescence at a 350 nm excitation wavelength and a 510 nm emission wavelength, using a fluorescent plate reader (SpectraMax
20 Gemini XS, Molecular Device). The concentration of a synthetic compound inhibiting 50% of beta-secretase activity, *i.e.*, IC_{50} and K_i were determined by comparing the measurement value with that of a control group with no addition of the synthetic compound.

Experimental Example 2: Enzymatic activity of recombinant cathepsin D

To determine enzymatic activity of cathepsin D and inhibition efficiency of synthetic compounds, a Fluorescence Resonance Energy Transfer (FRET) enzyme activity assay was carried out using cathepsin D (Calbiochem, #219401) and fluorescence-labeled cathepsin D-specific
5 substrate (Bachem #M-2455). This will be briefly illustrated in the following.

As a cathepsin D-specific substrate, a 10-aa peptide was synthesized with inclusion of Mca as a fluorophore and Dnp as a quenching group linked thereto. When this fluorescence-labeled substrate is reacted with cathepsin D, the cathepsin D functional region is cleaved and the quenching group is detached, and Mca emits fluorescence at 393 nm upon excitation with 328 nm light,
10 whereby the level of fluorescence is measured to accurately and conveniently determine the extent of the cleavage reaction.

Each of synthetic compounds at a concentration of 10 mM was dissolved in DMSO and then stored at 20°C. For determination of activity, a 10 mM DMSO solution was first added to the right row of a 96-well plate, and then nine successive doubling dilutions were carried out with an
15 equal volume of DMSO. 10 μl of the diluted compound solution was added to a 96-well assay plate containing 7 μM of a fluorescence-labeled cathepsin D substrate dissolved in 50 μl of reaction buffer (50 mM sodium acetate, pH 4.0) and 10 μl of 25% DMSO, so that DMSO was adjusted to a final concentration of 5%, and the inhibitor was treated through nine successive doubling dilutions from 250 μM . 30 μl of the purified cathepsin D fusion protein solution was
20 added to a final concentration of 0.75 ng/mL, followed by reaction at 37°C for 1 hour. An amount of the reaction product was measured by the magnitude of fluorescence at a 328 nm excitation wavelength and a 393 nm emission wavelength, using a fluorescent plate reader (SpectraMax

Gemini XS, Molecular Device). The concentration of a synthetic compound inhibiting 50% of cathepsin D activity, *i.e.*, IC_{50} and K_i were determined by comparing the measurement value with that of a control group with no addition of the synthetic compound.

5 Experimental Example 3: Secreted alkaline phosphatase (SEAP) activity assay

Step A: Establishment of permanent cell line expressing SEAP-APP_{sw}-KK

A gene which expresses SEAP and Swedish mutant form of APP (CRE-SEAP-APP695sweKK) under the control of cAMP response element (CRE) was cloned into pcDNA3.1(+)-Neo (Invitrogen) which is a mammalian expression vector. Neuro-2a cells (ATCC
10 Accession No. CCL-131) were cultured in a Dulbecco's minimum essential medium (DMEM, GIBCO-BRL) supplemented with 10% FBS, and then transferred to a 6-well culture plate. When the cells reached confluence, they were transfected with the CRE-SEAP-APP695sweKK expression vector using Lipofectamine 2000 (Life Technologies). Individual clones were isolated and then cultured again on a 6-well culture plate. Following selection of clones, 100 clones showing
15 acceptable growth rates were cultured on a 24-well culture plate for 3 days, followed by culture in DMSO/10 μ M Forskolin medium for 6 hours. 50 μ l/well of the culture was aliquoted and reacted with 50 μ l of AttoPhos (Promega). The magnitude of fluorescence was measured at room temperature for 30 min, at a 450 nm excitation wavelength and a 580 nm emission wavelength, using a fluorescent plate reader (SpectraMax Gemini XS, Molecular Device). Among the selected
20 clones, 4 clones showing high SEAP activity in forskolin/DMSO and exhibiting the greatest SEAP activity by forskolin were selected. The selected clones were sub-cultured on a 96-well culture plate

for one day at an equal cell density (2×10^4 cells/96-well), followed by treatment with DMSO/10 μ M Forskolin. After 6 hours, activity of SEAP secreted into the medium was assayed. Clone #159 showing the highest activity of SEAP was selected.

