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(71) Applicants (for all designated States except US): **NATIONAL CANCER CENTER** [KR/KR]; 111, Jeongbalsanro, Ilsandong-gu, Goyang-si, Gyeonggi-do 410-769 (KR). **ANYGEN CO., LTD.** [KR/KR]; Unit 306, Siheomsengsan-dong, Gwangju Techno Park, 958-3 Daecheon-dong, Buk-gu, Gwangju 143-701 (KR).

(72) Inventors; and

(71) Applicants : **KIM, Young-Chul** [KR/KR]; C-304, Gysu Apt., 1, Oryong-dong, Buk-gu, Gwangju 500-480 (KR). **LEE, Ju-Yeon** [KR/KR]; 501, Ga dong, Yeonhwabilla, Hadong-eup, Hadong-gun, Gyeongsangnam-do 667-800 (KR). **KIM, Soo Youl** [KR/KR]; 4-806 Jamwon Hanshin Apt., Jamwon-dong, Seocho-gu, Seoul 137-030 (KR). **LEE, Byung Il** [KR/KR]; 801-203 Bamgasi Kuny-

oung Villa 8 Danji, Jeongbalsan-dong, Ilsandong-gu, Goyang-si, Gyeonggi-do 410-761 (KR).

(74) Agent: **SHIN, Dong In**; #503, V-Charmant Bldg., 1601-1 Seocho 3-dong, Seocho-gu, Seoul 137-953 (KR).

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(54) Title: NOVEL PYRAZOLODIAZEPINE COMPOUNDS AS A TRANSGLUTAMINASE INHIBITOR, THE PREPARATION METHOD THEREOF AND A COMPOSITION CONTAINING THE SAME

(57) Abstract: The present invention relates to the novel pyrazolodiazepine compounds which potently inhibit the activity of transglutaminase in a dose dependent manner. Accordingly, it has been confirmed that the inventive compounds show potent inhibiting effect on the hyper-activated transglutaminase when the transglutaminase is over-expressed. Therefore the compounds can be useful in treating or preventing the disease caused by increased activation of transglutaminase.



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Description

Title of Invention: NOVEL PYRAZOLODIAZEPINE COMPOUNDS AS A TRANSGLUTAMINASE INHIBITOR, THE PREPARATION METHOD THEREOF AND A COMPOSITION CONTAINING THE SAME

Technical Field

- [1] The present invention relates to novel pyrazolodiazepine compound showing potent inhibiting activity of transglutaminase, the preparation method thereof and a composition comprising the same.

[2]

Background Art

- [3] Transglutaminase is an enzyme having protective response in normal condition such as blood coagulation, injury recovery etc. However, it plays an important role in the pathological mechanism of various diseases in case that the expression of transglutaminase is not appropriately regulated (Soo-Youl, KIM; New Target Against Inflammatory Diseases; Transglutaminase 2. *Archivum Immunologiae & Therapia experimentalis* 52,pp332-337,2004).
- [4] In particular, the expression of transglutaminase is remarkably increased in the diseases associated with inflammation, for example, rheumatoid arthritis, diabetes, inflammatory myositis, atherosclerosis, stroke, liver cirrhosis, breast cancer, Alzheimer, Parkinson's disease, Huntington's disease, encephalitis and celiac disease and the like.
- [5] In metastasis, chemo-resistance and radio-resistance, the increased expression of NF- κ B and transglutaminase had been found apparently (Soo-Youl KIM, Transglutaminase 2 in inflammation, *Front Biosci.*,11,pp3026-3035,2006).
- [6] Till now, there has not been found the relationship between the chemo-resistance to chemo-therapy and the function of transglutaminase, however, cancer cells has been found to be disappeared by the treatment of anti-cancer agent when the expression of transglutaminase was inhibited in human breast cancer cell showing chemo-resistance (Antonyak et al., Augmentation of tissue transglutaminase expression and activation by epidermal growth factor inhibit doxorubicin-induced apoptosis in human breast cancer cells, *J.Biol.Chem.*,2004, Oct. 1:279(40) pp.41461-7;Dae-Seok Kim et al,Reversal of drug resistance in Breast Cancer Cells by Transglutaminase 2 Inhibition and NuclearFactor-KB Inactivation, *Cancer Res.*, 2006, in press).
- [7] Recently, the pathological mechanism of transglutaminase through the molecular biological study on activated transglutaminase has been found and the feasibility of

- transglutaminase inhibition has been further embodied (Key Chung PARK, Kyung Cheon CHUNG, Yoon-Seong KIM, Jongmin LEE, Tong H, JOH and Soo-Youl KIM, Transglutaminase 2 induces nitric oxide synthesis in BV-2 microglia, *Biochem. Biophys. Res. Commun.* 323, pp1055-1062, 2004 ; Jongmin LEE, Yoon-seong KIM, Dong-Hee CHOI, Moon S. BANG, Tay R, HAN, Tong H. JOH, and Soo-Youl KIM, Transglutaminase 2 induces NF- κ B activation *via* a novel pathway in BV-2 microglia, *J. Biol. Chem.*, 279, pp53725-53735, 2004).
- [8] Inflammation is mainly caused by NF- κ B activation, induced by various kinases according to NF- κ B signaling pathway system. However, it has been reported that NF- κ B activation occurs without the help of various kinases and the significance of kinase inhibitor has been diminished (Tergaonkar et al., Ikappa B kinase-independent IkappaB α degradation pathway: functional NF-kappaB activity and implications for cancer therapy. *Mol. Cell Biol.*, 2003, Nov; 23(22), pp8070-83).
- [9] Through previous researches, the present inventors has found that transglutaminase activates NF- κ B by cross-linking to I κ B without various kinases such as IKK, NAK et al (Jongmin LEE et al., Transglutaminase 2 induces NF- κ B activation *via* a novel pathway in BV-2 microglia, *J. Biol. Chem.* 279, pp53725-53735, 2004), which means that NF- κ B can be activated only by the increase of cellular calcium ion since transglutaminase is a calcium-ion dependent enzyme.
- [10] Since the expression of various inflammatory factors including transglutaminase as well as I- κ B α , its inhibitor, is increased due to NF- κ B activation in inflammatory response, the consecutive activation of NF- κ B does not occur by I κ B in normal condition however it occurs in chronic inflammatory disease. Interestingly, it has been found that NF- κ B activation due to TNF- α or LPS, induces the expression of transglutaminase.
- [11] Accordingly, it has been supposed that abnormally activated transglutaminase maintain the inflammation response through the direct activation of NF- κ B in inflammation or the maintenance of its activation in inflamed cell. Those malignant circuit may be main factor which give rises to cancer metastasis and drug resistance in cancer tissue (Jongmin LEE et al., Transglutaminase 2 induces NF- κ B activation *via* a novel pathway in BV-2 microglia, *J. Biol. Chem.* 279, pp53725-53735, 2004).
- [12] Accordingly, transglutaminase inhibitor may be an important substance breaking the consecutive circuit of NF- κ B, and the fact may be basic evidence to prove the feasible efficacy of transglutaminase inhibitor which may be alternative with conventional steroids (Sohn J., Kim, T. I. Yoon, Y. H. and Kim S.Y.: transglutaminase inhibitor: A new Anti-inflammatory Approach in Allergic Conjunctivitis, *J.Clin.Invest.* 111, pp121-8,2003).
- [13] There has been reported that amine compounds such as cystamine or putrescine etc

are transglutaminase inhibitors (*Nature Genetics* 18 pp111-117, 1998; *Nature Medicine* 8, pp143-149, 2002). Besides the compounds, the other transglutaminase inhibitors, for example, monodansyl cadaverine (*J. Med. Chem.* 15, pp674-675, 1972); w-dibenzylaminoalkylamine (*J. Med. Chem.*, 18, pp278-284, 1975); 3-halo-4,5-dihydroisoxazole (*Mol. Pharmacol.*, 35, pp701-706, 1989); 2-[(2-oxopropyl)thio]imidazolium derivatives (*Blood*, 75, pp1455-1459, 1990) etc, are reported till now, however, they have been reported to show toxicity which gives rise to non-specifically inhibiting the other enzyme in vivo.

- [14] Accordingly, there has been still needed to develop more safe and effective transglutaminase-specific inhibitors till now. Recently, Sohn et al have found that peptide type transglutaminase-specific inhibitors have potent efficacy equivalent to that of steroid (Sohn J., Kim, T. I. Yoon, Y. H. and Kim S.Y.: transglutaminase inhibitor: A new Anti-inflammatory Approach in Allergic Conjunctivitis, *J. Clin. Invest.* 111, pp121-8, 2003) and the synthesized peptides which mimic the catalytic region of transglutaminase from anti-flammin protein (PLA2inhibitor) and elafin (potent transglutaminase substrate) have been developed as a transglutaminase-specific inhibitor (Nara K. et al., 1994. Elastase inhibitor elafin is a new type of protein inhibitor which has a transglutaminase-mediated anchoring sequence termed "cementoin". *J. Biochem. (Tokyo)*, 115: pp.441-448).
- [15] The increased expression of transglutaminase has been found in the disease associated with inflammation, for example, degenerative rheumatoid arthritis, diabetes, autoimmune myositis, atherosclerosis, stroke, liver cirrhosis, Malignant Breast Tumor, encephalitis, celiac disease etc.
- [16] However, there have been not disclosed or suggested on the usefulness of pyrazolodiazepine compounds as transglutaminase inhibitors in any of above-described literatures or patents, the disclosures of which are incorporated herein by reference in their entirety.
- [17] To investigate novel pyrazolodiazepine compounds having potent inhibiting effect on transglutaminase, the inventors of the present invention have intensively screened various pyrazolodiazepine compounds showing potent inhibiting activity of transglutaminase etc, and finally completed present invention by confirming that the inventive compounds could be useful as a medicament for treating the disease caused by increased activation of transglutaminase, preferably, neuronal system or cancer, more preferably, neuronal diseases such as Alzheimer's disease, multi-infarct dementia, the combined disease with Alzheimer's disease and multi-infarct dementia, Parkinson's disease, hypo-thyroidal syndrome, alcoholic dementia, or Huntington's disease; cancer diseases such as colorectal tumor, small intestine tumor, rectal cancer, anal cancer, esophageal cancer, pancreatic cancer, stomach cancer, renal cell carcinoma, uterine

cancer, breast cancer, lung cancer, lymphoma, thyroid cancer, prostate cancer, leukemia, skin cancer, colon carcinoma, brain cancer, bladder cancer, ovarian cancer, or gallbladder cancer etc.

[18]

[19] These and other objects of the present invention will become apparent from the detailed disclosure of the present invention provided hereinafter.

[20]

Disclosure of Invention

Technical Problem

[21] The present invention provides novel pyrazolodiazepine compounds and the pharmacologically acceptable salts thereof showing potent inhibiting effect on transglutaminase activity.

[22]

[23] The present invention also provides a pharmaceutical composition comprising novel pyrazolodiazepine compounds and the pharmacologically acceptable salts thereof as an active ingredient in an effective amount to treat and prevent the disease caused by increased activation of transglutaminase.

[24]

[25] The present invention also provides a use of pyrazolodiazepine compounds and the pharmacologically acceptable salts thereof for the preparation of medicament to treat and prevent the disease caused by increased activation of transglutaminase.

[26]

[27] The present invention also provides a method of treating or preventing the disease caused by increased activation of transglutaminase in a mammal comprising administering to said mammal an effective amount of novel pyrazolodiazepine compounds and the pharmacologically acceptable salts thereof, together with a pharmaceutically acceptable carrier thereof.

[28]

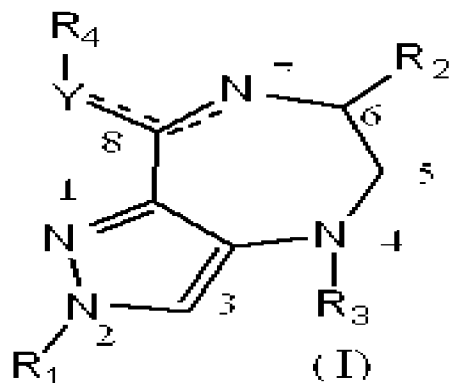
Solution to Problem

[29] Thus, the present invention provides novel pyrazolodiazepine compounds represented by the following general formula (I), the isomers thereof, and the pharmaceutically acceptable salts thereof:

[30]

[31] ChemistryFigure 1

[Chem.1]



[32]

[33] wherein

[34] Y is S, S-R₄ or N-R₄;

[35] R₁ is a hydrogen atom, C₁-C₆ alkyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom;

[36] R₂ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R', benzyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R' is protected or non-protected with an amine group;

[37] R₃ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R'', phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R'' is a ketone group or ester group substituted with C₁-C₆ alkyl group substituted with at least one halogen atom;

[38] R₄ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S which is substituted or un-substituted with protective group or benzyl group, C₁-C₆ alkyl group substituted or un-substituted with at least one R'', wherein R'' is a ketone group, ester group, amide group or carboxylic acid substituted with at least one selected from a hydrogen atom, halogen atom, amine group or C₁-C₆ alkyl group;

[39] (-----) denotes a single bond or double bond.

[40] As preferable compounds of general formula (I), the compounds of the present invention wherein R₁ is hydrogen atom, straight or branched butyl or pentyl group or phenethyl group; R₂ is a hydrogen atom, C₄-C₆ alkyl group substituted with Boc'-protected or non-protected amine group, benzyl group or phenethyl group; R₃ is a

hydrogen atom, straight or branched butyl or pentyl group substituted or un-substituted with ketone group wherein ketone group is substituted or un-substituted with C₁-C₂ alkyl group substituted with at least one halogen atom; R₄ is a hydrogen atom, C₂-C₆ alkyl group substituted or un-substituted with hetero aryl group selected from piperidine, pyrrolidine, piperazine, pyrazole, or morpholine which is substituted or un-substituted with Boc'-protective group or benzyl group, C₂-C₆ alkyl group substituted with ketone group, ester group, amide group or carboxylic acid substituted with at least one selected from a hydrogen atom, halogen atom, amine group or C₁-C₆ alkyl group.

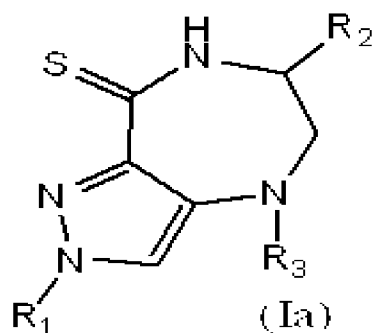
[41]

[42] In a preferred embodiment of the present invention, present invention also provides novel pyrazolodiazepine-8(2H) thione compounds represented by the following general formula (Ia), the isomers thereof, and the pharmaceutically acceptable salts thereof:

[43]

[44] ChemistryFigure 2

[Chem.2]



[45]

[46] R₁ is a hydrogen atom, C₁-C₆ alkyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom;

[47] R₂ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R', benzyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R' is protected or non-protected with an amine group;

[48] R₃ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R'', phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R'' is a ketone group or ester group substituted with C₁-C₆ alkyl group substituted with at least one halogen

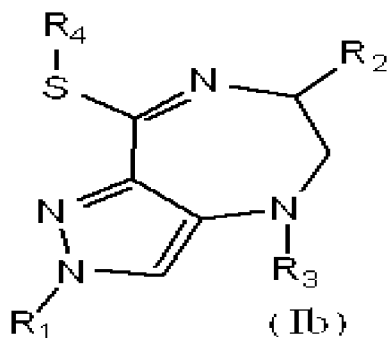
atom.

[49] In a preferred embodiment of the present invention, present invention also provides novel pyrazolodiazepine-8-yl thio compounds represented by the following general formula (Ib), the isomers thereof, and the pharmaceutically acceptable salts thereof:

[50]

[51] ChemistryFigure 3

[Chem.3]



[52]

[53] R₁ is a hydrogen atom, C₁-C₆ alkyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom;

[54] R₂ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R', benzyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R' is protected or non-protected with an amine group;

[55] R₃ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R'', phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R'' is a ketone group or ester group substituted with C₁-C₆ alkyl group substituted with at least one halogen atom;

[56] R₄ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S which is substituted or un-substituted with protective group or benzyl group, C₁-C₆ alkyl group substituted or un-substituted with at least one R'', wherein R'' is ketone group, ester group, amide group or carboxylic acid substituted with at least one selected from a hydrogen atom, halogen atom, amine group or C₁-C₆ alkyl group.

