Description

Title of Invention: NOVEL MEDICAMENT FOR TREATING INFLAMMATORY DISEASE

Technical Field

[0001] The present invention relates to a medicament for treating and/or preventing inflammatory disease, in more detail, a medicament for treating and/or preventing inflammatory disease, comprising a quinolone compound as an active ingredient.

Background Art

[0002] Many refractory inflammatory diseases such as systemic lupus erythematosus (SLE) and psoriasis have problems of treatment, for example, the medication can only achieve a remission, the patients have multiple relapses, there is no curative therapy, existing drugs have severe side effects, etc., thus it has been desired to develop a new drug without such problems.

[0003] As one of the treatments for inflammatory diseases, anti-IL-17A antibody exhibits therapeutic effect for psoriasis (Non-Patent Literature 1), but the effect is just neutralizing effect for IL-17A, and it has no effect for reducing IL-17-producing cells. And, many studies for reducing IL-17-producing cells have been also done, for example, RORγt inhibitor or bacteriotherapy, but any effective drug-development has not been attained yet (Non-Patent Literatures 2 - 6).

[0004] Patent Literature 1 discloses specific quinolone antimicrobials which exhibit the antibacterial activity against Clostridium difficile living in intestinal tract.

Citation List

Patent Literature

[0005] [PL 1] WO2013/029548

Non-Patent Literature

[0006] [NPL 1] Langley RG, et al., N. Engl. J. Med. 2014; 371(4): 326-338.

[NPL 2] Bassolas-Molina H, et al., Front. Immunol. 2018; 9: 2307.

[NPL 3] Ogita T, et al., J. Biomed. Biotechnol. 2011;2011:378417.

[NPL 4] Mu Q, Tavella VJ, et al., Sci. Rep. 2017; 7(1): 13675.

[NPL 5] Wu HJ, Ivanov II, et al., Immunity. 2010; 25; 32(6): 815-827.

[NPL 6] Krebs CF, et al., Immunity. 2016; 45(5): 1078-1092.

Summary of Invention

Technical Problem

[0007] The main purpose of the present invention is to provide a novel medicament for treating and/or preventing refractory inflammatory disease.

Solution to Problem

[0008] The present inventors have extensively studied and then have found that a known quinolone antimicrobial, 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-(2-amino-3-cyano-5-pyridyl)-4-oxo-3-quinoline-carboxylic acid can reduce IL-17-producing cells which are known to be related to exacerbation of many inflammatory diseases, and it is effectable for treating refractory inflammatory diseases. Based upon the new findings, the present invention has been completed.

[0009] The present invention includes the following embodiments.

(Item 1)

A medicament for treating and/or preventing inflammatory disease, comprising 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-(2-amino-3-cyano-5-pyridyl)-4-oxo-3-quinoline-carboxylic acid or a pharmaceutically acceptable salt thereof or a metabolite thereof as an active ingredient.

[0010] (Item 2)

The medicament of Item 1, wherein the metabolite is (2S,3S,4S,5R,6R)-6-((7-amino-5-cyanopyridin-3-yl)-1-cyclopropyl-6-fluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carbonyl)oxo)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid, 7-(6-amino-5-carbamoylpyridin-3-yl)-1-cyclopropyl-6-fluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, or ethyl 7-(6-amino-5-cyanopyridin-3-yl)-1-cyclopropyl-6-fluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate.

[0011] (Item 3)

The medicament of Item 1 or 2, wherein the inflammatory disease is an inflammatory disease related to IL-17-producing cell.

[0012] (Item 4)

The medicament of any one of Items 1 to 3, wherein the inflammatory disease is systemic lupus erythematosus, rheumatoid arthritis, scleroderma, multiple sclerosis, or psoriasis.

[0013] (Item 5)

The medicament of any one of Items 1 to 4, which is for oral administration.

[0014] (Item 6)

The medicament of any one of Items 1 - 5, wherein the daily dose of the active ingredient is 7.5 mg - 24000 mg.

[0015] (Item 7)

A method for treating and/or preventing inflammatory disease, comprising admin-

istering a therapeutically effective amount of 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-(2-amino-3-cyano-5-pyridyl)-4-oxo-3-quinoline-carboxylic acid or a pharmaceutically acceptable salt thereof or a metabolite thereof to a patient in need thereof.