5 Step B: SEAP activity assay

The clone N2A SEAP-APP^{sw}-KK #159 expressing CRE-SEAP-APP695^{swe}KK was seeded at a density of 2×10^4 cells/80 μ l to each well of a 96-well culture plate. 10mM Forskolin and DMSO were diluted 100 times with the culture medium and then 10 μ l/well of the dilution was added to the 96-well plate.

10 Each of synthetic compounds at a concentration of 10 mM was dissolved in DMSO and then stored at 20°C. For determination of activity, a 10 mM DMSO solution was first added to the right row of a 96-well plate, and then seven successive 3-fold dilutions were carried out with an equal volume of DMSO. 10 μ l of the serial compound dilution was diluted 10 times with 90 μ l of the culture. DMSO was adjusted to a final concentration of 1.1% and the inhibitor was treated
15 through seven successive doubling dilutions from 100 μ M. After treatments were complete, the cells were cultured in a 6% CO₂ incubator at 37°C for 5 hours. In order to measure an amount of SEAP secreted into the medium, the culture was first subjected to heat inactivation at 65°C for 30 min to thereby abolish activity of other alkaline phosphatases. 50 μ l of the heat-inactivated culture and 50
20 μ l of AttoPhos (Promega) were reacted at room temperature for 30 min. The magnitude of fluorescence was measured at a 450 nm excitation wavelength and a 580 nm emission wavelength, using a fluorescent plate reader (SpectraMax Gemini XS, Molecular Device). The concentration of a

synthetic compound inhibiting 50% of SEAP activity, *i.e.*, IC_{50} was determined by comparing the measurement value with that of a control group with no addition of the synthetic compound.

Experimental Example 4: Assay of amyloid beta in neurons of transgenic mice

5 Step A: Culture of primary neurons of transgenic mice (APP/PS1dE9)

Offspring mice from interbreeding of transgenic (APP/PS1dE9) male and female mice were included in experiments. The brains of 3 or 4-day-old mice were dissected, and the hippocampus tissue and cerebral membrane were removed at 4°C and minced, followed by treatment with DNase (Sigma, D5025) and protease (Sigma, P5147) and placement in an incubator
10 at 37°C for 20 to 25 min. After being separated into cells, 4×10^5 cells/well were seeded onto a 24-well plate coated with poly-L-lysine and containing a medium (27.6 mL Neurobasal + 1.5 mL FBS + 600 μ l B27 + 300 μ l of 200 mM L-glutamine). The cells were cultured in an incubator at 37°C for 7 days, and then used for subsequent experiments.

15 Step B: Ab40 activity assay

Each of synthetic compounds at a concentration of 10 mM was dissolved in DMSO and then stored at 20°C. For determination of activity, a 10 mM DMSO solution was first added to the right row of a 96-well plate, and then six successive 3-fold dilutions were carried out with an equal volume of DMSO. The serial compound dilution was diluted 250 times with a medium (29.1 mL

Neurobasal + 600 μl of B27 + 300 μl of 200 mM L-glutamine + 7.5 μl of 100 mM L-glutamate). 350 μl of the dilution was added to the cultured primary neurons and the cells were then cultured in an incubator at 37°C for 8 hours. An expression level of the beta-amyloid peptide secreted into the medium was measured by sandwich ELISA (Biosource, #KHB3482) using two
5 types of antibodies specific for the beta-amyloid peptide, which will be briefly illustrated below.