[57]

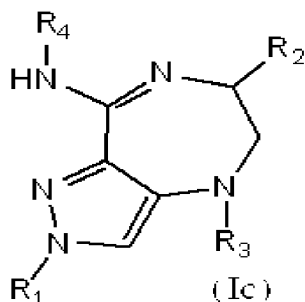
[58] In a preferred embodiment of the present invention, present invention also provides

novel pyrazolodiazepine-8-amine compounds represented by the following general formula (Ic), the isomers thereof, and the pharmaceutically acceptable salts thereof:

[59]

[60] ChemistryFigure 4

[Chem.4]



[61]

[62] R₁ is a hydrogen atom, C₁-C₆ alkyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom;

[63] R₂ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R', benzyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R' is protected or non-protected with an amine group;

[64] R₃ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R'', phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R'' is a ketone group or ester group substituted with C₁-C₆ alkyl group substituted with at least one halogen atom;

[65] R₄ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S which is substituted or un-substituted with protective group or benzyl group, C₁-C₆ alkyl group substituted or un-substituted with at least one R'', wherein R'' is ketone group, ester group, amide group or carboxylic acid substituted with at least one selected from a hydrogen atom, halogen atom, amine group or C₁-C₆ alkyl group;

[66]

[67] As preferable compounds of general formula (Ia)-(Ic), the compounds of the present invention wherein R₁ is hydrogen atom, straight or branched butyl or pentyl group or phenethyl group; R₂ is a hydrogen atom, C₄-C₆ alkyl group substituted with Boc'-protected or non-protected amine group, benzyl group or phenethyl group; R₃ is a

hydrogen atom, straight or branched butyl or pentyl group substituted or un-substituted with ketone group wherein ketone group is substituted or un-substituted with C₁-C₂ alkyl group substituted with at least one halogen atom; R₄ is a hydrogen atom, C₂-C₆ alkyl group substituted or un-substituted with heteroaryl group selected from piperidine, pyrrolidine, piperazine, pyrazole, or morpholine which is substituted or un-substituted with Boc'-protective group or benzyl group, C₂-C₆ alkyl group substituted with ketone group, ester group, amide group or carboxylic acid substituted with at least one selected from a hydrogen atom, halogen atom, amine group or C₁-C₆ alkyl group.

- [68] The most preferred compound of general formula (Ia)-(Ic) is one selected from the group consisting of;
- [69] Among the group of general formula (Ia),
- [70] (R)-1-(6-benzyl-2-phenethyl-8-thioxo-5,6,7,8-tetrahydropyrazolo[4,3-e][1,4]diazepine-4(2H)-yl)-2,2,2-trifluoroethanone(LDD843; Example 1);
- [71] (R)-1-(6-benzyl-2-phenethyl-4,5,6,7-tetrahydropyrazolo[4,3-e][1,4]diazepine-8(2H)-thione (LDD847; Example 2);
- [72] (S)-*tert*-butyl-4-(2,4-diisobutyl-8-thioxo-2,4,5,6,7,8-hexahydropyrazolo[4,3-e][1,4]diazepine-6-yl)butylcarbamate(LDD1008; Example 11);
- [73] (S)-6-(4-aminobutyl)-2,4-diisobutyl-4,5,6,7-hexahydropyrazolo[4,3-e][1,4]diazepine-8(2H)-thione (LDD998; Example 12);
- [74]
- [75] Among the group of general formula (Ib),
- [76] (S,E)-*tert*-butyl-3-(6-4-(*tert*-butoxy-carbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanoate(LDD1007; Example 14);
- [77] (S,E)-*tert*-butyl-2-(6-4-(*tert*-butoxy-carbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)acetate(LDD1006; Example 15);
- [78] (S,E)-3-(6-4-(aminobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanoic acid(LDD997; Example 16);
- [79] (S,E)-3-(6-4-(*tert*-butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanoic acid(LDD1005; Example 17);
- [80] (S,E)-ethyl-3-(6-4-(*tert*-butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanoate(LDD1004; Example 18);
- [81] (S,E)-*tert*-butyl-4-(8-(3-amino-3-oxopropylthio)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-6-ylthio)butyl carbamate(LDD1003; Example 19);
- [82] (S,E)-*tert*-butyl-4-(8-(3-hydrazinyl-3-oxopropylthio)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-6-yl)butyl carbamate(LDD1002; Example 20);
- [83] (S,E)-3-(6-(4-amniobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanhydrazide (LDD1001; Example 21);

- [85] (S,E)-3-(6-(4-amniobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanamide (LDD1000; Example 22);
- [86]
- [87] Among the group of general formula (Ic),
- [88] (R,E)-6-benzyl-2-phenethyl-N-(2-(piperidin-1-yl)ethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD844; Example 3);
- [89] (R,E)-6-benzyl-2-phenethyl-N-(2-(pyrrolidin-1-yl)ethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD845; Example 4);
- [90] (R,E)-6-benzyl-2-phenethyl-N-(2-(piperazin-1-yl)ethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD846; Example 5);
- [91] (R,E)-6-benzyl-N-(2-morpholinoethyl)-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD848; Example 6);
- [92] (R,E)-6-benzyl-N-methyl-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD849; Example 7);
- [93] (S)-*tert*-butyl-3-((R,E)-6-benzyl-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylamino)pyrrolidin-1 carboxylate(LDD850; Example 8);
- [94] (R,E)-6-benzyl-N-(1-benzylpiperidin-4-yl)-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD854; Example 9);
- [95] (R,E)-6-benzyl-2-phenethyl-N-(piperidin-4-yl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD1010; Example 10);
- [96] (S,E)-*tert*-butyl-4-(2,4-diisobutyl-8-(2-morpholinoethylamino)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-6-yl)butylcarbamate(LDD1009; Example 13).
- [97]
- [98] The inventive compounds represented by general formula (I) can be transformed into their pharmaceutically acceptable salt and solvates by the conventional method well known in the art. For the salts, acid-addition salt thereof formed by a pharmaceutically acceptable free acid thereof is useful and can be prepared by the conventional method. For example, after dissolving the compound in the excess amount of acid solution, the salts are precipitated by the water-miscible organic solvent such as methanol, ethanol, acetone or acetonitrile to prepare acid addition salt thereof and further the mixture of equivalent amount of compound and diluted acid with water or alcohol such as glycol monomethylether, can be heated and subsequently dried by evaporation or filtrated under reduced pressure to obtain dried salt form thereof.
- [99]
- [100] As a free acid of above-described method, organic acid or inorganic acid can be used. For example, organic acid such as methansulfonic acid, *p*-toluensulfonic acid, acetic acid, trifluoroacetic acid, citric acid, maleic acid, succinic acid, oxalic acid, benzoic acid, lactic acid, glycolic acid, gluconic acid, galacturonic acid, glutamic acid, glutaric

acid, glucuronic acid, aspartic acid, ascorbic acid, carbonylic acid, vanillic acid, hydroiodic acid and the like, and inorganic acid such as hydrochloric acid, phosphoric acid, sulfuric acid, nitric acid, tartaric acid and the like can be used herein.

[101]

[102] Further, the pharmaceutically acceptable metal salt form of inventive compounds may be prepared by using base. The alkali metal or alkali-earth metal salt thereof can be prepared by the conventional method, for example, after dissolving the compound in the excess amount of alkali metal hydroxide or alkali-earth metal hydroxide solution, the insoluble salts are filtered and remaining filtrate is subjected to evaporation and drying to obtain the metal salt thereof. As a metal salt of the present invention, sodium, potassium or calcium salt are pharmaceutically suitable and the corresponding silver salt can be prepared by reacting alkali metal salt or alkali-earth metal salt with suitable silver salt such as silver nitrate.

[103]

[104] The pharmaceutically acceptable salt of the compound represented by general formula (I) comprise all the acidic or basic salt which may be present at the compounds, if it does not indicated specifically herein. For example, the pharmaceutically acceptable salt of the present invention comprise the salt of hydroxyl group such as the sodium, calcium and potassium salt thereof; the salt of amino group such as the hydrogen bromide salt, sulfuric acid salt, hydrogen sulfuric acid salt, phosphate salt, hydrogen phosphate salt, dihydrophosphate salt, acetate salt, succinate salt, citrate salt, tartrate salt, lactate salt, mandelate salt, methanesulfonate(mesylate) salt and *p* - toluenesulfonate(tosylate) salt etc, which can be prepared by the conventional method well known in the art.

[105]

[106] There may exist in the form of optically different diastereomers since the compounds represented by general formula (I) have unsymmetrical centers, accordingly, the compounds of the present invention comprise all the optically active isomers, R or S stereoisomers and the mixtures thereof. Present invention also comprises all the uses of racemic mixture, more than one optically active isomer or the mixtures thereof as well as all the preparation or isolation method of the diastereomer well known in the art.

[107]

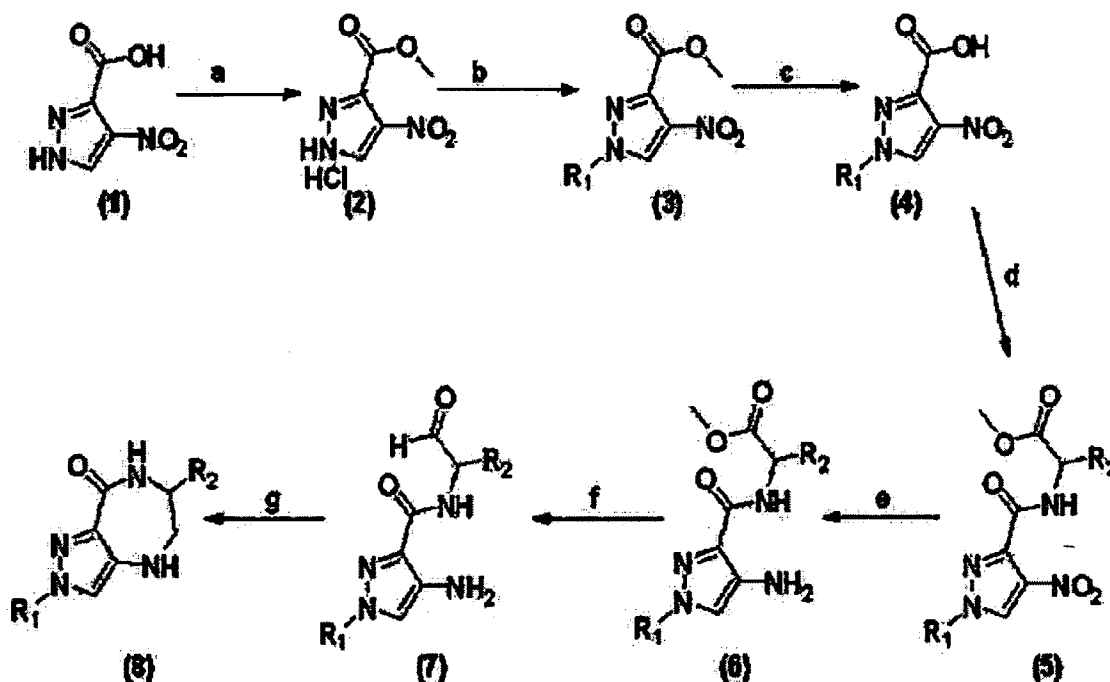
[108] The compounds of the invention of formula (I) may be chemically synthesized by the methods which will be explained by following reaction chemistry figures hereinafter, which are merely exemplary and in no way limit the invention. The reaction chemistry figures show the steps for preparing the representative compounds of the present invention, and the other compounds also may be produced by following the steps with appropriate modifications of reagents and starting materials, which are envisaged by

those skilled in the art.

[109]

[110] ChemistryFigure 5

[Chem.5]



[111]

[112] a. AcCl, MeOH., b. R_1 -X, NaH, DMF., c. 1N NaOH, MeOH., d. amino acid ester, EDC, HOBt, TEA, DCM., e. Pd/C, H_2 , MeOH., f. DIBAL, toluene., g. $NaBH(OAc)_3$, 1% AcOH, DCM.

[113]

[114] As depicted in the above Chemistry figure 5, the chemistry figure 5 explains the process for preparing pyrazolodiazepin-8-one derivatives, an intermediate for synthesizing novel pyrazolodiazepine compounds represented by the following general formula (I), from starting materials well-known in the art as follows:

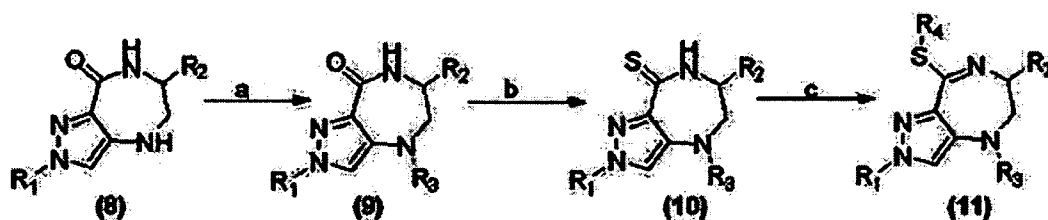
[115] 4-nitropyrazolo-1-carboxylic acid (1) as a starting material is reacted with esterifying reagent such as acetyl chloride under reaction solvent such as methanol to obtain ester (2) at step 1; the amine moiety of ester pyrazole ring prepared in step 1, is performed to selective alkylation reaction by reacting with R_1 -X under sodium hydride and reaction solvent such as DMF etc to introduce R_1 substituents such as phenethyl group, isobutyl group, propyl group etc thereto at step 2; the substituted compound (3) prepared in step 2, is performed to hydrolysis reaction under alkali condition using by alkali such as

NaOH and reaction solvent such as methanol etc to hydrolyze the ester and then the reaction solution is neutralized with acid solution such as hydrochloric acid etc to obtain carboxylate compound (4) at step 3; the compound (4) is performed to coupling reaction with coupling reagent such as the condition using amino acid ester ($\text{CH}_3 - \text{OCO}-\text{C}(\text{R}_2)-\text{NH}_2$), EDC and HOBr under reaction solvent such as TEA and DCM to obtain amide compound (5) at step 4; the nitro group of compound (5) prepared in step 4, is reduced with reducing agent such as the condition Pd/C catalyst and hydrogen gas under reaction solvent such as methanol etc to reduce primary amine to amine group of the compound (6) at step 5; the ester group of the compound (6) is performed to selective reducing reaction by reacting with DIBAL-H under ultra cold condition such as -78°C in inactive solvent such as toluene etc to obtain aldehyde (7) through selective reducing reaction at step 6; the DIBAL-H is removed from the solution using by potassium-sodium tartrate saturated water solution in order to accelerate the imine reaction and the compound (7) prepared in step 6 is performed to intramolecular cyclic amine reaction using by reacting agent such as $\text{NaB}(\text{OAc})_3\text{H}$ etc to afford scaffold(8).

[116]

[117] ChemistryFigure 6

[Chem.6]



[118] a. R_3 -aldehyde, $\text{NaBH}(\text{OAc})_3$, 1% AcOH, DCM., b. Lawesson's reagent, toluene., c. $\text{R}_4\text{-X}$, DBU, DCM.

[119]

[120] As depicted in the above Chemistry figure 6, the chemistry figure 6 explains the process for preparing pyrazolodiazepin-8-thio derivatives, an intermediate for synthesizing novel pyrazolodiazepine compounds represented by the following general formula (I), from starting materials well-known in the art as follows:

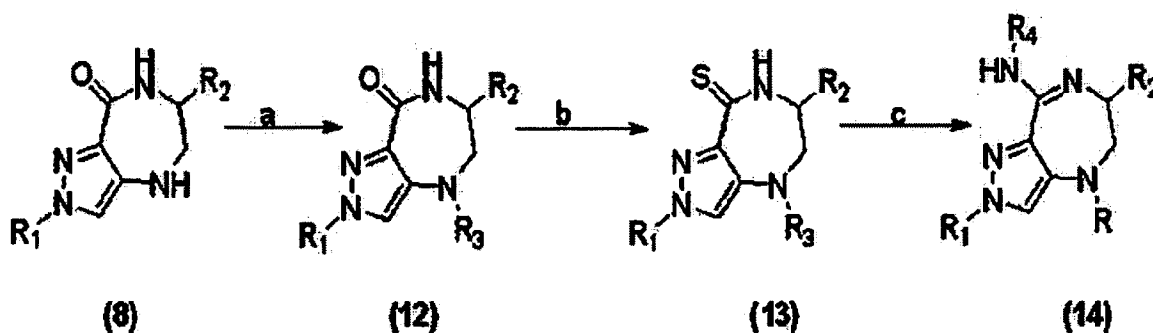
[121] The amine moiety of scaffold, i.e., pyrazolo[1[4,3-e][1,4]diazepin-8(2H)-one (8) prepared in reaction chemistry figure 5 as a starting material is performed to reductive alkylation using by R_3 -aldehyde having aldehyde substituent under the reaction solvent such as 1% acetic acid and DCM to introduce R_3 substituent to the amine moiety in

order to obtain tertiary amine compound (9) at step 1; the compound (9) is reacted with Lawesson's reagent under reaction solvent such as toluene etc to obtain pyrazolodiazepin-8-thione compound (10) at step 2; the compound (10) is performed to selective S-alkylation reaction modifying Eschenmoser coupling reaction by reacting with DBU and R_4-X under reaction solvent such as DCM etc to obtain R_4 -substituent-introduced pyrazolodiazepin-8-thio derivatives (11).