[0016] (Item 8)

Use of 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-(2-amino-3-cyano-5-pyridyl)-4-oxo-3-quinoline-carboxylic acid or a pharmaceutically acceptable salt thereof or a metabolite thereof in the manufacture of a medicament for treating and/or preventing inflammatory disease.

[0017] (Item 9)

1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-(2-amino-3-cyano-5-pyridyl)-4-oxo-3-quinoline-carboxylic acid or a pharmaceutically acceptable salt thereof or a metabolite thereof for use in treating and/or preventing inflammatory disease.

Effect of Invention

[0018] The present compound can reduce IL-17-producing cells which are known to be related to exacerbation of many inflammatory diseases. Thus, it is expected to a novel medicament for treating and/or preventing refractory inflammatory diseases such as systemic lupus erythematosus, rheumatoid arthritis, scleroderma, multiple sclerosis, and psoriasis. Furthermore, the present compound is a poorly absorbable drug, and thereby it is distributed in a high concentration in the intestinal tract when it is orally administered, but it has low blood transferability. Thus, the present compound also has a merit, i.e., a low risk of generalized side effect, which is a problem in existing quinolone antibacterial agents.

Brief Description of Drawings

[0019] [Fig. 1] Fig. 1 shows the result of Example 1.

[Fig. 2] Fig. 2 shows the result of Example 2.

[Fig. 3] Fig. 3 shows the result of Example 2.

[Fig. 4] Fig. 4 shows the result of Example 8.

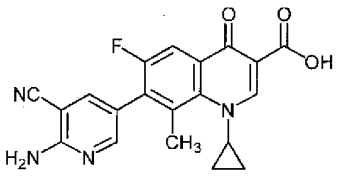
[Fig. 5] Fig. 5 shows the result of Example 8.

Description of Embodiments

[0020] The present compound 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-(2-amino-3-cyano-5-pyridyl)-4-oxo-3-quinoline-carboxylic acid has the structure of formula (1), which is disclosed as Compound number 2-18 in Patent Literature 1 that also discloses its process and its antibacterial activity for Clostridium difficile. And, the metabolites thereof are (2S,3S,4S,5R,6R)-6-((7-amino-5-cyanopyridin-3-yl)-1-cyclopropyl-6-fluoro-8-methyl-

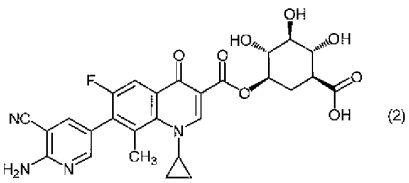
4-oxo-1,4-dihydroquinoline-3-carbonyl)oxo)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid,
7-(6-amino-5-carbamoylpyridin-3-yl)-1-cyclopropyl-6-fluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, and ethyl
7-(6-amino-5-cyanopyridin-3-yl)-1-cyclopropyl-6-fluoro-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate which have the following structures of formulae (2) - (4), respectively, which are included in the present compound.

[Chem.1]



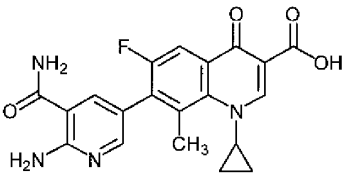
(1)

[Chem.2]



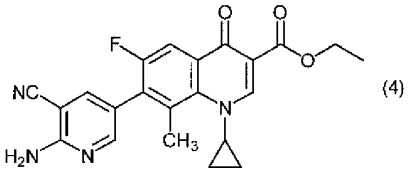
(2)

[Chem.3]



(3)

[Chem.4]



(4)

[0021] The compound of the present invention may be in the form of hydrate and/or solvate, and hence the present compound also encompasses a hydrate and/or solvate thereof.

In addition, the compound of the present invention in which any one or more ^{1}H atoms are replaced by $^{2}H(D)$ atoms is also within the scope of the present invention.

There may exist a polymorphism in a crystal of the compound of the present invention or a pharmaceutically acceptable salt thereof, and hence such crystal poly-

morphism is also within the scope of the present invention.

[0022] The "pharmaceutically acceptable salt" includes, as an acid addition salt, a salt with inorganic acid such as hydrochloride, hydrobromide, hydroiodide, sulfate, perchlorate, and phosphate; a salt with organic acid such as oxalate, malonate, maleate, fumarate, lactate, malate, citrate, tartrate, benzoate, trifluoroacetate, acetate, methanesulfonate, p-toluenesulfonate, and trifluoromethanesulfonate; and a salt with amino acid such as glutamate and aspartate; and as a salt with a base, an alkali metal salt such as sodium salt and potassium salt; alkaline-earth metal salt such as calcium salt; and an ammonium salt.