50 μl of the water-soluble beta-amyloid or cell culture diluted to different concentrations and 50 μl of reporter antibodies were mixed and added to an antibody-coated plate, followed by reaction at room temperature for 3 hours (or one or more days at 4°C). The culture plate was washed five times with a 1-fold volume of a wash solution (Biosource, #KHB3482), and 100 μl of
10 horseradish peroxidase (HRP)-conjugated antibodies diluted 100 times with an antibody dilution solution (Biosource, #KHB3482) was added thereto, followed by reaction at room temperature for 30 min. Then, HRP-conjugated antibodies were removed and the plate was washed five times with a 1-fold volume of a wash solution, to which 100 μl of a chromogene solution (Biosource, #KHB3482) was added, followed by reaction at room temperature for 30 min. Then, 100 μl of a
15 stop solution (Biosource, #KHB3482) was added thereto, followed by reaction at room temperature for 30 min. The optical density (absorbance) was measured at 450 nm using a microplate reader (SpectraMax 340, Molecular Device). The concentration of a synthetic compound inhibiting 50% of intracellular beta-secretase activity, *i.e.*, IC₅₀ was determined by comparing the measurement value with that of a control group treated with 0.4% DMSO and with no addition of the synthetic
20 compound.

Experimental Example 5: *In vivo* assay of beta-secretase activity

The inhibition degree of intracellular beta-secretase activity by synthetic compounds can be determined by using a cell line producing a beta-amyloid peptide from an amyloid precursor protein (APP).

5 Step A: Establishment of permanent cell line expressing amyloid precursor protein (APP)

A mutant form of APP gene (APP751NFEV), expression of which is under the control of Tet-response element (TRE), was cloned into a PBI-L vector (ClonTech) as a mammalian expression vector that expresses a luciferase gene under the control of Tet-response element (TRE). Neuro-2a cells (ATCC Accession No. CCL-131) were cultured in a Dulbecco's minimum essential
10 medium (DMEM, GIBCO-BRL) supplemented with 10% FBS, and then transferred to a 6-well culture plate. When the cells reached confluence, they were transfected with the pBI-L APP751 NFEV expression vector using Lipofectamine 2000 (Life Technologies). Individual clones were isolated and then cultured again on a 6-well culture plate. Following selection of clones, 100 clones showing acceptable growth rates were cultured on a 96-well culture plate for 1 day. The culture
15 medium was replaced with a medium containing 1 $\mu\text{g/mL}$ of doxycycline, followed by culture for 24 hours. 50 μl of Bright-Glo luciferase reagent (Promega) was added to each well which was then left at room temperature for 15 min. Then, luminescence of each well was measured using a luminometer (Victor). Following selection of clones, 4 clones showing the highest expression level of luciferase were sub-cultured on a 24-well culture plate for one day at an equal cell density (3 X
20 10^5 cells/mL/24-well). The culture medium was replaced with Opti-MEM (GIBCO-BRL) containing 1 $\mu\text{g/mL}$ of doxycycline, followed by culture for 24 hours. An amount of the beta-amyloid peptide secreted into the medium was measured using an ELISA method using antibodies

specific for the beta-amyloid peptide. As a result, clone #79 showing the highest growth rate and beta-amyloid peptide expression level was selected.

Step B: ELISA assay of water-soluble beta-amyloid precursor protein (sAPP)

5 3×10^5 cells/well of Neuro-2a APP751 NFEV # 79 with permanent expression of a mutant form of APP were seeded onto a 24-well culture plate. The cells were cultured in a 6% CO₂ incubator at 37°C for 24 hours. When the cells reached confluence, the culture medium was replaced with Opti-MEM (GIBCO-BRL) containing 300 μl of 1 $\mu\text{g/mL}$ of doxycycline and the beta-secretase inhibitor (compounds of Examples) diluted to concentrations of individual steps,
10 followed by culture for 24 hours. An expression level of water-soluble beta-amyloid precursor secreted into the medium was measured using a human sAPP assay kit (IBL) according to the manufacturer's instructions. This will be briefly illustrated in the following.

100 μl of the water-soluble beta-amyloid precursor or cell culture diluted to different concentrations was added to an antibody-coated plate, followed by reaction at room temperature for
15 4 hours (or one or more days at 4°C). The culture plate was washed seven times with a wash solution (PBS + 0.05% Tween 20), and 100 μl of HRP-conjugated antibodies diluted 30 times with an antibody dilution solution (PBS + 1% BSA + 0.05% Tween 20) was added thereto, followed by reaction at room temperature for 30 min. Then, the plate was washed nine times with a wash solution, to which 100 μl of a TMB solution was added, followed by reaction at room
20 temperature for 30 min. Then, 100 μl of a 1N sulfuric acid (H₂SO₄) solution was added thereto, followed by reaction at room temperature for 30 min. The optical density (absorbance) was

measured at 450 nm using a microplate reader (SpectraMax 340, Molecular Device). The concentration of a synthetic compound inhibiting 50% of intracellular beta-secretase activity, *i.e.*, IC₅₀ was determined by comparing the measurement value with that of a control group treated with 1% DMSO and with no addition of the synthetic compound.