[122]

[123] ChemistryFigure 7

[Chem.7]



[124]

[125] a. R_3 -anhydride, DCM. or R_3 -aldehyde, $NaBH(OAc)_3$, 1% AcOH, DCM., b. Lawesson's reagent, toluene., c. R_4-NH , ethanol.

[126]

[127] As depicted in the above chemistry figure 7, the chemistry figure 7 explains the process for preparing pyrazolodiazepin-8-amine derivatives, an intermediate for synthesizing novel pyrazolodiazepine compounds represented by general formula (I), from starting materials well-known in the art as follows:

[128] The 4-amine moiety of scaffold, i.e., pyrazolo[1,4]diazepin-8(2H)-one (8) prepared in reaction scheme 1 as a starting material is introduced with R_3 substituent by reacting under the condition with R_3 -aldehyde, trifluoroacetyl anhydride in the presence of 1% acetic acid and DCM solvent or the alternative condition of R_3 -anhydride and DCM to obtain tertiary amine compound (12) at step 1; the compound (12) is heated with Lawesson's reagent under reaction solvent such as toluene etc to obtain pyrazolodiazepin-8-thione compound (13) at step 2; the pyrazolodiazepin-8-thione compound (13) prepared in step 2 is reacted with abundant amount of amine (R_4-NH) in reaction solvent such as ethanol etc to obtain pyrazolodiazepin-8-amine compound (14) at step 2.

[129]

[130] Accordingly, present invention also provide a method for preparing novel pyrazolodiazepine compounds represented by general formula (I) as described above.

[131]

[132] The novel pyrazolodiazepine compounds prepared by the above-described method represented by general formula (I) shows potent inhibiting activity of transglutaminase, therefore, the inventive could be useful as a medicament for treating the disease caused by increased activation of transglutaminase, preferably, neuronal diseases such as Alzheimer's disease, multi-infarct dementia, the combined disease with Alzheimer's disease and multi-infarct dementia, Parkinson's disease, hypo-thyroidal syndrome, alcoholic dementia, or Huntington's disease; cancer diseases such as colorectal tumor, small intestine tumor, rectal cancer, anal cancer, esophageal cancer, pancreatic cancer, stomach cancer, renal cell carcinoma, uterine cancer, breast cancer, lung cancer, lymphoma, thyroid cancer, prostate cancer, leukemia, skin cancer, colon carcinoma, brain cancer, bladder cancer, ovarian cancer, or gallbladder cancer etc, more preferably, rheumatoid arthritis, diabetes, inflammatory myositis, atherosclerosis, stroke, liver cirrhosis, breast cancer Alzheimer, Parkinson disease, Huntington disease, encephalitis or celiac disease etc, neuronal system or cancer.

[133]

[134] Accordingly, it is another object of the present invention to provide the pharmaceutical composition comprising an effective amount of the compound represented by general formula (I) or the pharmaceutically acceptable salt thereof as an active ingredient in the amount effective to treat or prevent the disease caused by increased activation of transglutaminase, together with pharmaceutically acceptable carriers or diluents.

[135]

[136] It is another object of the present invention to provide a transglutaminase inhibitor comprising an effective amount of the compound represented by general formula (I) or the pharmaceutically acceptable salt thereof as an active ingredient.

[137]

[138] Through the determination of the activity of transglutaminase which binds [1,4-¹⁴C] putrescine to succinylated casein *in vitro* test, the inventive compounds potently inhibit the activity of transglutaminase in a dose dependent manner. Accordingly, it has been confirmed that the inventive compounds show potent inhibiting effect on the hyper-activated transglutaminase when the transglutaminase is over-expressed.

[139]

[140] In accordance with the other aspect of the present invention, there is also provided a use of the compound represented by general formula (I) or the pharmaceutically acceptable salt thereof for manufacture of medicines employed for treating or preventing the disease caused by increased activation of transglutaminase in mammals including human as an active ingredient in the amount effective to treat or prevent the disease

caused by increased activation of transglutaminase.

[141]

[142] In accordance with the other aspect of the present invention, there is also provided a method of treating or preventing the disease caused by increased activation of transglutaminase in a mammal comprising administering to said mammal an effective amount of novel derivatives represented by general formula (I) and the pharmacologically acceptable salt thereof, together with a pharmaceutically acceptable carrier thereof into the mammals including human suffering from said disease.

[143] In accordance with the other aspect of the present invention, there is also provided a method of inhibiting hyper-activated transglutaminase in a mammal comprising administering to said mammal an effective amount of novel derivatives represented by general formula (I) and the pharmacologically acceptable salt thereof, together with a pharmaceutically acceptable carrier thereof into the mammals including human suffering from said disease.

[144] The term “prevent” disclosed herein means all the performing behaviors which inhibit or delay the occurrence of all the diseases caused by the increased activity of transglutaminase; and the term “treat” disclosed herein means all the performing behaviors which turn all the diseases caused by the increased activity of transglutaminase for the better condition or change all the diseases caused by the increased activity of transglutaminase for favorable condition.

[145]

[146] The term “the disease caused by increased activation of transglutaminase” disclosed herein comprise all the diseases caused by over-expressed transglutaminase the disease caused by increased activation of transglutaminase, specifically, neuronal disease or cancer disease.

[147]

[148] The term “neuronal disease” disclosed herein comprises the diseases involved in the neuronal cell death or injury, especially, CNS neuronal diseases caused by neuronal cell death or injury such as Alzheimer’s disease, multi-infarct dementia, the combined disease with Alzheimer’s disease and multi-infarct dementia, Parkinson’s disease, hypo-thyroidal syndrome, alcoholic dementia, or Huntington’s disease etc. the principle symptoms of the diseases comprise cognition function disorder, the disorders in speaking, discretion, abstract ability, spatial and time ability, and learning ability, resulting in death. The inventive compounds can be useful in treating and preventing the disease's caused by increased activation of transglutaminase, for example, Huntington's disease caused by over-expressed transglutaminase in brain (*Nature Medicine*, 8, pp143-149, 2002); Alzheimer's disease caused by over-expressed transglutaminase in cerebellar cortex and cerebral cortex (*The Journal of Biological*

Chemistry, 274(43), issue of October 22, pp30715-30721); Parkinson's disease caused by aggregated α -synuclein by the action of transglutaminase (*PNAS*, February 18, 2003, 100(4), pp2047-2052) etc, however, the disease are not intended to limit thereto in the present invention and comprise all the diseases caused by the over-expressed transglutaminase.

- [149] The term "cancer disease" disclosed herein comprises the cancer diseases showing remarkably increased expression of transglutaminase such as metastasis, chemotherapy-resistant cancer or radio-therapy-resistant cancer etc, specifically, cancer diseases showing remarkably increased expression of transglutaminase such as colorectal tumor, small intestine tumor, rectal cancer, anal cancer, esophageal cancer, pancreatic cancer, stomach cancer, renal cell carcinoma, uterine cancer, breast cancer, lung cancer, lymphoma, thyroid cancer, prostate cancer, leukemia, skin cancer, colon carcinoma, brain cancer, bladder cancer, ovarian cancer, or gallbladder cancer etc, however, the disease are not intended to limit thereto in the present invention and comprise all the diseases caused by the over-expressed transglutaminase.
- [150] The composition comprising inventive compounds and the treating method using thereby could be applied to human disease as well as mammal such as cow, horse, sheep, pig, goat, camel, antelope, dog, cat etc which may be suffered from the disease caused by increased activation of transglutaminase.
- [151] Accordingly, (I) shows potent inhibiting activity of transglutaminase, therefore, the inventive could be useful as a medicament for treating the disease caused by increased activation of transglutaminase, preferably, neuronal diseases such as Alzheimer's disease, multi-infarct dementia, the combined disease with Alzheimer's disease and multi-infarct dementia, Parkinson's disease, hypo-thyroidal syndrome, alcoholic dementia, or Huntington's disease; cancer diseases such as colorectal tumor, small intestine tumor, rectal cancer, anal cancer, esophageal cancer, pancreatic cancer, stomach cancer, renal cell carcinoma, uterine cancer, breast cancer, lung cancer, lymphoma, thyroid cancer, prostate cancer, leukemia, skin cancer, colon carcinoma, brain cancer, bladder cancer, ovarian cancer, or gallbladder cancer etc, more preferably, rheumatoid arthritis, diabetes, inflammatory myositis, atherosclerosis, stroke, liver cirrhosis, breast cancer Alzheimer, Parkinson's disease, Huntington's disease, encephalitis or celiac disease etc, neuronal system or cancer.
- [152]
- [153]
- [154] Accordingly, it is another object of the present invention to provide the pharmaceutical composition comprising an effective amount of the compound represented by general formula (I) or the pharmaceutically acceptable salt thereof as an active ingredient in amount effective to treat or prevent the disease caused by increased ac-

tivation of transglutaminase, preferably, neuronal disease or cancer disease, more preferably, neuronal diseases such as Alzheimer's disease, multi-infarct dementia, the combined disease with Alzheimer's disease and multi-infarct dementia, Parkinson's disease, hypo-thyroidal syndrome, alcoholic dementia, or Huntington's disease; cancer diseases such as colorectal tumor, small intestine tumor, rectal cancer, anal cancer, esophageal cancer, pancreatic cancer, stomach cancer, renal cell carcinoma, uterine cancer, breast cancer, lung cancer, lymphoma, thyroid cancer, prostate cancer, leukemia, skin cancer, colon carcinoma, brain cancer, bladder cancer, ovarian cancer, or gallbladder cancer etc, together with pharmaceutically acceptable carriers or diluents.

[155]

[156] The compound according to the present invention can be provided as a pharmaceutical composition containing pharmaceutically acceptable carriers, adjuvants or diluents. For example, the compound of the present invention can be dissolved in oils, propylene glycol or other solvents which are commonly used to produce an injection. Suitable examples of the carriers include physiological saline, polyethylene glycol, ethanol, vegetable oils, isopropyl myristate, etc., but are not limited to them.

[157]

[158] Hereinafter, the following formulation methods and excipients are merely exemplary and in no way limit the invention.

[159]

[160] The compound of the present invention in pharmaceutical dosage forms may be used in the form of their pharmaceutically acceptable salts, and also may be used alone or in appropriate association, as well as in combination with other pharmaceutically active compounds.

[161]

[162] The compound of the present invention may be formulated into preparations for injections by dissolving, suspending, or emulsifying them in aqueous solvents such as normal saline, 5% Dextrose, or non-aqueous solvent such as vegetable oil, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol. The formulation may include conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[163]

[164] The desirable dose of the inventive compound varies depending on the condition and the weight of the subject, severity, drug form, route and period of administration, and may be chosen by those skilled in the art. However, in order to obtain desirable effects, it is generally recommended to administer at the amount ranging 0.0001 - 100 mg/kg, preferably 0.001 - 10 mg/kg by weight/day of the inventive compound of the present invention. The dose may be administered in single or divided into several times per

day. In terms of composition, the compound should be present between 0.0001 to 10% by weight, preferably 0.0001 to 1% by weight based on the total weight of the composition.

[165]

[166] The pharmaceutical composition of present invention can be administered to a subject animal such as mammals (rat, mouse, domestic animals or human) *via* various routes. All modes of administration are contemplated, for example, administration can be made by inhaled, orally, rectally or by intravenous, intramuscular, subcutaneous, intrathecal, epidural or intracerebroventricular injection.

[167]

[168] The present invention is more specifically explained by the following examples. However, it should be understood that the present invention is not limited to these examples in any manner.

[169]

[170]

Advantageous Effects of Invention

[171] The present invention relates to the novel pyrazolodiazepine compounds which potently inhibit the activity of transglutaminase in a dose dependent manner. Accordingly, it has been confirmed that the inventive compounds show potent inhibiting effect on the hyper-activated transglutaminase when the transglutaminase is over-expressed. Therefore the compounds can be useful in treating or preventing the disease caused by increased activation of transglutaminase.

[172]

Best Mode for Carrying out the Invention

[173] It will be apparent to those skilled in the art that various modifications and variations can be made in the compositions, use and preparations of the present invention without departing from the spirit or scope of the invention.

[174]

[175] The present invention is more specifically explained by the following examples. However, it should be understood that the present invention is not limited to these examples in any manner.

[176]

Mode for the Invention

[177] EXAMPLES

[178] The following Reference Example, Examples and Experimental Examples are intended to further illustrate the present invention without limiting its scope.

[179]

[180] **Reference Example 1. Experiment preparation and apparatus**

[181]

[182] 1-1. Analytic apparatus

[183] ¹H-NMR (300 MHz) and ¹³C-NMR(300MHz) were used in the experiment and coupling constant was expressed as Hz. MS spectrum was used in the experiment.

[184]

[185] 1-2. TLC and column chromatography

[186] TLC(Thin layer chromatography; Merck F254, E. Merck Co.) and Silica gel column chromatography (Merck EM9385, 230-400 mesh) were used in the experiment. The result of TLC was identified by using UV lamp (254nm) or spraying agent such as anisaldehyde, KMnO₄ reagent etc.

[187]

[188] 1-3. Reagent

[189] All the reagents used in the experiments are procured from Sigma-Aldrich Co. ltd, Lancaster co. ltd or Fluka Co. ltd and the solvent used in the experiments are 1st grade from Sigma-Aldrich Co. ltd, merck co. ltd or junsei Chemical Co. Ltd. THF solvent prepared by adding Na metal and benzophenone to the solvent and refluxing under argon atmosphere with heating to the extent that the solvent has been changed to blue color and CH₂Cl₂ prepared by adding CaH₂ to the solvent and refluxing under argon atmosphere with heating were used in the experiment. Ethylacetate and hexane used in the experiment were purified with refluxing under argon atmosphere with heating.

[190]

[191]

[192] **Reference Example 2. Preparation of intermediate: pyrazolodiazepin-8-one derivative**

[193]

[194] 2-1. Preparation of Ester (2)

[195] 20 g of 4-nitropyrazolo-1-carboxylic acid (1) (127.32 mM) as a starting material was dropwisely added to the reaction solution dissolving 11.10 ml of acetyl chloride (381.96 mM) in 350 ml of methanol to react together and finally 23.69 g of methyl-4-nitro-1H-pyrazole-3-carboxylate hydrochloride was obtained (yield: 90%).

[196]

[197] 2-2. Preparation of R₁-substituted derivatives(3)

[198] 12 g of the ester (2) prepared in step 2-1 (58.09 mM) was performed to selective alkylation reaction by adding 11.7 ml of phenethyl bromide (88.01mM) with 5.8 g of sodium hydride (145.22 mM) thereto and reacting in 250 ml of DMF to introduce various R₁-substituents such as phenethyl group, isobutyl group, propyl group etc and finally 10.54 g of methyl-4-nitro-1H-phenethyl-1H-pyrazole-3-carboxylate (3) was

obtained (yield: 69%).

[199]

[200] 2-3. Preparation of carboxylate (4)

[201] 5.09 g of methyl-4-nitro-1H-phenethyl-1H-pyrazole-3-carboxylate (3) prepared in step 2-2 (13.99 mM) was performed to hydrolysis reaction by adding 6 g of NaOH thereto and reacting in 150 ml of methanol under alkali condition at room temperature for 30mins to hydrolyze the solution was neutralized with 150 ml of 1N hydrochloric acid to remove the resulting salt from the solution. Finally 3.50 g of 4-nitro-1H-phenethyl-1H-pyrazole-3-carboxylate (4) was obtained (yield: 97%).

[202]

[203] 2-4. Preparation of amides (5)

[204] 3.50 g of 4-nitro-1H-phenethyl-1H-pyrazole-3-carboxylate (4) prepared in step 2-3 (13.40 mM) was performed to coupling reaction by adding 2.88 g of H-Phe-OMe HCl (16.08 mM) thereto and reacting with 3.08 g of (ethyl-N'', N''-dimethylamino) propylcarbodiimide (EDC; 16.08 mM) and 2.17 g of N-hydroxybenzotriazole (HOBt; 16.08 mM) as a coupling reagent in 2.13 ml of TEA (Triethylamine; 16.08 mM) under 100 ml of DCM (dichloromethane) to afford 4.24 g of 2-(4-nitro-1-phenethyl-1H-pyrazole-3-carboxamido)-3-phenylpropanoate (5) (yield: 75%).

[205]

[206] 2-5. Preparation of amines (6)

[207] 4 g of 2-(4-nitro-1-phenethyl-1H-pyrazole-3-carboxamido)-3-phenylpropanoate (5) prepared in step 2-4 (9.47 mM) was performed to reducing reaction by reacting with 600 mg of Pd/C catalyst and H₂ gas under 150 ml of methanol to reduce the nitro group of amide (5) to amine (NH₂) and finally, 3.52 g of methyl-2-(4-amino-1-phenethyl-1H-pyrazole-3-carboxamido)-3-phenylpropanoate (6) (yield : 95%).

[208]

[209] 2-6. Preparation of amides (7)

[210] 3.52 g of methyl-2-(4-amino-1-phenethyl-1H-pyrazole-3-carboxamido)-3-phenylpropanoate (6) prepared in step 2-5 (8.97 mM) was performed to selective reducing reaction by reacting with 2M DIBAL-H(diisobutylaluminium hydride) at -78°C in 9 ml of toluene dropwisely for 2.5 hours and then the remaining DIBAL was removed by adding methanol. The resulting precipitate formed by reacting with DIBAL was further removed by potassium-sodium tartrate saturated water solution to obtain 2.9 g of 4-amino-N-(1-oxo-3-phenylpropan-2-yl)-1-phenethyl-1H-pyrazole-3-carboxamide (7).

[211]

[212] 2-7. Preparation of scaffold (8)

[213] 2.9 g of

4-amino-N-(1-oxo-3-phenylpropan-2-yl)-1-phenethyl-1H-pyrazole-3-carboxamide (7) prepared in step 2-6 was added with 1% AcOH DCM solvent and reacted for 1 hour to accelerate the spontaneous iminization reaction and 2.8 g of NaB(OAc)₃H was added thereto to stir for 12 hour at room temperature in order to obtaining 1.45 g of 6-benzyl-2-phenethyl-4,5,6,7-tetrahydropyrazolo[4,3-e][1,4]diazepin-8(2H)-one as a scaffold of pyrazolodiazepine-8-one (8) (yield:52%).

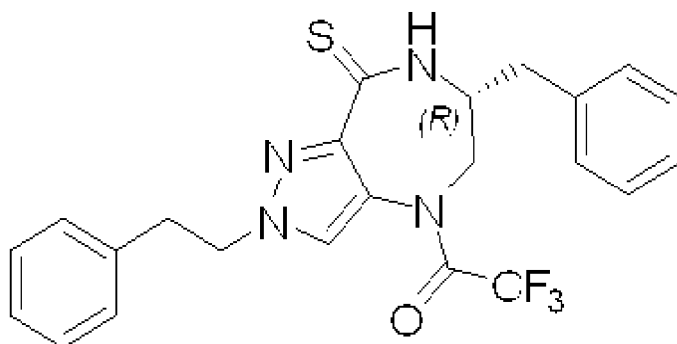
[214]

[215]

[216] **Example 1. (R)-1-(6-benzyl-2-phenethyl-8-thioxo-5,6,7,8-tetrahydropyrazolo**
 [217] **[4,3-e][1.4]diazepin-4(2H)-yl)-2,2,2-trifluoroethanone (LDD843)**

[218] ChemistryFigure 8

[Chem.8]



[219]

[220] 1-1. Preparation of (R)-6-benzyl-2-phenethyl-4-(2,2,2,-trifluoroacetyl)

[221] -4,5,6,7-tetrahydropyrazolo[4,3-e][1,4]diazepin-8(2H)-one (LDD843-1)

[222]

[223] 520 mg of

((R)-6-benzyl-2-phenethyl-4,5,6,7-tetrahydropyrazolo[4,3-e][1,4]diazepin-8(2H)-one (8) (1.5 mM) as a starting material was dissolved in DCM(dichloromethane) and 420 mg of trifluoroacetic anhydride (3.0 mM) was added thereto to stir for 4 hours at room temperature. At the end of the reaction, remaining TFA was removed with water and solvent was removed with evaporation to afford 521 mg of (R)-6-benzyl-2-phenethyl-4-(2,2,2,-trifluoroacetyl)-4,5,6,7-tetrahydropyrazolo[4,3-e][1,4]diazepin-8(2H)-one (LDD843-1) showing following physicochemical property (yield: 78.5%):

[224]

[225] ^1H -NMR (300MHz, CDCl_3) δ (ppm) 8.15(1H, s), 7.38-7.07(10H, m), 7.10(1H, NH), 4.44(2H, $J=6.9\text{Hz}$, t), 4.36(1H, $J=15\text{Hz}$, m), 3.94(1H, m), 3.80(1H, m), 3.22(2H, $J=6.9\text{Hz}$, t), 3.05(1H, $J=5.7\text{Hz}$, 13.8Hz, dd), 2.83(1H, $J=13.9\text{Hz}$, 9.3Hz, dd);

[226] MS (ESI), m/z : 443.2 ($[\text{M}+\text{H}]^+$)

[227]

[228] 1-2. Preparation of (R)-1-(6-benzyl-2-phenethyl-8-thioxo-5,6,7,8-tetrahydropyrazolo[4,3-e][1,4]diazepin-4(2H)-yl)-2,2,2-trifluoroethanone (LDD843)

[230]

[231] 430 mg of

(R)-6-benzyl-2-phenethyl-4-(2,2,2-trifluoroacetyl)-4,5,6,7-tetrahydropyrazolo[4,3-e][1,4]diazepin-8(2H)-one (LDD843-1) prepared in step 1-1 of Example 1, was reacted with 471 mg of Lawesson's reagent (227439; Aldrich Co.) in toluene at 70 °C for 2 hours to afford 427 mg of

(R)-1-(6-benzyl-2-phenethyl-8-thioxo-5,6,7,8-tetrahydropyrazolo[4,3-e][1,4]diazepin-4(2H)-yl)-2,2,2-trifluoroethanone (LDD843) showing following physicochemical property (yield: 98.5%):

[232]

[233] ^1H -NMR(300MHz, CDCl_3) δ (ppm) 8.15(1H,s), 7.84(1H,NH), 7.38-7.07(10H,m), 4.46(2H, $J=6.9\text{Hz}$,t), 4.00(1H,m), 3.92(2H,m), 3.22(2H, $J=6.9\text{Hz}$,t), 3.03(2H,m);

[234] MS(ESI), m/z : 459.2($[\text{M}+\text{H}]^+$)

[235]

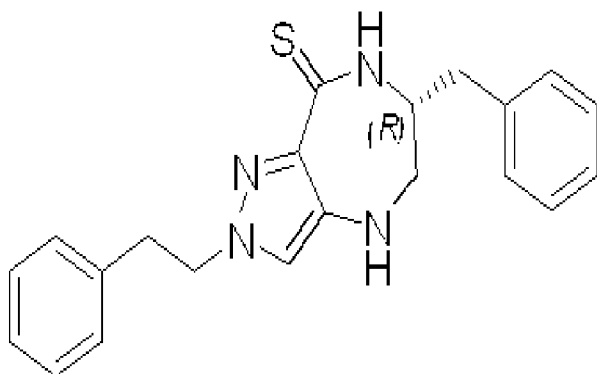
[236]

[237] **Example 2. (R)-6-benzyl-2-phenethyl-4,5,6,7-tetrahydropyrazolo[4,3-e][1,4]diazepin-8(2H)-thione (LDD847)**

[239]

[240] ChemistryFigure 9

[Chem.9]



[241]

[242] To prepare the purposed compound, after the similar method disclosed in Example 1 was performed, 1N-NaOH was added thereto to afford 10 mg of (R)-6-benzyl-2-phenethyl-4,5,6,7-tetrahydropyrazolo[4,3-e][1,4]diazepin-8(2H)-thione (LDD847) showing following physicochemical property (yield: 99%):

[243]

[244] $^1\text{H-NMR}$ (300MHz, CDCl_3)(ppm) 8.16 (1H, NH) 7.34-7.16 (10H, m), 6.80 (1H, s), 4.38 (2H, $J=7.5\text{Hz}$, t), 3.86 (1H, m), 3.41 (1H, $J=13.2\text{Hz}$, d), 3.25 (1H, m), 3.20 (2H, $J=7.5\text{Hz}$, t), 2.96 (2H, m);

[245] MS (ESI), m/z : 361.0 ($[\text{M-H}]^+$)

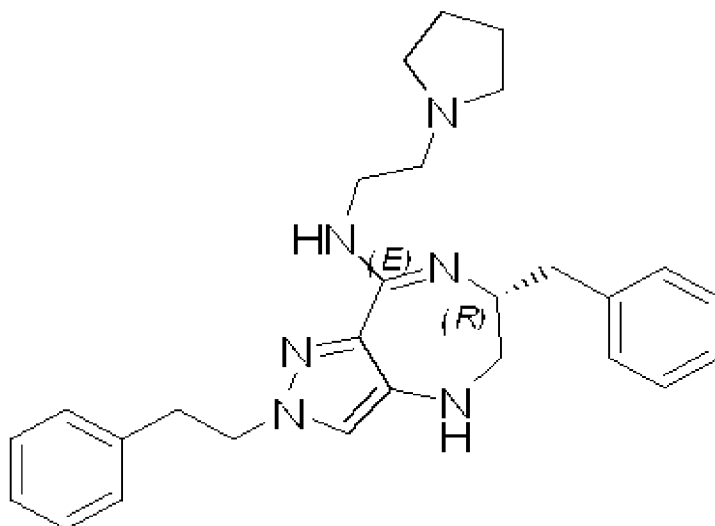
[246]

[247] **Example 3. (R,E)-6-benzyl-2-phenethyl-N-(2-(piperidin-1-yl)ethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-8(2H)-amine (LDD844)**

[249]

[250] ChemistryFigure 10

[Chem.10]



[251]

[252] To prepare the purposed compound, after the similar method disclosed in Example 1 was performed, the solution was reacted with 30% 2-piperidine ethyl amine in THF at room temperature to introduce 2-piperidine ethyl amine substituent and de-protection was performed. Finally, 14 mg of (R,E)-6-benzyl-2-phenethyl-N-(2-(piperidin-1-yl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-8-amine (LDD844) showing following physicochemical property (yield: 52.2%) was obtained:

[253]

[254] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm) 7.35-7.13(10H,m), 7.18(1H,s), 5.19(1H,NH),

4.38(1H,m), 4.30(2H,J=7.2Hz,t), 3.70(2H,brs), 3.43(1H,m), 3.21(2H,J=7.2Hz,t),
3.06(1H,J=13.5Hz,d), 2.89(2H,J=6.6Hz,J=13.2Hz,J=40.2Hz,ddd), 2.67(2H,brs)
1.53-1.48(5H,m);

[255] MS (ESI), m/z : 457.2 ([M+H]⁺)

[256]

[257]

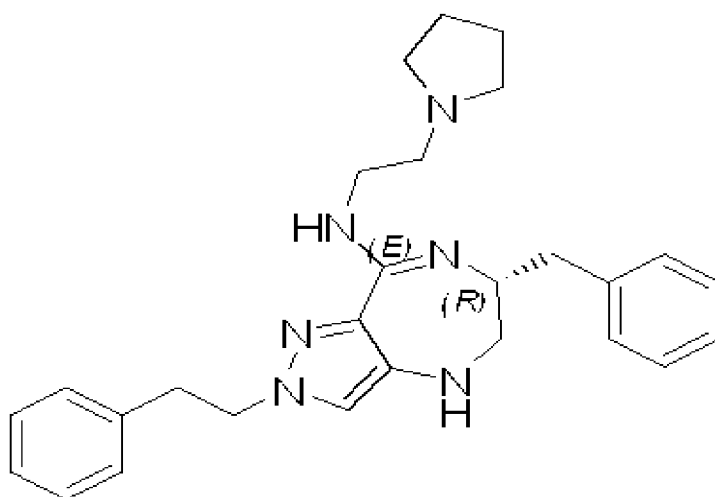
[258] **Example 4. (R,E)-6-benzyl-2-phenethyl-N-(2-(pyrrolidin-1-yl)ethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-8-amine (LDD845)**

[259]

[260]

[261] ChemistryFigure 11

[Chem.11]



[262]

[263] To prepare the purposed compound, after the similar method disclosed in Example 1 was performed, the solution was reacted with 30% 2-pyrrolidine ethyl amine in THF at room temperature for 12 hours to introduce 2-pyrrolidine ethyl amine substituent and de-protection was performed. Finally, 7 mg of (R,E)-6-benzyl-2-phenethyl-N-(2-(pyrrolidin-1-yl)ethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-8-amine (LDD845) showing following physicochemical property (yield: 36.8%) was obtained:

[264]

[265] ¹H-NMR (300MHz, CDCl₃) δ(ppm) 7.34-7.14 (10H,m), 7.17(1H,s), 5.13(1H,NH), 4.35(1H,m), 4.30(2H,J=7.2Hz,t), 3.79(2H,m), 3.41(1H,m), 3.20(2H,J=7.2Hz,t), 3.09(1H,J=10.8Hz,d), 2.99-2.78(5H,m), 2.64(3H,m), 1.78(4H,m);

[266] MS (ESI), m/z : 429.4 ([M+H]⁺)

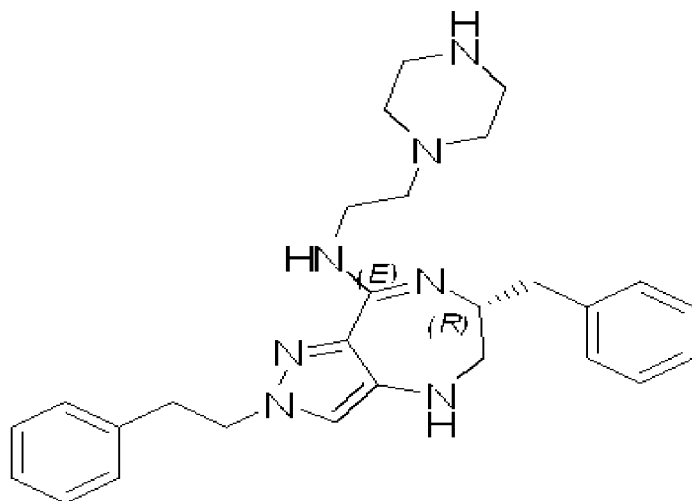
[267]

[268] **Example 5. (R,E)-6-benzyl-2-phenethyl-N-(2-(piperazin-1-yl)ethyl)-2,4,5,6-tet**
 [269] **rahydropyrazolo[4,3-e][1.4]diazepin-8-amine (LDD846)**

[270]

[271] ChemistryFigure 12

[Chem.12]



[272]

[273] To prepare the purposed compound, after the similar method disclosed in Example 1 was performed, the solution was reacted with 30% 2-piperazine ethyl amine in THF at room temperature for 12 hours to introduce 2-piperazine ethyl amine substituent and de-protection was performed. Finally, 5.5 mg of (R,E)-6-benzyl-2-phenethyl-N-(2-(piperazin-1-yl)ethyl)-2,4,5,6-tetrahydropyrazolo [4,3-e][1.4]diazepin-8-amine (LDD846) showing following physicochemical property (yield: 27.8%) was obtained:

[274]

[275] ¹H-NMR (300MHz, CDCl₃) δ(ppm) 7.36-7.12(10H,m), 7.05(1H,s), 4.40(2H,J=7.2Hz,t), 3.51(1H,m), 3.45(1H,m), 3.42(2H,J=6.3Hz,t), 3.21(2H,J=7.2Hz,t), 3.18-3.07(4H,m), 2.89(1H,m), 2.77(1H,m), 2.75-2.66(4H,m), 2.62(2H,J=6.3Hz,t);

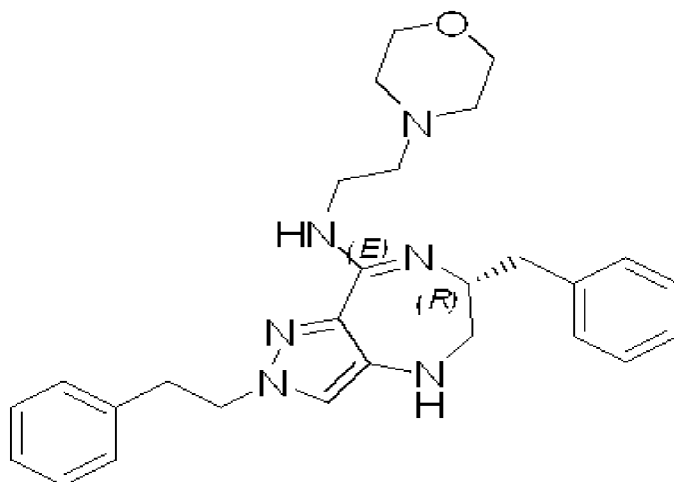
[276] MS (ESI), m/z : 458.3 ([M+H]⁺)

[277]

[278] **Example 6.**
(R,E)-6-benzyl-2-phenethyl-N-(2-morpholinethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-amine (LDD848)

[279] ChemistryFigure 13

[Chem.13]



[280]

[281] To prepare the purposed compound, after the similar method disclosed in Example 1 was performed, the solution was reacted with 30% 2-morpholine ethyl amine in THF at room temperature for 12 hours to introduce 2-morpholine ethyl amine substituent and de-protection was performed. Finally, 14 mg of (R,E)-6-benzyl-2-phenethyl-N-(2-morpholinethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-8-amine (LDD848) showing following physicochemical property (yield: 70.0 %) was obtained:

[282]