5

Experimental Example 6: Beta-amyloid assay

Quantitative analysis of beta-amyloid was carried out by ELISA using two types of antibodies (Human beta amyloid 1-40 colorimetric immunoassay kit, Biosource, California, USA). Two antibodies used in ELISA were an antibody that specifically recognizes the N-terminus of beta-secretase-cleaved beta amyloid and an antibody that binds to the C-terminus thereof. Two different
10 antibodies and the beta amyloid protein were reacted at room temperature for 3 hours (or one or more days at 4 °C). The plate was washed four times with a wash solution, and reacted with HRP-conjugated anti-rabbit IgG antibodies for 30 min. The plate was washed four times with a wash solution, and tetramethylbenzidine as the HRP substrate was added, followed by reaction at room
15 temperature for 30 min. The optical density (absorbance) was measured at 450 nm using a microplate reader (SpectraMax 340, Molecular Device). Reduction of beta amyloid was determined by comparing the measurement value with that of a control group.

Assay of sAPPbeta (secreted amyloid precursor protein beta) was also carried out analogously to the procedure described in ELISA of beta-amyloid.

20

Experimental Example 7: Animal tests (*in vivo* assay)

In order to investigate whether the activity of beta-secretase was inhibited, the inhibition degree of production of beta amyloid which is a beta-secretase cleavage product was examined in animals. The animals used in the experiments were transgenic mice harboring both a Swedish mutant form of beta-APP (chimeric mouse/human amyloid precursor protein 695swe) and a mutant form of presenilin 1 (presenilin 1-dE9) (Jankosky JL et al., Biomolecular engineering, 17(6), 157-165, 2001). The beta-secretase inhibitor was administered at a dose that is expected to result in a decrease of the amyloid protein via an intraperitoneal or subcutaneous route but does not cause toxicity. Following administration of the drug, the animals were anesthetized at a given time point, and blood and cerebral tissues were isolated. The blood was collected in a heparinized tube by cardiac puncture and centrifuged at 13,000 rpm for 10 min (Eppendorf) to separate the plasma. The plasma was stored together with the excised brain tissues (cerebral cortex and hippocampus) at 80 °C until use. For analysis, the plasma was diluted 5 times, and inhibitory effects on amyloid formation were examined by ELISA as described above. A 4-fold volume of PBS was added to the excised brain tissues which were then homogenized with a sonicator. The homogenate was reacted with guanidine at room temperature for 4 hours. For extraction of the amyloid protein with guanidine-buffer, guanidine was adjusted to a final concentration of 5M using 8.2 M guanidine/82 mM Tris HCl (pH 8.0). The beta amyloid protein was extracted and diluted 1:500 in BSAT-DPBS (Dulbecco's phosphate buffered saline with 5% BSA and 0.03% Tween-20), followed by analysis.

20

Experimental Example 8: Dosing and formulation

A drug compound was dissolved in 10% hydroxypropyl-beta-cyclodextrin (HPCD) and was then administered to a subject, typically at a dose of 15 to 100 mg/kg/day, once or three to five times.

The compound of the present invention had a K_i value of about 0.001 to 500 μM , preferably about 0.001 to 1 μM and more preferably about 0.001 to 0.1 μM . K_i values of representative Example compounds are given in Table 6 below.

[Table 6]

Example No.	Enzymatic activity (K_i)
1	0.284 μM
3	0.051 μM
13	0.024 μM
14	0.010 μM
27	0.053 μM
28	0.009 μM
29	0.018 μM
30	0.002 μM

In addition, IC_{50} of intracellular SEAP activity was in a range of about 0.1 to 100 μM , preferably 0.1 to 10 μM , and more preferably 0.1 to 1.0 μM . IC_{50} values of representative Example compounds relating to intracellular SEAP activity are given in Table 7 below.

5

[Table 7]

Example No.	SEAP activity (IC_{50})
13	0.986 μM
3	1.285 μM
27	1.973 μM
28	0.429 μM
29	0.578 μM
30	0.152 μM

Generally, the compounds of Examples in accordance with the present invention exhibited selectivity for Cat. D. Representatively, the compounds of Examples 12, 27 and 29 exhibited

approx. 10-fold, 15.2-fold and 29.5-fold higher selectivity, respectively, as compared to a control group.

Further, these compounds also exhibited excellent cell activity on primary neurons. The compound of Example 13 exhibited an IC_{50} value of about 0.050 μ M.

5 Blood-brain barrier (BBB) permeability of some compounds was excellent. The compound of Example 3 exhibited effective reduction of Ab40 in the brain and plasma of double mutant transgenic mice, in conjunction with effective decreases of sAPPb levels in the brain.

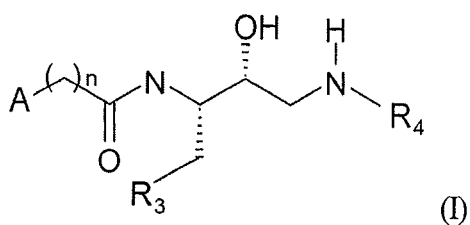
INDUSTRIAL APPLICABILITY

10 As apparent from the foregoing, compounds of Formula I in accordance with the present invention exhibit excellent inhibitory effects on human beta-secretase. Therefore, these compounds can be used as drugs for improvement of cognitive functions or for prevention and treatment of neurodegenerative diseases such as Alzheimer's disease.

15 Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

WHAT IS CLAIMED IS

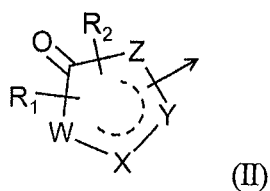
1. A compound represented by Formula I:



wherein:

n is 0 to 2;

A is represented by Formula II:



- 10 wherein W, X, Y and Z are each independently C or N atom, one or two of which being N;

R₁ is -(CR₆R₇)_m-R₈ wherein m is 0 to 2, R₆ is selected from the group consisting of hydrogen, alkyl and alkoxy, R₇ is selected from the group consisting of hydrogen, alkyl and -

C(O)NHR₆, and R₈ is selected from the group consisting of alkyl, alkoxy, arylalkoxy, cycloalkyl, heterocycle, heteroaryl, -C(O)R₆ and -C(O)NR₆R₇ wherein R₆ and R₇ are as defined above;

R₂ is selected from the group consisting of hydrogen, alkyl and -(CR₆R₇)_m-R₉ wherein m is 0 to 2, R₆ and R₇ are as defined above, and R₉ is aryl;

5 R₃ is selected from the group consisting of hydrogen, alkyl, alkoxy and -(CR₆R₇)_p-R₁₀ wherein p is 0 to 2, R₆ and R₇ are as defined above, and R₁₀ is selected from the group consisting of cycloalkyl, aryl, heterocycle and heteroaryl;

R₄ is selected from the group consisting of hydrogen, alkyl, cycloalkyl and -(CH₂)_p-A'R₁₁ wherein p is as defined above, A' is aryl or heteroaryl and R₁₁ is selected from the group consisting
10 of hydrogen, halogen, hydroxy, alkyl, alkoxy and -NR₆R₇ wherein R₆ and R₇ are as defined above;
and

R₆ and R₇ may be taken together to form a cyclic or acyclic structure,

wherein the alkyl, alkoxy, aryl, cycloalkyl, heterocycle, and heteroaryl may be unsubstituted or substituted with at least one substituent selected from the group consisting of
15 halogen, amino, alkylamino, dialkylamino, alkylacylamino, C₁-C₄ alkyl, hydroxy, C₁-C₄ alkoxy, aryl alkoxy and oxo, and

the heteroaryl and heterocycle are each independently a 4 to 8-membered ring containing 1 to 3 hetero atoms selected from the group consisting of O, N and S, and having 0 to 2 double bonds; or a pharmaceutically acceptable salt or isomer thereof.