[283] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm) 7.37-7.07(10H,m), 7.04(1H,s), 4.56(1H,m), 4.32(2H,J=7.2Hz,t), 3.83(2H,m), 3.70(4H,brs), 3.42(1H,m), 3.21(2H,J=7.2Hz,t), 3.06(2H,brm), 2.77(1H,m), 2.70(2H,brm), 2.50(4H,brs);

[284] MS(ESI), m/z : 459.0([M-H] $^+$)

[285]

[286] **Example 7.**

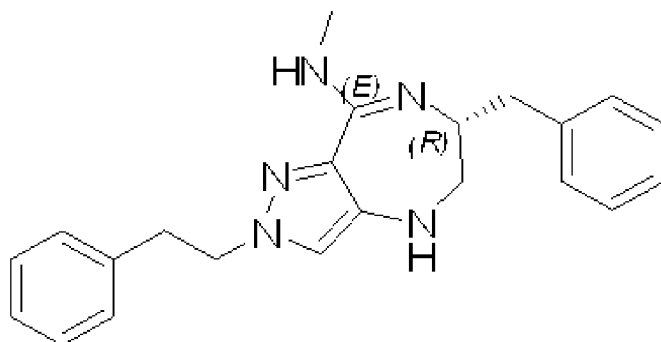
(R,E)-6-benzyl-N-methyl-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-8-amine (LDD849)

[287]

[288]

[289] ChemistryFigure 14

[Chem.14]



[290]

[291] To prepare the purposed compound, after the similar method disclosed in Example 1 was performed, the solution was reacted with 30% 2-morpholine ethyl amine in THF at room temperature for 12 hours to introduce 2-morpholine ethyl amine substituent at position 8 and de-protection was performed. Finally, 4.7 mg of (R,E)-6-benzyl-N-methyl-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-8-amine (LDD849) showing following physicochemical property (yield: 30.0 %) was obtained:

[292]

[293] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm) 7.35-7.07(10H,m), 6.94(1H,s), 4.65(1H,NH), 4.30(2H,J=7.2Hz,t), 4.10(1H,s), 3.40(3H,J=5.7Hz,d), 3.28(1H,m), 3.15(2H,J=7.2Hz,t), 2.99(1H,J=10.2Hz,d), 2.72(1H,J=10.2Hz,J=12.6Hz,dd);

[294] MS (ESI), m/z : 360.3 ($[\text{M}+\text{H}]^+$)

[295]

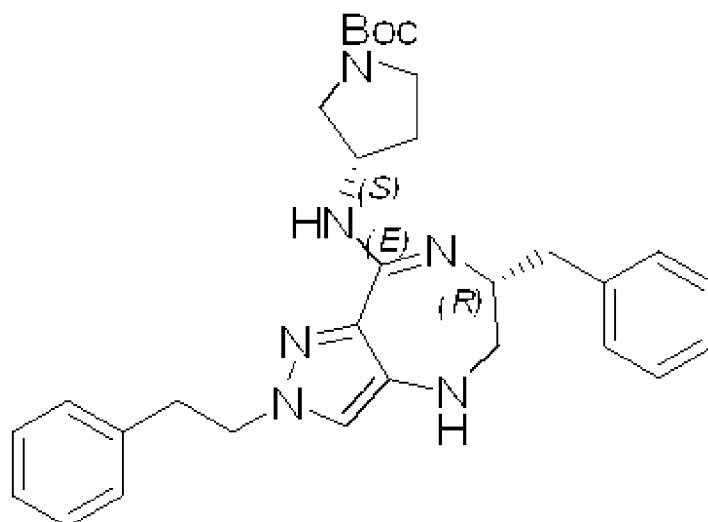
[296] **Example 8. (S)- *tert* -butyl-3-((R,E)-6-benzyl-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-8-amino)pyrrolidin-1-carboxylate(LDD850)**

[297]

[298] ChemistryFigure 15

[299]

[Chem.15]



[300]

[301] To prepare the purposed compound, after the similar method disclosed in Example 1 was performed, the solution was reacted with 30% 1-Boc pyrrolidine amine in THF at room temperature for 12 hours to introduce 1-Boc pyrrolidine amine substituent at position 8 and de-protection was performed. Finally,

[302] 5.6 mg of (S)-*tert*-butyl-3-((R,E)-6-benzyl-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-amino)pyrrolidin-1-carboxylate (LDD850) showing following physicochemical property (yield:25%) was obtained:

[303]

[304] ¹H-NMR (300MHz, CDCl₃) δ(ppm) 7.36-7.12(10H,m), 6.93(1H,s), 5.21(1H,NH), 4.68(1H,m), 4.33(2H,J=7.2Hz,t), 4.06(1H,m), 3.79(1H,m), 3.58(1H,m), 3.49(1H,m), 3.43(1H,m), 3.41(1H,m), 3.20(1H,m), 3.17(2H,J=7.2Hz,t), 3.08(1H,m), 3.04(1H,J=10.2Hz,d), 2.79(1H,J=10.2Hz,J=12.6Hz,dd), 2.04(1H,m), 1.82(1H,m), 1.48(9H,s);

[305] MS (ESI), m/z : 515.2 ([M+H]⁺)

[306]

[307]

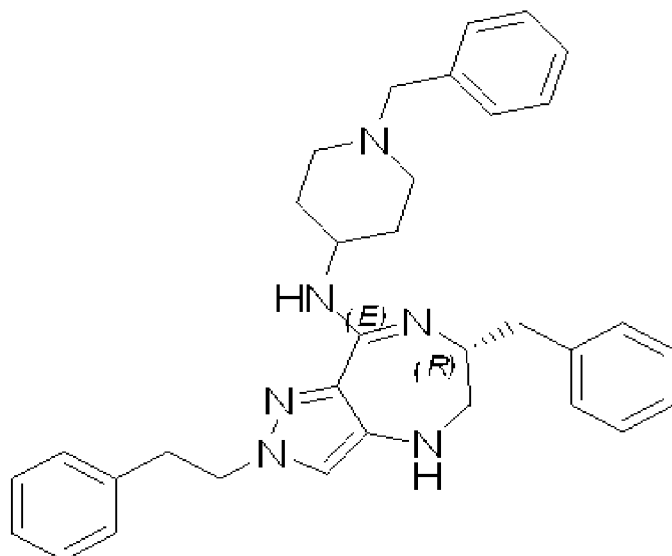
[308] **Example 9. ((R,E)-6-benzyl-N-(1-benzylpiperidin-4-yl)-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-amine (LDD854)**

[309]

[310] ChemistryFigure 16

[311]

[Chem.16]



[312]

[313] To prepare the purposed compound, after the similar method disclosed in Example 1 was performed, the solution was reacted with 30% 1-benzyl piperidine amine in THF at room temperature for 12 hours to introduce 1-benzyl piperidine amine substituent at position 8 and de-protection was performed. Finally, 15 mg of (R,E)-6-benzyl-N-(1-benzylpiperidin-4-yl)-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-amine (LDD854) showing following physicochemical property (yield: 69 %) was obtained:

[314]

[315] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm) 4.63(2H,m), 4.33(2H,J=7.2Hz,t), 4.11(1H,J=4.8Hz,d), 3.62(2H,s), 3.28(1H,m), 3.15(2H,J=7.2Hz,t), 3.11(1H,m), 3.04(1H,J=13.2Hz,d), 2.95(2H,brm), 2.72(1H,J=11.1Hz,J=13.2Hz,dd), 2.56(2H,brm), 2.17(2H,J=14.1Hz,m), 1.73(2H,brm);

[316] MS (ESI), m/z : 519.2 ($[\text{M}+\text{H}]^+$)

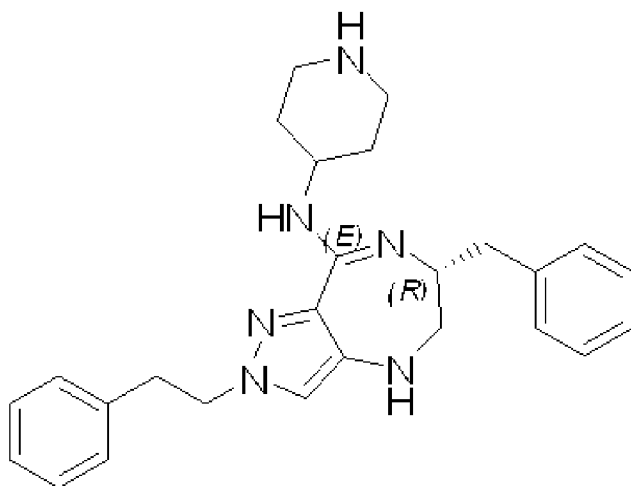
[317]

[318] **Example 10. (R,E)-6-benzyl-2-phenethyl-N-(piperidin-4-yl)-2,4,5,6-tetrahydro**
 [319] **pyrazolo[4,3-e][1.4]diazepin-8-amine (LDD1010)**

[320]

[321] ChemistryFigure 17

[Chem.17]



[322]

[323] To prepare the purposed compound, after the similar method disclosed in Example 1 was performed, the solution was reacted with 30% piperidine amine in THF at room temperature for 12 hours to introduce piperidine amine substituent at position 8 and de-protection was performed. Finally, 2 mg of (R,E)-6-benzyl-2-phenethyl-N-(piperidin-4-yl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-amine (LDD1010) showing following physicochemical property (yield: 11%) was obtained:

[324]

[325] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm) 7.39-7.11(10H,m), 6.94(1H,s), 4.63(2H,m), 4.33(2H,J=7.2Hz,t), 4.11(1H,J=4.8Hz,d), 3.28(1H,m), 3.15(2H,J=7.2Hz,t), 3.11(1H,m), 3.04(1H,J=13.2Hz,d), 2.95(2H,brm), 2.72(1H,J=11.1Hz,J=13.2Hz,dd), 2.56(2H,brm), 2.17(2H,J=14.1Hz,m), 1.73(2H,brm);

[326] MS (ESI), m/z : 429.2 ($[\text{M}+\text{H}]^+$)

[327]

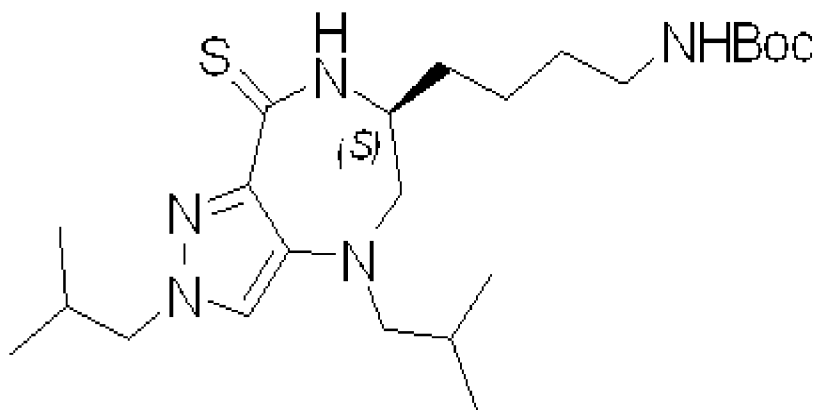
[328] **Example 11. (S)- *tert*-butyl-4-(2,4-diisobutyl-8-thioxo-2,4,5,6,7,8-hexahydropyrazolo[4,3-e][1.4]diazepin-6-yl)butylcarbamate(LDD1008)**

[329]

[330]

[331] ChemistryFigure 18

[Chem.18]



[332]

[333] 11-1. Preparation of pyrazolodiazepine-8-one intermediate (LDD1008-1)

[334] 6-benzyl-2-phenethyl-4,5,6,7-tetrahydropyrazolo[4,3-e][1,4]diazepin-8(2H)-one prepared in Reference Example 2 was modified and 800 mg of (S)-*tert*-butyl-4-(2,4-diisobutyl-8-oxo-2,4,5,6,7,8-hexahydropyrazolo[4,3-e][1,4]diazepin-6-yl)butylcarbamate of which C-2 position was introduced with isobutyl group and C-6 position was introduced with Boc-aminobutyl group was obtained (yield: 40%).

[335]

[336] 11-2. Preparation of (S)-*tert*-butyl-4-(2,4-diisobutyl-8-thioxo-2,4,5,6,7,8-hexahydropyrazolo[4,3-e][1,4]diazepin-6-yl)butylcarbamate (LDD 10 08)

[337] (S)-*tert*-butyl-4-(2,4-diisobutyl-8-oxo-2,4,5,6,7,8-tetrahydropyrazolo[4,3-e][1,4]diazepin-6-yl)butylcarbamate prepared in step 11-1, was dissolved in 1% AcOH in DCM and isobutylaldehyde was added to stir for 30 mins. NaB(OAc)₃H was added thereto to stir for 12 hours at roomtemperature and performed to reducing reaction in order to substituting C-4 position with isobutyl group. The solution was further reacted with Lawesson's reagent (227439;Aldrich Co.ltd) under toluene at 70°C for 2 hours to afford 586 mg of (S)-*tert*-butyl-4-(2,4-diisobutyl-8-thioxo-2,4,5,6,7,8-hexahydropyrazolo[4,3-e][1,4]diazepin-6-yl)butylcarbamate(LDD1008)showing following physicochemical property (yield: 67 %) was obtained:

[338]

[339] ¹H-NMR (300MHz, CDCl₃) δ(ppm)8.05(1H,NH), 6.78(1H,s), 4.56(1H,NH), 3.92(2H,J=7.5Hz,J=13.2Hz,J=25.8Hz,ddd), 3.60(1H,m), 3.32(2H,J=3.9Hz,d), 3.10(2H,m), 2.89(2H,m), 2.26(1H,m), 1.98(1H,m), 1.66(2H,m), 1.49(4H,m), 1.43(9H,s), 0.93(6H,J=6.3Hz,d), 0.90(6H,J=6.6Hz,d);

[340] MS (ESI), m/z : 450.2 ([M-H]⁺)

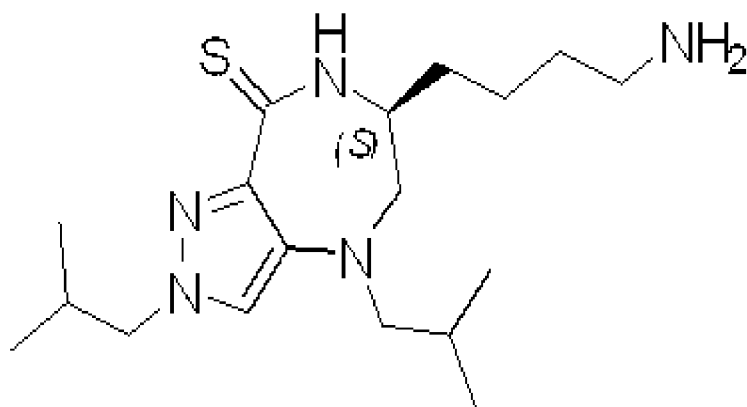
[341]

[342] **Example 12. (S)-6-(4-aminobutyl)-2,4-diisobutyl-4,5,6,7-tetrahydropyrazolo[4,**
 [343] **3-e][1.4]diazepin-8(2H)-thione (LDD998)**

[344]

[345] ChemistryFigure 19

[Chem.19]



[346]

[347] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 20% TFA in DCM

[348] at room temperature for 30 mins and de-protection was performed to remove Boc-substituent. Finally, 5.2 mg of

(S)-6-(4-aminobutyl)-2,4-diisobutyl-4,5,6,7-tetrahydropyrazolo[4,3-e][1.4]diazepin-8(2H)-thione (LDD998) showing following physicochemical property (yield: 45 %) was obtained:

[349]

[350] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm) 8.48(1H, NH), 6.83(1H, s), 3.93(2H, $J=7.5\text{Hz}$, $J=13.2\text{Hz}$, $J=25.8\text{Hz}$, ddd), 3.66(1H, m), 3.34(2H, $J=3.9\text{Hz}$, d), 2.90(2H, m), 2.80(2H, m), 2.28(1H, m), 2.01(1H, m), 1.66(2H, m), 1.49(4H, m), 0.93(6H, $J=6.3\text{Hz}$, d), 0.90(6H, $J=6.6\text{Hz}$, d);

[351] MS(ESI), m/z : 350.2([M+H] $^+$)

[352]

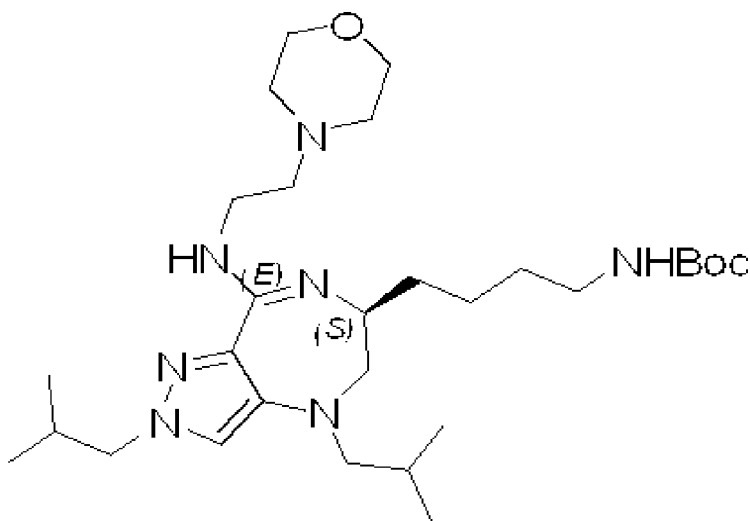
[353]

[354] **Example 13. (S,E)- *tert* -butyl-(2,4-diisobutyl-8-(2-morpholinoethylamino)-2,4,5,**
 [355] **,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-6-yl)-butylcarbamate(LDD1009)**

[356]

[357] ChemistryFigure 20

[Chem.20]



[358]

[359] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 30% 2-morpholinethylamine in THF at room temperature for 12 hours to introduce 2-morpholinethylamine substituent at position 8 and finally, 7 mg of (S,E)-*tert*-butyl-(2,4-diisobutyl-8-(2-morpholinoethylamino)-2,4,5,6-tetrahydropyr

[360] azolo[4,3-e][1,4]diazepin-6-yl)-butylcarbamate(LDD1009) showing following physicochemical property (yield: 58%) was obtained:

[361]

[362] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm)6.78(1H,s),4.56(1H,NH),3.92(2H,J=7.5Hz,J=13.2 Hz,J=25.8Hz,ddd),3.70(4H,brs),3.60(1H,m),3.32(2H,J=3.9Hz,d),3.10(2H,m),3.06(2H,brm),2.70(2H,brm),2.50(4H,brs),2.89(2H,m),2.26(1H,m),1.98(1H,m),1.66(2H,m),1.49 (4H,m),1.43(9H,s),0.93(6H,J=6.3Hz,d),0.90(6H,J=6.6Hz,d);

[363] MS (ESI), m/z : 548.0 ($[\text{M}+\text{H}]^+$)

[364]

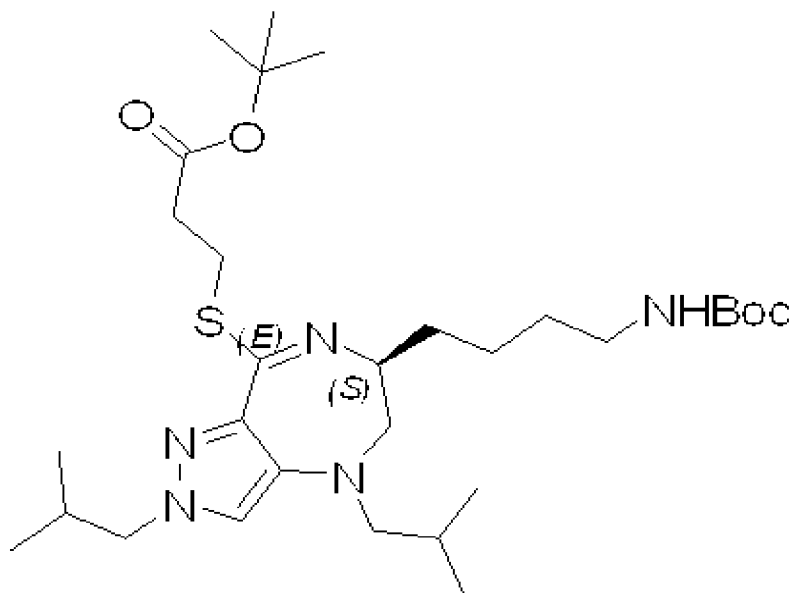
[365]

[366] **Example14. (S,E)- *tert* -butyl-3-(6-(4-(*tert* -butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-8-ylthio)propanoate (LDD1007)**

[368]

[369] ChemistryFigure 21

[Chem.21]



[370]

[371] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 3-bromo-*tert*-butylpropanoate(20equivalents) in DCM using by 1,8-diazabicyclo[5,4,0]undec-7-ene(BDU) as a base at 40°C for 24 hours to introduce *tert*-butyl propanoate substituent at position 8 and finally, 9 mg of (S,E)-*tert*-butyl-3-(6-(4-(*tert*-butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo

[372] [4,3-*e*][1.4]diazepin-8-ylthio)propanoate (LDD1007) showing following physico-chemical property (yield: 78%) was obtained:

[373]

[374] ¹H-NMR(300MHz, Acetone-d₆) δ(ppm) 7.11(1H, s), 5.83(1H, NH), 3.79(2H, J=7.2Hz, d), 3.56(1H, m), 3.22(1H, J=13.2Hz, d), 3.06(2H, m), 2.89(1H, m), 2.85(2H, J=2.7Hz, J=7.5Hz, dd), 2.52(2H, J=7.8Hz, t), 2.21(1H, m), 1.95(1H, m), 1.66(2H, m), 1.56(4H, m), 1.39(9H, s), 1.34(9H, s), 0.93(6H, J=6.3Hz, d), 0.90(6H, J=6.6Hz, d);

[375] MS (ESI), m/z : 580.3 ([M+H]⁺)

[376]

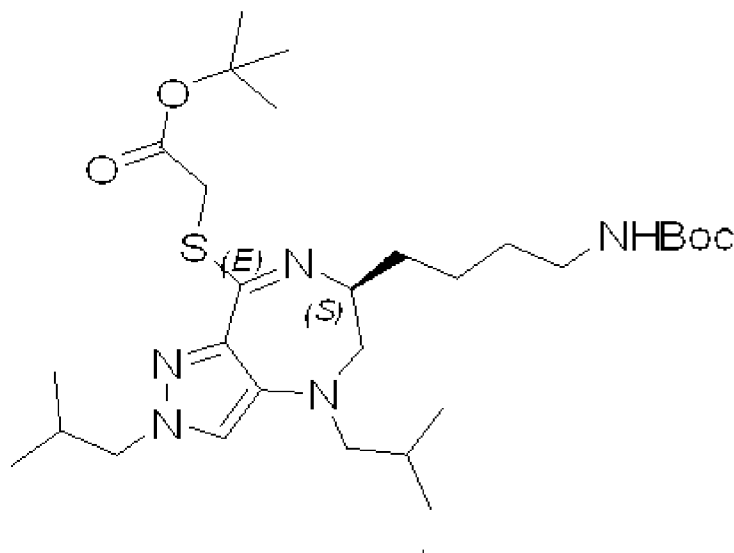
[377]

[378] **Example 15. (S,E)-*tert*-butyl-2-(6-(4-(*tert*-butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-*e*][1.4]diazepin-8-ylthio)acetate(LDD1006)**

[381]

[382] ChemistryFigure 22

[Chem.22]



[383] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 4 microgram of 2-bromo-*tert*-butylacetate(1 equivalent)in DCM using by BDU as a base at room temperature for 2 hours to introduce *tert*-butyl propionate substituent at position 8 and finally, 4 mg of (S,E)-*tert*-butyl-2-(6-(4-(*tert*-butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio) acetate (LDD1006) showing following physico-chemical property (yield: 66%) was obtained:

[384]

[385] $^1\text{H-NMR}$ (300MHz,CDCl₃) δ (ppm)6.71(1H,s),4.69(1H,NH),3.79(2H,J=7.5Hz,d),3.75(2H,J=15.6Hz,J=36.3Hz,q),3.58(1H,m),3.14(2H,m),3.13(1H,m),2.96(1H,m),2.80(2H,m),2.22(1H,m),1.93(1H,m),1.66(2H,m),1.56(4H,m),1.45(9H,s),1.44(9H,s),0.93(6H,J=6.3Hz,d),0.90(6H,J=6.6Hz,d);

[386] MS(ESI), m/z : 566.3([M+H]⁺)

[387]

[388]

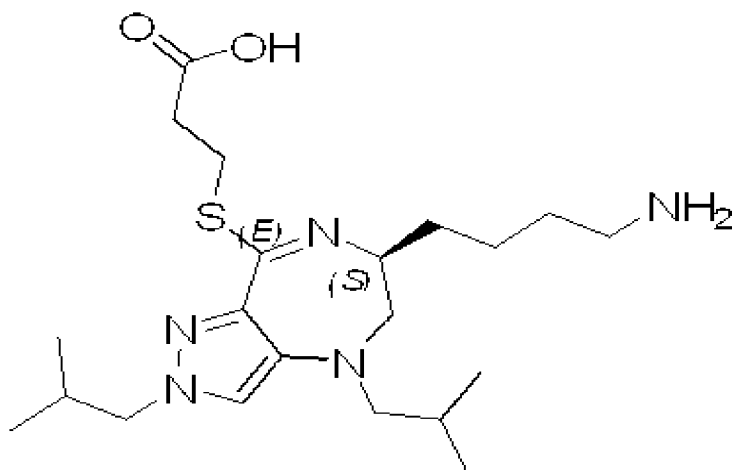
[389] **Example 16. (S,E)-3-(6-(4-aminobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio)propanoate (LDD997)**

[390]

[391] ChemistryFigure 23

[392]

[Chem.23]



[393]

[394] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 74 microliter of 3-bromo-*tert*-butyl propanoate in DCM using by BDU as a base at 40°C for 48 hours to introduce *tert*-butyl propanoate substituent at position 8 and then reacted with 50% TFA in DCM at room temperature for 1 hour. After the endof the reaction, the solvent was concentrated and the solution was allowed to resin column chromatography (SAX:strong anion exchanger resin) to remove TFA salt. Finally, 7 mg of (S,E)-3-(6-(4-aminobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio)propanoate (LDD997) showing following physicochemical property (yield: 99 %) was obtained:

[395]

[396] ¹H-NMR(300MHz,CDCl₃)δ(ppm)6.72(1H,s),3.83(2H,J=6.6Hz,d),3.57(1H,m),3.27(2H,brn),3.14(2H,J=12.9Hz,d),2.98(2H,m),2.81(2H,m),2.60(2H,brn),2.21(1H,m),1.95(1H,m),1.66(2H,m),1.56(4H,m),0.93(6H,J=6.3Hz,d),0.90(6H,J=6.6Hz,d);

[397] MS (ESI), m/z : 422.3 ([M-H]⁺)

[398]

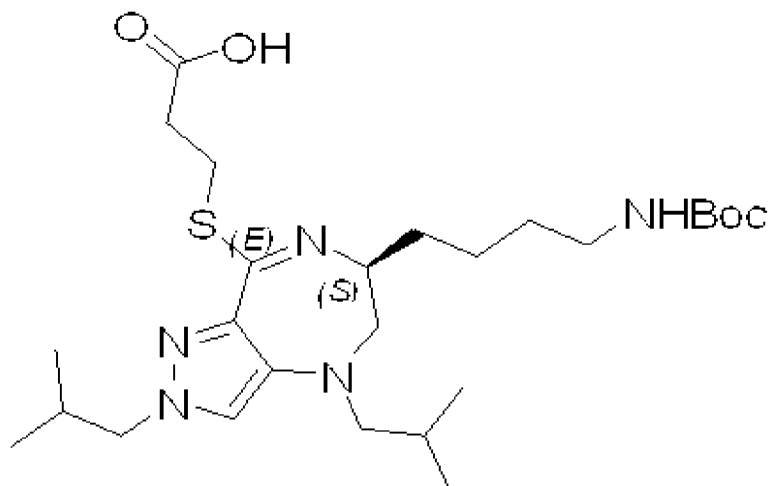
[399]

[400] **Example 17. (S,E)-3-(6-(4-(*tert*-butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio)propanoate(LDD1005)**

[402]

[403] ChemistryFigure 24

[Chem.24]



[404]

[405] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 33 mg of 3-bromo-*tert*-butyl propionate (10 equivalents) in DCM using by BDU as a base at roomtemperature for 12 hours to introduce propionate substituentat position 8 and finally, 10 mg of (S,E)-3-(6-(4-(*tert*-butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio)propanoate

[406] (LDD1005) showing following physicochemical property (yield: 87%) was obtained:

[407]

[408] ¹H-NMR(300MHz,CDCl₃)δ(ppm)6.82(1H,s),4.98(1H,NH),3.97(1H,m),3.83(2H,J=7.2Hz,d),3.27(4H,brm),3.12(2H,brm),2.89(4H,m),2.21(1H,m),2.01(1H,m),1.66(2H,m),1.56(4H,m),1.43(9H,s),0.93(6H,J=6.3Hz,d),0.90(6H,J=6.6Hz,d);

[409] MS (ESI), m/z : 522.3 ([M-H]⁺)

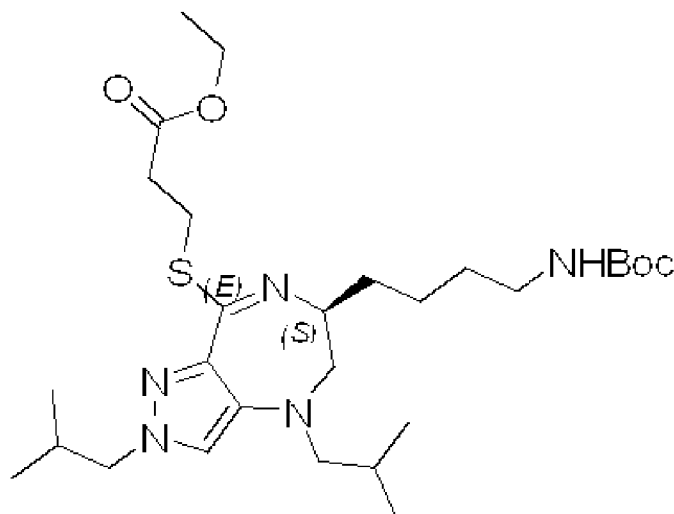
[410]

[411] **Example 18. (S,E)-ethyl-3-(6-(4-(*tert* -butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio)propanoate(LDD1004**

[412] **)**

[414] ChemistryFigure 25

[Chem.25]



[415]

[416] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 60 microgram of 2-bromo-ethyl propanoate (5 equivalents) in DCM using by BDU as a base at room temperature for 12 hours to introduce ethyl propanoate substituent at position 8 and finally, 20 mg of (S,E)-ethyl-3-(6-(4-(*tert*-butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydro pyrazolo[4,3-e][1.4]diazepin-8-ylthio)propanoate(LDD1004) showing following physicochemical property (yield: 64%) was obtained:

[417]

[418] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm)6.72(1H,s),4.70(1H,NH),4.16(2H,J=7.2Hz,q),3.83(2H,J=7.2Hz,d),3.63(1H,m),3.27(5H,brn),3.09(1H,m),2.81(2H,J=5.7Hz,t),2.68(2H,J=7.5Hz,t),2.18(1H,m),1.94(1H,m),1.66(2H,m),1.56(4H,m),1.37(9H,s),1.24(3H,J=7.2Hz,t),0.93(6H,J=6.3Hz,d),0.90(6H,J=6.6Hz,d);

[419] MS(ESI), m/z : 552.3([M+H] $^+$)

[420]

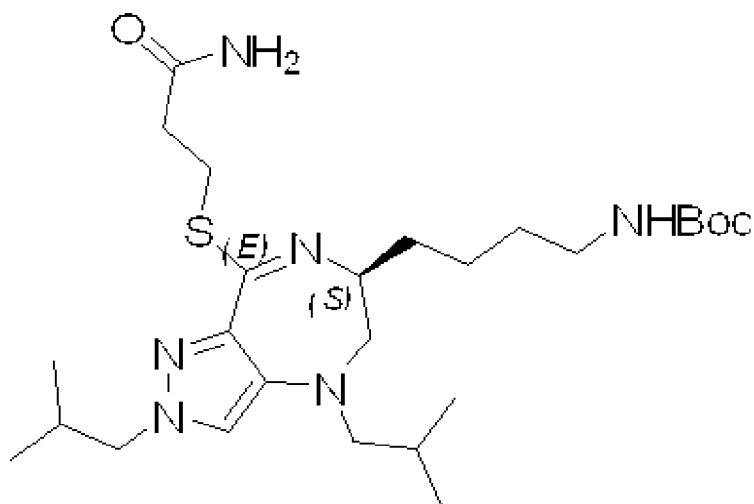
[421]

[422] **Example 19. (S,E)- *tert* - butyl-4-(8-(3-amino-3-oxopropylthio)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-6-yl)butylcarbamate(LDD1003)**

[423]

[424] ChemistryFigure 26

[Chem.26]



[425]

[426] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 33 mg of 3-bromo-propionate (10 equivalents) in DCM using by BDU as a base at room temperature for 12 hours to introduce propionate substituent at position 8 and reacted with HATU (2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate Methanaminium) used as a coupling reagent under 2 M ammonia at room temperature for 12 hours to afford 5.2 mg of (S,E)-*tert*-