2. The compound according to claim 1, wherein

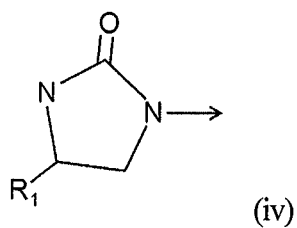
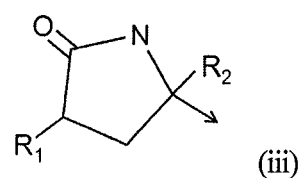
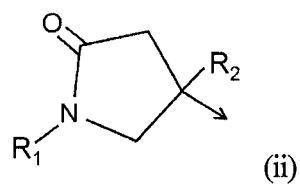
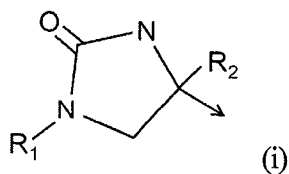
R_1 is $-(CR_6R_7)_m-R_8$ wherein m is 0 to 2, R_6 is hydrogen or C_1-C_8 alkyl, R_7 is selected from the group consisting of hydrogen, C_1-C_8 alkyl and $-C(O)NHR_6$ wherein R_6 is as defined above, and
 5 R_8 is selected from the group consisting of C_1-C_8 alkyl, C_1-C_8 alkoxy, arylalkoxy, cycloalkyl, heteroaryl, $-C(O)R_6$ and $-C(O)NR_6R_7$ wherein R_6 and R_7 are as defined above;

R_2 is selected from the group consisting of hydrogen, C_1-C_8 alkyl and $-(CR_6R_7)_m-R_9$ wherein m is 0 to 2, R_6 and R_7 are as defined above and R_9 is phenyl;

R_3 is selected from the group consisting of hydrogen, C_1-C_8 alkyl, C_1-C_8 alkoxy and $-(CR_6R_7)_p-R_{10}$ wherein p is 0 to 2, R_6 and R_7 are as defined above and R_{10} is selected from the group
 10 consisting of cycloalkyl, aryl, heterocycle and heteroaryl; and

R_4 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, 5 or 6-membered cycloalkyl and $-(CH_2)_p-A'R_{11}$ wherein p is as defined above, A' is aryl or heteroaryl and R_{11} is
 15 selected from the group consisting of hydrogen, halogen, hydroxy, C_1-C_8 alkyl, C_1-C_8 alkoxy and $-NR_6R_7$ wherein R_6 and R_7 may be taken together to form a cyclic structure.

3. The compound according to claim 1, wherein A is any one of heterocycles of Formulae (i) to (iv):



5

4. The compound according to claim 1, wherein R_1 is $-(CR_6R_7)_m-R_8$ wherein m is 0 to 2, R_6 is hydrogen or C_1 - C_8 alkyl, R_7 is selected from the group consisting of hydrogen, C_1 - C_8 alkyl and $-C(O)NHR_6$ wherein R_6 is as defined above, and R_8 is selected from the group consisting of C_1 - C_8 alkyl, C_1 - C_8 alkoxy, arylalkoxy, cycloalkyl, heteroaryl, $-C(O)R_6$ and $-C(O)NR_6R_7$.

10

5. The compound according to claim 1, wherein R_1 is $-(CHR_7)_m-R_8$ wherein m is 0 or 1, R_7 is hydrogen, C_1 - C_4 alkyl or $-C(O)NHR_6$ wherein R_6 is as defined above, and R_8 is selected from the

group consisting of C₁-C₈ alkyl, arylalkoxy, cycloalkyl, -C(O)R₆ and -C(O)NR₆R₇ wherein R₆ and R₇ are as defined above.

6. The compound according to claim 5, wherein R₁ is selected from the group consisting of
- 5 hydrogen, benzyl, dimethylamino-carbonyl, propyl-carbonyl, phenethyl, butyl, pentyl, ethylcarbamoyl, trifluorobenzyl, 4-trifluoromethylbenzyl, 2-trifluoromethylbenzyl, phenoxyethyl, 2,4,5-trifluorobenzyl, 4-fluorobenzyl, 3,4-difluorobenzyl, 2,4-difluorobenzyl, 3,5-difluorobenzyl, 3,5-dimethoxybenzyl, 3-methoxybenzyl, 3,5-dibromophenyl, cyclohexylmethyl and 2-acetylaminophenyl.

10

7. The compound according to claim 1, wherein R₂ is selected from the group consisting of hydrogen, C₁-C₈ alkyl and benzyl.

8. The compound according to claim 7, wherein R₂ is selected from the group consisting of
- 15 hydrogen, methyl and benzyl.

9. The compound according to claim 1, wherein R₃ is selected from the group consisting of hydrogen, C₁-C₈ alkyl, cycloalkyl and aryl, each of which being unsubstituted or substituted with

one or more substituents selected from the group consisting of halogen, hydroxy, C₁-C₄ alkyl, C₁-C₄ alkoxy and arylalkoxy.

10. The compound according to claim 9, wherein R₃ is phenyl unsubstituted or substituted
5 with one or more substituents selected from the group consisting of fluorine, chlorine and methyl.

11. The compound according to claim 10, wherein R₃ is phenyl or 3,5-difluorobenzyl.

12. The compound according to claim 1, wherein R₄ is selected from the group consisting of
10 hydrogen, C₁-C₆ alkyl, cycloalkyl and -(CH₂)-A'R₁₁ wherein A' is aryl or heteroaryl and R₁₁ is
selected from the group consisting of hydrogen, halogen, hydroxy, alkyl, alkoxy and -NR₆R₇
wherein R₆ is selected from the group consisting of hydrogen, C₁-C₃ alkyl and C₁-C₃ alkoxy and R₇
is selected from the group consisting of hydrogen, C₁-C₄ alkyl and -C(O)NHR₆.

15 13. The compound according to claim 12, wherein R₄ is selected from the group consisting
of 3-dimethylaminobenzyl, 3-isopropylbenzyl, 3-trifluoromethoxybenzyl, 3-trifluoromethylbenzyl,
3-ethylbenzyl, 3-t-butylbenzyl, ethyl and cyclohexyl.

14. The compound according to claim 1, wherein the compound of Formula I is any one selected from:

(3S)-1-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

5 (3R)-1-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(2S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide;

10 (2R)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide;

2-((3S)-1-benzyl-5-oxopyrrolidin-3-yl)-N-[(2R,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]acetamide;

2-((3R)-1-benzyl-5-oxopyrrolidin-3-yl)-N-[(2R,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]acetamide;

15 (4S)-1-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-1-[2-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]amino-2-oxoethyl]-N,N-dimethyl-2-oxoimidazolidine-4-carboxamide;

(4S)-1-butanoyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

5 (4S)-N-[4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-phenethylimidazolidine-4-carboxamide;

N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-(1-methyl-oxopyrrolidin-3-yl)acetamide;

1-butyl-N-[4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

10

(3S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxo-1-phenethylpyrrolidine-3-carboxamide;

(3R)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxo-1-phenethylpyrrolidine-3-carboxamide;

15 1-(2-amino-2-oxoethyl)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(3S)-1-benzyl-N-[(2S,3R)-3-hydroxy-1-phenyl-4-[(3-trifluoromethoxyphenyl)methylamino]butan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-N-ethyl-2-oxoimidazolidine-1,4-dicarboxamide;

(3S)-1-[(2S)-3-(4-chlorophenyl)-1-ethylamino-1-oxopropan-2-yl]-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(3R)-1-[(2S)-3-(4-chlorophenyl)-1-ethylamino-1-oxopropan-2-yl]-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(3S)-1-benzyl-N-{(2S,3R)-3-hydroxy-1-phenyl-4-[(3-trifluoromethoxyphenyl)methylamino]butan-2-yl}-2-oxoimidazolidine-4-carboxamide;

(3S)-2,4-dibenzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide;

(3R)-2,4-dibenzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide;

(2S)-4-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-2-carboxamide;

(4S)-1,4-dibenzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(4-trifluoromethylphenyl)methyl]imidazolidine-4-carboxamide;

5 (4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(4-trifluoromethoxyphenyl)methyl]imidazolidine-4-carboxamide;