[427] o[4,3-e][1.4]diazepin-6-yl)butylcarbamate(LDD1003) showing following physico-chemical property (yield: 88%) was obtained:

[428]

[429] ¹H-NMR(300MHz,CDCl₃)δ(ppm)6.72(1H,s),4.72(1H,NH),3.83(2H,J=7.2Hz,d),3.63(1H,m),3.27(2H,m),3.13(3H,m),2.97(1H,m),2.81(2H,m),2.64(2H,m),2.22(1H,m),1.94(1H,m),1.63-1.46(6H,brm),1.43(9H,s),0.93(6H,J=6.3Hz,d),0.90(6H,J=6.6Hz,d);

[430] MS (ESI), m/z : 545.0 ([M+Na]⁺)

[431]

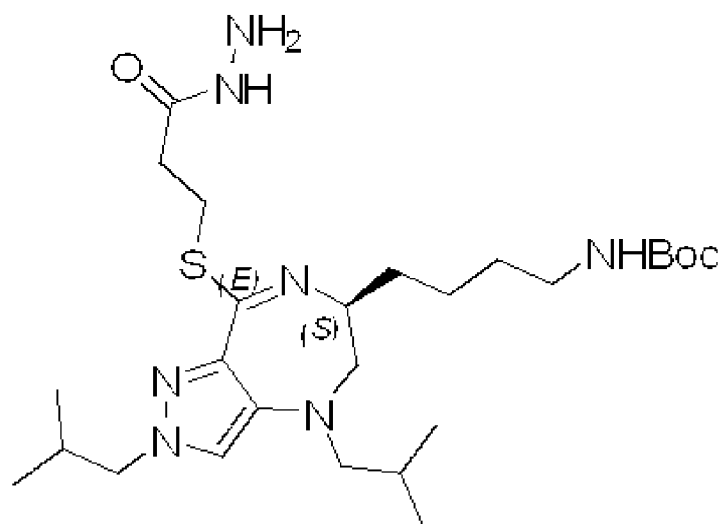
[432]

[433] **Example 20. (S,E)-*tert*-butyl-4-(8-(3-hydrazinyl-3-oxopropylthio)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-6-yl)butylcarbamate(LDD1002)**

[436]

[437] ChemistryFigure 27

[Chem.27]



[438]

[439] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 60 microgram of 2-bromo-ethyl propanoate (5 equivalents) in DCM using by BDU as a base at room temperature for 4 hours to introduce ethyl propanoate substituent at position 8 and reacted with 30% hydrazine hydrate in ethanol at room temperature for 12 hours to introduce propane hydrazide substituent at position 8. Finally, 3.6 mg of (S,E)-tert - butyl-4-(8-(3-hydrazinyl-3-oxopropylthio)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-6-yl)butylcarbamate (LDD1002) showing following physico-chemical property (yield: 63%) was obtained:

[440]

[441] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm)6.72(1H,s),4.80(1H,NH),3.90(2H,NH),3.83(2H,J=7.2Hz,d),3.63(1H,m),3.27(2H,J=7.2Hz,t),3.20(3H,m),3.02(1H,m),2.81(2H,m),2.62(2H,m),2.17(1H,m),1.91(1H,m),1.63-1.46(6H,brm),1.43(9H,s),0.93(6H,J=6.3Hz,d),0.90(6H,J=6.6Hz,d);

[442] MS(ESI), m/z : 538.3([M+H]⁺)

[443]

[444]

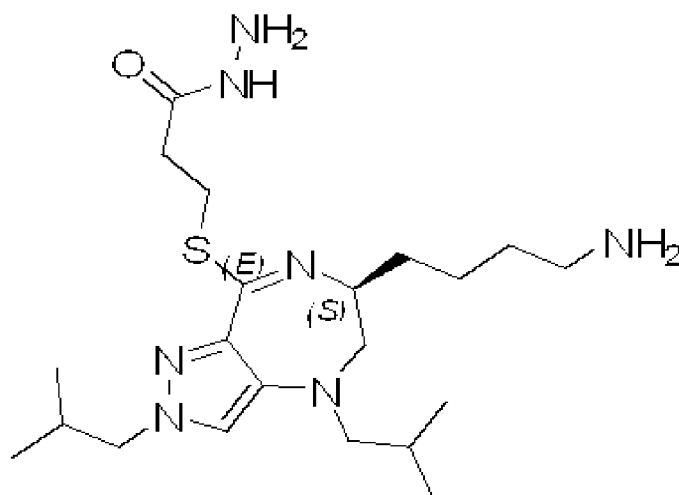
[445] **Example 21. (S,E)-3-(6-(4-aminobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepin-8-ylthio)propanehydrazide (LDD1001)**

[446]

[447]

[448] ChemistryFigure 28

[Chem.28]



[449]

[450] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 60 microgram of 3-bromo-ethyl propanoate (5 equivalents) in DCM using by BDU as a base at room temperature for 4 hours to introduce ethyl propanoate substituent at position 8 and reacted with 30% hydrazine hydrate in ethanol at room temperature for 4 hours to introduce propane hydrazide substituent at position 8. The solution was reacted with 20% TFA in DCM at room temperature for 30 mins. After the end of the reaction, the solvent was concentrated and the solution was allowed to resin column chromatography (SAX: strong anion exchanger resin) to remove TFA salt. Finally, 2 mg of (S,E)-3-(6-(4-aminobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio)propanehydrazide (LDD1001) showing following physicochemical property (yield: 71 %) was obtained:

[451]

[452] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm)6.72(1H,s),3.90(2H,NH),3.83(2H,J=7.2Hz,d),3.63(1H,m),3.27(2H,J=7.2Hz,t),3.20(3H,m),3.02(1H,m),2.81(2H,m),2.62(2H,m),2.17(1H,m),1.91(1H,m),1.63-1.46(6H,brm),0.93(6H,J=6.3Hz,d),0.90(6H,J=6.6Hz,d);

[453] MS(MALDI), m/z : 438.5([M+H] $^+$)

[454]

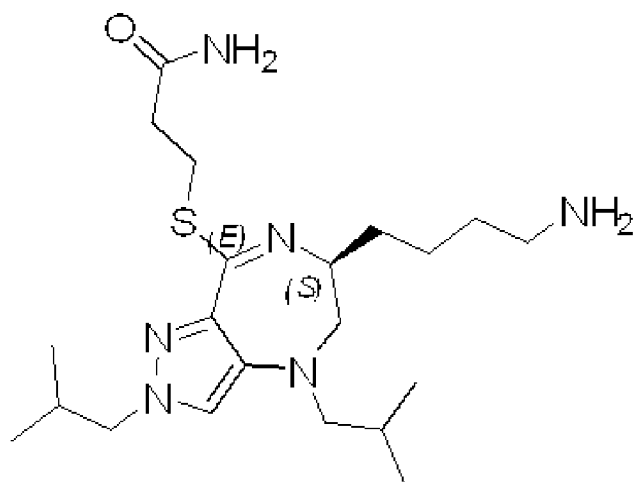
[455]

[456] **Example 22. (S,E)-3-(6-(4-aminobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio)propanamide (LDD1000)**

[457]

[458] ChemistryFigure 29

[Chem.29]



[459]

[460] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, the solution was reacted with 33 mg of 3-bromo-propionate (10 equivalents) in DCM using by BDU as a base at room temperature for 12 hours to introduce propionate substituent at position 8 and reacted with HATU (2-(1H-7-Azabenzotriazol-1-yl)--1,1,3,3- tetramethyl uronium hexafluorophosphate Methanaminium) used as a coupling reagent under 2 M ammonia in dioxane at room temperature for 12 hours to introduce 3-amino-3-oxopropyl substituent at position 8. The solution was reacted with 20% TFA in DCM at room temperature for 30 mins. After the end of the reaction, the solvent was concentrated and the solution was allowed to resin column chromatography (SAX: strong anion exchanger resin) to remove TFA salt. 4 mg of (S,E)-3-(6-(4-aminobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio)propanamide (LDD1000) showing following physicochemical property (yield: 80 %) was obtained:

[461]

[462] ¹H-NMR(300MHz,CDCl₃)δ(ppm)6.72(1H,s),6.67(1H,NH),5.99(1H,NH),3.83(2H,J=7.2Hz,d),3.63(1H,m),3.27(2H,m),3.15(1H,J=6.3Hz,d),2.97(1H,m),2.81(2H,m),2.64(2H,m),2.22(1H,m),1.94(1H,m),1.63-1.46(6H,brm),0.93(6H,J=6.3Hz,d),0.90(6H,J=6.6Hz,d);

[463] MS(MALDI), m/z : 423.3([M+H]⁺)

[464]

[465]

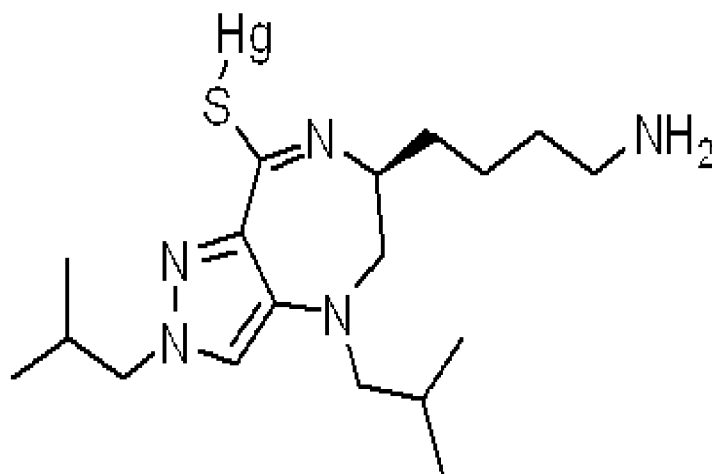
[466] **Example 23. (S,E)-3-(6-(4-aminobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio)mercury (LDD887)**

[467]

[468]

[469] ChemistryFigure 30

[Chem.30]



[470]

[471] To prepare the purposed compound, after the similar method disclosed in Example 11-2 was performed, (S)-tert-butyl-4-(2,4-diisobutyl-8-thioxo-2,4,5,6,7,8-hexahydropyrazolo[4,3-e][1.4]diazepin-6-yl)butylcarbamate was dissolved in ethanol and reacted with 1.2 equivalents of mercury chloride (HgCl_2) at room temperature for 2 hours. After the end of the reaction, the remaining mercury was removed with filtering and the solvent was removed with evaporator. The resultant was reacted with 20% TFA in DCM to de-protect Boc-substituent to afford 20 mg of (S,E)-3-(6-(4-aminobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1.4]diazepin-8-ylthio) mercury (LDD887) showing following physicochemical property (yield: 78 %) was obtained:

[472]

[473] $^1\text{H-NMR}$ (300MHz, CDCl_3) δ (ppm) 7.14(1H,s), 4.10(1H,m), 4.01(2H, $J=7.5\text{Hz}$, d), 3.34(2H,m), 3.10(2H,m), 2.91(2H,m), 2.28(1H,m), 2.01(1H,m), 1.66(2H,m), 1.49(4H,m), 0.93(6H, $J=6.3\text{Hz}$, d), 0.90(6H, $J=6.6\text{Hz}$, d);

[474] MS(ESI), m/z : 350.6([$\text{M}+\text{H}$] $^+$)

[475]

[476] **Experimental Example 1. Inhibition test of transglutaminase activity (In vitro)**

[477]

[478] To determine the inhibitory effect of the inventive compounds prepared in Examples on the activity of transglutaminase, the test for determining the effect of transglutaminase on the binding activity of [$1,4\text{-}^{14}\text{C}$] putrescine to succinylated casein was de-

terminated according to the modified method disclosed in the literature (Sohn,J.,Kim,T.I.,Yoon,Y.H.,and Kim,S.Y,: Transglutaminase inhibitor; a new an-inflammatory approach in allergic conjunctivitis, *J. Clin. Invest.* 111, pp121-8, 2003).

[479]

[480] 1g of succinylated casein procured from Calbiochem (Cat. No. 573464) was dissolved in 50 ml of reaction buffer solution (0.1 M tris-acetate (pH 8.0), 10 mM CaCl_2 , 0.15 M NaCl, 1.0 mM EDTA) containing 5 mM DTT(DL-Dithiothreitol) and stored at -80°C before the use. The stock solution of [1,4- ^{14}C] putrescine procured from GE healthcare (Cat. No. CFA301) was diluted to the extent that the radiological dosage reached to 5 microCi/ml. Transglutaminase procured from Sigma-Aldrich (Cat. No. T5398) was diluted with distilled water to the extent that the final concentration reached to 1 unit/ml. The stock solution of the inventive compounds prepared in Examples was prepared by dissolving in DMSO at the concentration of 10 mM and was diluted with DMSO to make various concentration of test sample.

[481] 450 microliter of succinylated casein solution and 50 microliter of [1,4- ^{14}C] putrescinedihydrochloride solution were mixed together to use as a substrate. Each sample was mixed with 96 microliter of reaction buffer solution, 3 microliter of selphalab stock solution and 1 microliter of transglutaminase stock solution and incubated for 10 mins at 37°C . 500 microliter of substrate solution β and 100 microliter of sample solution mixed together and the mixture was incubated for 1 hour at 37°C . The reaction was stopped by adding 4.5 ml of cold 7.5% TCA thereto and the solution was store for 1 hour at 4°C . TCA-protein precipitate was filtered with GF/glass fiber filter, washed with cold 7.5% TCA and dried. The radioactivity of the cross-linked protein was determined using by Liquid scintillation counter (LS-6500, Beckman Coulter) and the data was corrected by using DMSO-control group as a standard.

[482] The determined data was transformed into the activity of transglutaminase and the test was repeated three times. The inhibitory activity of each compound on the activity of transglutaminase was shown in following Tables 1 to 11. IC_{50} was calculated according to generally used non-linear regression method.

[483]

[484] Table 1

[Table 1]

[Table]

Concentration	Mean	SD(Standard deviation)
0 nM	1.000	0.000
50 nM	0.699	0.090
100 nM	0.358	0.040
250 nM	0.092	0.031
500 nM	0.005	0.002
IC ₅₀ = 75.5 ±1..3 nM P<0.0001 R= 0.9999		

[485]

[486] Table 2

[Table 2]

[Table]

Concentration	Mean	SD(Standard deviation)
0 nM	1.000	0.000
20 nM	1.118	0.061
100 nM	1.008	0.120
500 nM	1.111	0.079
2,500 nM	1.174	0.106
10,000 nM	1.119	0.050

[487]

[488] Table 3

[Table 3]

[Table]

Concentration	Mean	SD(Standard deviation)
0 nM	1.000	0.000
20 nM	1.015	0.078
100 nM	1.027	0.065
500 nM	1.148	0.057
2,500 nM	1.047	0.046
10,000 nM	1.094	0.071

[489]

[490] Table 4

[Table 4]

[Table]

Concentration	Mean	SD(Standard deviation)
0 nM	1.000	0.000
20 nM	1.007	0.082
100 nM	1.050	0.024
500 nM	1.042	0.052
2,500 nM	0.980	0.075
10,000 nM	0.805	0.112

[491]

[492] Table 5

[Table 5]

[Table]

Concentration	Mean	SD(Standard deviation)
0 nM	1.000	0.000
20 nM	0.932	0.091
100 nM	1.006	0.046
500 nM	0.961	0.066
2,500 nM	0.835	0.085
10,000 nM	0.438	0.055
IC ₅₀ = 8.34 ±0.80 microM P= 0.0005 R= 0.9995 R ² = 0.9990		

[493]

[494] Table 6

[Table 6]

[Table]

Concentration	Mean	SD(Standard deviation)
0 nM	1.000	0.000
20 nM	0.969	0.091
100 nM	1.006	0.134
500 nM	1.029	0.028
2,500 nM	1.059	0.088
10,000 nM	0.853	0.074

[495]

[496] Table 7

[Table 7]

[Table]

Concentration	Mean	SD(Standard deviation)
0 nM	1.000	0.000
20 nM	0.998	0.010
100 nM	0.986	0.113
500 nM	1.000	0.098
2,500 nM	1.007	0.032
10,000 nM	0.786	0.067

[497]

[498] Table 8

[Table 8]

[Table]

Concentration	Mean	SD(Standard deviation)
0 microM	1.000	0.000
0.2 microM	0.957	0.082
1 microM	0.921	0.089
5 microM	0.873	0.044
25 microM	0.457	0.027
IC ₅₀ = 22.1 ±2.9 microM P= 0.0046 R= 0.9993 R ² = 0.9987		

[499]

[500]

[501] Table 9

[Table 9]

[Table]

Concentration	Mean	SD(Standard deviation)
0 microM	1.000	0.000
0.2 microM	0.932	0.095
1 microM	0.879	0.085
5 microM	0.858	0.057
25 microM	0.784	0.035

[502]

[503]

[504] Table 10

[Table 10]

[Table]

Concentration	Mean	SD(Standard deviation)
0 microM	1.000	0.000
0.2 microM	1.040	0.144
1 microM	1.115	0.025
5 microM	0.916	0.091
25 microM	0.288	0.021
IC ₅₀ = 16.28 ± 2.89 microM P= 0.0111 R= 0.9825 R ² = 0.9652		

[505]

[506] Table 11

[Table 11]

[Table]

Concentration	Mean	SD(Standard deviation)
0 microM	1.000	0.000
0.2 microM	0.907	0.063
1 microM	1.133	0.069
5 microM	1.016	0.054
25 microM	0.373	0.059
IC ₅₀ = 24.07 microM		

[507]

[508]

[509] At the result, the inventive compounds, especially, compound LDD-887 (IC₅₀: 75.5±1.3 nM), compound LDD-998(IC₅₀: 8.34±0.80 microM-without DTT), compound LDD-1000(IC₅₀: 22.1±2.9 microM-DTT w/o before activation), and compound LDD-1001(IC₅₀: 24.07 microM-DTT w/o before activation), inhibited the activity of transglutaminase in a dose dependent manner.