(4S)-1-benzyl-N-[(2S,3R)-1-(3,5-difluorophenyl)-4-(3-dimethylaminophenyl)methylamino-3-hydroxybutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(3S)-1-benzyl-N-[(2S,3R)-1-(3,5-difluorophenyl)-4-(3-dimethylaminophenyl)methylamino-3-hydroxybutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

10

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-(2-phenoxyethyl)imidazolidine-4-carboxamide;

(4R)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-(2-phenoxyethyl)imidazolidine-4-carboxamide;

15 (4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(2-trifluoromethylphenyl)methyl]imidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-1-(3,5-difluorophenyl)-4-(3-dimethylaminophenyl)methylamino-3-hydroxybutan-2-yl]-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

(4S)-1-benzyl-N-[(2S,3R)-4-(cyclohexylamino)-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4R)-1-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-4-methyl-2-oxoimidazolidine-4-carboxamide;

5 (4S)-1-benzyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-4-methyl-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-1-[(4-fluorophenyl)methyl]-2-oxoimidazolidine-4-carboxamide;

(4S)-1-[(3,4-difluorophenyl)methyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

10

(4S)-1-[(2,4-difluorophenyl)methyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

15 (4S)-1-benzyl-N-[(2S,3R)-3-hydroxy-1-phenyl-4-[(3-trifluoromethylphenyl)methylamino]butan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-1-benzyl-N-{(2S,3R)-3-hydroxy-1-phenyl-4-[(3-propan-2-ylphenyl)methylamino]butan-2-yl}-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-1-(3,5-difluorophenyl)-3-hydroxy-4-[(3-propan-2-yl)phenyl)methylamino]butan-2-yl]-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

5 (4S)-1-(3,5-dimethoxyphenyl)methyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-ethylphenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

10 (4S)-N-[(2S,3R)-4-(3-*t*-butylphenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

(4S)-1-[(3,5-difluorophenyl)methyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

15 (4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-1-[(3-methoxyphenyl)methyl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-1-[(4-methoxyphenyl)methyl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-*t*-butylphenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-1-(3,5-difluorophenyl)methyl-2-oxoimidazolidine-4-carboxamide;

(4S)-1-(3,5-difluorophenyl)methyl-N-[(2S,3R)-3-hydroxy-1-phenyl-4-[(3-propan-2-ylphenyl)methylamino]butan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-(3-*t*-butylphenyl)methylamino-1-(3,5-difluorophenyl)-3-hydroxybutan-2-yl]-1-(3,5-difluorophenyl)methyl-2-oxoimidazolidine-4-carboxamide;

5 (4S)-N-[(2S,3R)-4-ethylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-[(2,4,5-trifluorophenyl)methyl]imidazolidine-4-carboxamide;

(4S)-1-(3,5-dibromo)phenyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

10 (4S)-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxo-1-phenylimidazolidine-4-carboxamide;

(3S)-1-cyclohexylmethyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

(3R)-1-cyclohexylmethyl-N-[(2S,3R)-4-(3-dimethylaminophenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-5-oxopyrrolidine-3-carboxamide;

15 (4S)-N-[(2S,3R)-4-(3-*t*-butylphenyl)methylamino-3-hydroxy-1-phenylbutan-2-yl]-2-oxoimidazolidine-4-carboxamide;

(4S)-N-[(2S,3R)-4-[(3-dimethylaminophenyl)methylamino]-3-hydroxy-1-phenylbutan-2-yl]-5-oxo-1-pentylpyrrolidine-4-carboxamide; and

(4R)-N-[(2S,3R)-4-[(3-dimethylaminophenyl)methylamino]-3-hydroxy-1-phenylbutan-2-yl]-5-oxo-1-pentylpyrrolidine-4-carboxamide.

15. A pharmaceutical composition for inhibiting beta-secretase, comprising a therapeutically
5 effective amount of a compound of Formula I of claim 1 or a pharmaceutically acceptable salt or isomer thereof as an active ingredient and a pharmaceutically acceptable carrier.

16. The composition according to claim 15, wherein the composition is used for the improvement of cognitive functions or treatment or prevention of neurodegenerative diseases.

10

17. The composition according to claim 16, wherein the composition is used for the treatment of Alzheimer's disease.