[510]

[511]

[512] Hereinafter, the formulating methods and kinds of excipients will be described, but the present invention is not limited to them. The representative preparation examples were described as follows.

[513]

[514] The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

[515]

[516] Hereinafter, the formulating methods and kinds of excipients will be described, but the present invention is not limited to them. The representative preparation examples were described as follows.

[517]

[518] Preparation of powder

[519] Compound (LDD-997) 20mg

[520] Lactose 100mg

[521] Talc 10mg

[522] Powder preparation was prepared by mixing above components and filling sealed package.

[523]

[524] Preparation of tablet

[525] Compound (LDD-997) 10mg

[526] Corn Starch 100mg

[527] Lactose 100mg

[528] Magnesium Stearate 2mg

[529] Tablet preparation was prepared by mixing above components and entabletting.

[530]

[531] Preparation of capsule

[532] Compound (LDD-887) 10mg

[533] Corn starch 100mg

[534] Lactose 100mg

[535] Magnesium Stearate 2mg

[536] Tablet preparation was prepared by mixing above components and filling gelatin capsule by conventional gelatin preparation method.

[537]

[538] Preparation of injection

[539] Compound (LDD-1000) 10mg

[540] Distilled water for injection optimum

[541] amount

[542] pH controller optimum

[543] amount

[544] Injection preparation was prepared by dissolving active component, controlling pH to about 7.5 and then filling all the components in 2 ml ample and sterilizing by conventional injection preparation method.

[545]

[546] The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

[547]

[548]

[549]

[550]

[551]

[552]

[553]

Industrial Applicability

[554] As described in the present invention, the novel pyrazolodiazepine compounds of the present invention potently inhibit the activity of transglutaminase in a dose dependent manner. Accordingly, it has been confirmed that the inventive compounds show potent inhibiting effect on the hyper-activated transglutaminase when the transglutaminase is over-expressed. Therefore the compounds can be useful in treating or preventing the disease caused by increased activation of transglutaminase.

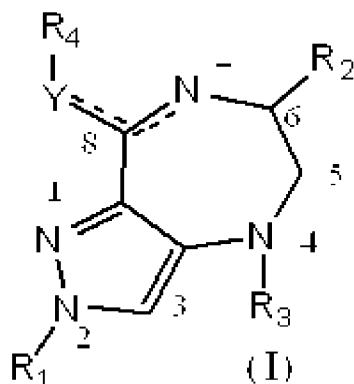
[555]

[556]

Claims

[Claim 1]

A novel pyrazolodiazepine compounds represented by the following general formula (I), the isomers thereof, and the pharmaceutically acceptable salt thereof:



wherein

Y is S, S-R₄ or N-R₄;

R₁ is a hydrogen atom, C₁-C₆ alkyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom;

R₂ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R', benzyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ hetero aryl group having at least one hetero atom selected from O, N or S, or halogen atom, where in R' is protected or non-protected with an amine group;

R₃ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R'', phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R'' is a ketone group or ester group substituted with C₁-C₆ alkyl group substituted with at least one halogen atom;

R₄ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with C₅-C₁₀ heteroaryl group having at least one hetero atom selected from O, N or S which is substituted or un-substituted with protective group or benzyl group, C₁-C₆ alkyl group substituted or un-substituted with at least one R'', wherein R'' is a ketone group, ester group, amide group or carboxylic acid substituted with at least one selected from a hydrogen atom, halogen atom, amine group or C₁-C₆ alkyl group;

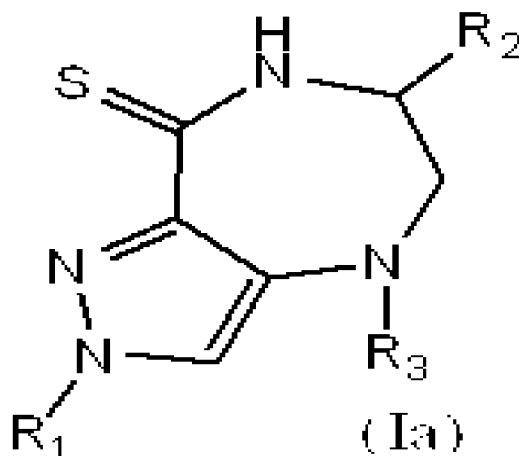
(-----) denotes a single bond or double bond.

[Claim 2]

The compound according to claim 1,
 wherein R₁ is hydrogen atom, straight or branched butyl or pentyl group or phenethyl group; R₂ is a hydrogen atom, C₄-C₆ alkyl group substituted with Boc'-protected or non-protected amine group, benzyl group or phenethyl group; R₃ is a hydrogen atom, straight or branched butyl or pentyl group substituted or un-substituted with ketone group where in ketone group is substituted or un-substituted with C₁-C₂ alkyl group substituted with at least one halogen atom; R₄ is a hydrogen atom, C₂-C₆ alkyl group substituted or un-substituted with heteroaryl group selected from piperidine, pyrrolidine, piperazine, pyrazole, or morpholine which is substituted or un-substituted with Boc'-protective group or benzyl group, C₂-C₆ alkyl group substituted with ketone group, ester group, amide group or carboxylic acid substituted with at least one selected from a hydrogen atom, halogen atom, amine group or C₁ - C₆ alkyl group.

[Claim 3]

The compound according to claim 1,
 Wherein said compound is a novel pyrazolodiazepine-8(2H) thione compounds represented by the following general formula (Ia), the isomers thereof, and the pharmaceutically acceptable salt thereof:



R₁ is a hydrogen atom, C₁-C₆ alkyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom;

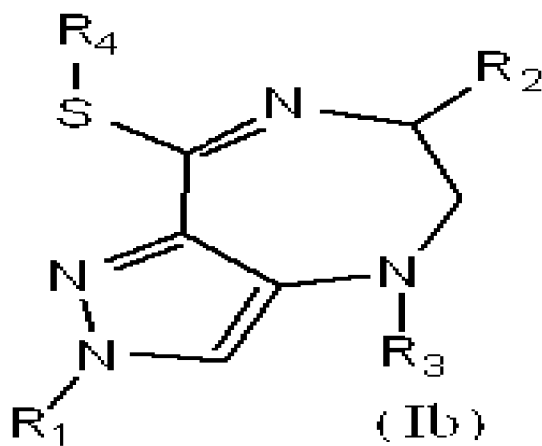
R₂ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R', benzyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one hetero atom selected from O, N or S, or halogen atom, wherein R' is protected or non-protected with an amine group;

R₃ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R'', phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R'' is a ketone group or ester group substituted with C₁-C₆ alkyl group substituted with at least one halogen atom.

[Claim 4]

The compound according to claim 1,

Wherein said compound is a novel pyrazolodiazepine-8-yl thio compounds represented by the following general formula (Ib), the isomers thereof, and the pharmaceutically acceptable salt thereof:



R₁ is a hydrogen atom, C₁-C₆ alkyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom;

R₂ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R', benzyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R' is protected or non-protected with an amine group;

R₃ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R'', phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R'' is a ketone group or ester group substituted with C₁-C₆ alkyl group substituted with at least one halogen atom;

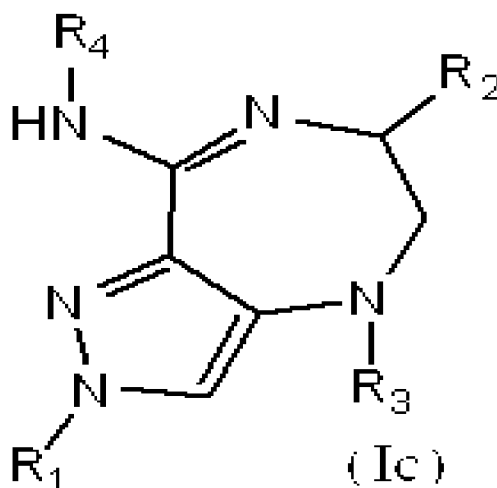
R₄ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S which is substituted or un-substituted with protective group or benzyl group, C₁-C₆ alkyl group substituted or un-substituted

with at least one R'', wherein R'' is ketone group, ester group, amide group or carboxylic acid substituted with at least one selected from a hydrogen atom, halogen atom, amine group or C₁-C₆ alkyl group.

[Claim 5]

The compound according to claim 1,

Wherein said compound is a novel pyrazolodiazepine-8-amine compounds represented by the following general formula (Ic), the isomers thereof, and the pharmaceutically acceptable salt thereof:



R₁ is a hydrogen atom, C₁-C₆ alkyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one hetero atom selected from O, N or S, or halogen atom;

R₂ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R', benzyl group, phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R' is protected or non-protected with an amine group;

R₃ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with R'', phenethyl group, C₅-C₁₀ aryl group, C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S, or halogen atom, wherein R'' is a ketone group or ester group substituted with C₁-C₆ alkyl group substituted with at least one halogen atom;

R₄ is a hydrogen atom, C₁-C₆ alkyl group substituted or un-substituted with C₅-C₁₀ heteroaryl group having at least one heteroatom selected from O, N or S which is substituted or un-substituted with protective group or benzyl group, C₁-C₆ alkyl group substituted or un-substituted with at least one R'', wherein R'' is ketone group, ester group, amide group or carboxylic acid substituted with at least one selected from a

[Claim 6]

hydrogen atom, halogen atom, amine group or C₁-C₆ alkyl group;
 The compound according to any one among claims 3 to 5,
 Wherein R₁ is hydrogen atom, straight or branched butyl or pentyl group or phenethyl group; R₂ is a hydrogen atom, C₄-C₆ alkyl group substituted with Boc'-protected or non-protected amine group, benzyl group or phenethyl group; R₃ is a hydrogen atom, straight or branched butyl or pentyl group substituted or un-substituted with ketone group wherein ketone group is substituted or un-substituted with C₁-C₂ alkyl group substituted with at least one halogen atom; R₄ is a hydrogen atom, C₂-C₆ alkyl group substituted or un-substituted with heteroaryl group selected from piperidine, pyrrolidine, piperazine, pyrazole, or morpholine which is substituted or un-substituted with Boc'-protective group or benzyl group, C₂-C₆ alkyl group substituted with ketone group, ester group, amide group or carboxylic acid substituted with at least one selected from a hydrogen atom, halogen atom, amine group or C₁ - C₆ alkyl group.

[Claim 7]

The compound according to claim 6,
 (R)-1-(6-benzyl-2-phenethyl-8-thioxo-5,6,7,8-tetrahydropyrazolo[4,3-e][1,4]diazepine-4(2H)-yl)-2,2,2-trifluoroethanone (LDD843; Example 1);
 (R)-1-(6-benzyl-2-phenethyl-4,5,6,7-tetrahydropyrazolo[4,3-e][1,4]diazepine-8(2H)-thione (LDD847; Example 2);
 (S)-*tert*-butyl-4-(2,4-diisobutyl-8-thioxo-2,4,5,6,7,8-hexahydropyrazolo[4,3-e][1,4]diazepine-6-yl)butylcarbamate (LDD1008; Example 11);
 (S)-6-(4-aminobutyl)-2,4-diisobutyl-4,5,6,7-hexahydropyrazolo[4,3-e][1,4]diazepine-8(2H)-thione (LDD998; Example 12);
 (S,E)-*tert*-butyl-3-(6-4-(*tert*-butoxy-carbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanoate (LDD1007; Example 14);
 (S,E)-*tert*-butyl-2-(6-4-(*tert*-butoxy-carbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)acetate (LDD1006; Example 15);
 (S,E)-3-(6-4-(aminobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanoic acid (LDD997; Example 16);
 (S,E)-3-(6-4-(*tert*-butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanoic acid (LDD1005; Example 17)
 (S,E)-ethyl-3-(6-4-(*tert*-butoxycarbonylamino)butyl)-2,4-diisobutyl-2,4

,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanoate(LDD 1004;

Example 18);

(S,E)-*tert*-butyl-4-(8-(3-amino-3-oxopropylthio)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-6-ylthio)butylcarbamate(LDD 1003; Example 19);

(S,E)-*tert*-butyl-4-(8-(3-hydrazinyl-3-oxopropylthio)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-6-yl)butylcarbamate(LDD 1002; Example 20);

Example 20);

(S,E)-3-(6-(4-amniobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanhydrazide(LDD1001; Example 21);

(S,E)-3-(6-(4-amniobutyl)-2,4-diisobutyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylthio)propanamide(LDD1000; Example 22);

(R,E)-6-benzyl-2-phenethyl-N-(2-(piperidin-1-yl)ethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD844; Example 3);

(R,E)-6-benzyl-2-phenethyl-N-(2-(pyrrolidin-1-yl)ethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD845; Example 4);

(R,E)-6-benzyl-2-phenethyl-N-(2-(piperazin-1-yl)ethyl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD846; Example 5);

(R,E)-6-benzyl-N-(2-morpholinoethyl)-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD848; Example 6);

(R,E)-6-benzyl-N-methyl-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD849; Example 7);

(S)-*tert*-butyl-3-((R,E)-6-benzyl-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-ylamino)pyrrolidin-1-carboxylate (LDD850; Example 8);

(R,E)-6-benzyl-N-(1-benzylpiperidin-4-yl)-2-phenethyl-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD854; Example 9);

(R,E)-6-benzyl-2-phenethyl-N-(piperidin-4-yl)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-8-amine (LDD1010; Example 10);

(S,E)-*tert*-butyl-4-(2,4-diisobutyl-8-(2-morpholinoethylamino)-2,4,5,6-tetrahydropyrazolo[4,3-e][1,4]diazepine-6-yl)butylcarbamate(LDD1009; Example 13).

[Claim 8]

A transglutaminase inhibitor comprising an effective amount of the compound represented by general formula (I) as set forth in claim 1 or the pharmaceutically acceptable salt thereof as an active ingredient

- [Claim 9] A pharmaceutical composition comprising an effective amount of the compound represented by general formula (I) as set forth in claim 1 or the pharmaceutically acceptable salt thereof as an active ingredient in amount effective to treat or prevent the disease caused by increased activation of transglutaminase, together with pharmaceutically acceptable carriers or diluents.
- [Claim 10] The pharmaceutical composition according to claim 9,
Wherein said "the disease caused by increased activation of transglutaminase" is neuronal disease or cancer disease.
- [Claim 11] The pharmaceutical composition according to claim 10,
Wherein said neuronal disease is Alzheimer's disease, multi-infarct dementia, the combined disease with Alzheimer's disease and multi-infarct dementia, Parkinson's disease, hypo-thyroidal syndrome, alcoholic dementia, or Huntington's disease.
- [Claim 12] The pharmaceutical composition according to claim 10,
Wherein said cancer disease is colorectal tumor, small intestine tumor, rectal cancer, anal cancer, esophageal cancer, pancreatic cancer, stomach cancer, renal cell carcinoma, uterine cancer, breast cancer, lung cancer, lymphoma, thyroid cancer, prostate cancer, leukemia, skin cancer, colon carcinoma, brain cancer, bladder cancer, ovarian cancer, or gallbladder cancer.
- [Claim 13] A use of the compound represented by general formula (I) as set forth in claim 1 or the pharmaceutically acceptable salt thereof for manufacture of medicines employed for treating or preventing the disease caused by increased activation of transglutaminase in mammals including human as an active ingredient in the amount effective to treat or prevent the disease caused by increased activation of transglutaminase.
- [Claim 14] A method of treating or preventing the disease caused by increased activation of transglutaminase in a mammal comprising administering to said mammal an effective amount of novel derivatives represented by general formula (I) as set forth in claim 1 and the pharmacologically acceptable salt thereof, together with a pharmaceutically acceptable carrier thereof into the mammals including human suffering from said disease.
- [Claim 15] A method of inhibiting hyper-activated transglutaminase in a mammal comprising administering to said mammal an effective amount of novel derivatives represented by general formula (I) as set forth in claim 1

and the pharmacologically acceptable salt thereof, together with a pharmaceutically acceptable carrier thereof into the mammals including human suffering from said disease.