[0023] The "inflammatory disease" herein is not limited as long as it is an inflammatory disease, preferably it means inflammatory diseases which are related to IL-17-producing cell. For example, it includes systemic lupus erythematosus, rheumatoid arthritis, scleroderma, multiple sclerosis, and psoriasis.

[0024] The present compound may be administered via any route selected from oral administration, parenteral administration and rectal administration. The daily dose depends on the compound, administration route, condition of patient, age of patient, etc. In case of oral administration, for example, it may be generally administered in a dose of about 0.125 mg - about 400 mg, preferably about 0.25 mg - about 200 mg, more preferably about 0.5 mg - about 100 mg, even more preferably about 1 mg - about 50 mg, per kg of human or mammal's body weight, in one to several portions. For example, the daily dose of human includes about 7.5 mg - about 24000 mg, preferably about 15 mg - about 12000 mg, more preferably about 30 mg - about 6000 mg, even more preferably about 60 mg - about 3000 mg.

[0025] The dosage form in the present invention includes tablet, capsule, granule, powder, syrup, suspension, injection, suppository, eyedrop, ointment, liniment, patch, and inhalant. These dosage forms can be prepared in a conventional manner. If the dosage form is a liquid one, it may be a formulation to prepare a solution or suspension in use by mixing it with water, appropriate water-solution, or other appropriate solvent. The tablet and the granule may be coated in a well-known manner. The dosage form may be prepared in known manner with pharmaceutically acceptable additives.

The additives used herein include, according to the intended use, excipients, disintegrating agents, binders, fluidizer, lubricants, coating agents, colorants, solubilizers, solubilizing agents, thickeners, dispersants, stabilizing agents, sweeteners, and flavors. For example, they include lactose, mannitol, calcium hydrogen phosphate, microcrystalline cellulose, low-substituted hydroxypropylcellulose, cornstarch, partly pregelatinized starch, carmellose calcium, croscarmellose sodium, crospovidone, sodium starch glycolate, hydroxypropylcellulose, hydroxypropyl methylcellulose, polyvinyl alcohol, light anhydrous silicic acid, magnesium stearate, calcium stearate, sodium

stearyl fumarate, polyethylene glycol, propylene glycol, titanium oxide, talc, iron sesquioxide, and yellow ferric oxide.

[0026] In case that the present compound is formulated into a single dosage form, the dosage form may include the present compound in 0.1 - 85 % (w/w) per the whole composition, but the present invention is not limited thereto. Preferably, it is 10 - 70 % (w/w) per the whole composition.

[0027] In addition, the present compound may be used in combination with another drug or as a combination with another drug in order to enhance the effect and/or relieve side effects. The other drug which can be used in combination includes, for example, steroids such as prednisolone and budesonide, immunosuppressants such as azathioprine, cyclophosphamide, and tacrolimus, biopharmaceuticals such as rituximab and belimumab.

Examples

[0028] The present invention is explained in more detail in the following by referring to Examples, however, the present invention should not be limited thereto. The present compound used herein (hereinafter, referred to as "test substance") and the reference drug were gained as shown below.

Test substance [1-cyclopropyl-6-fluoro-1,4-dihydro-8-methyl-7-(2-amino-3-cyano-5-pyridyl)-4-oxo-3-quinoline-carboxylic acid]: gained from Otsuka Pharmaceutical Co., Ltd.

[0029] Example 1. Effect on IL-17A-producing cell in normal mouse

The test substance was administered to a normal BALB/c mouse to evaluate the influence of the test substance on IL-17A-producing CD4-positive T cell in mesenteric lymph node, inguinal lymph node, and spleen.

(Preparation of test substance)

The test substance was weighed and suspended in 5 % aqueous gum arabic to adjust the concentration to 2 mg/mL. The prepared suspension was stored at 4°C in shade.

(Drug administration)

The test substance was orally administered to a normal BALB/c mouse in a dose of 20 mg/kg once a day for 21 days. The setting of each group was shown in Table 1.

Table 1

	Group	Dose	n
1	Solvent control group	-	3
2	Test substance administration group	20 mg/kg/day	3

[0030] (Preparation of mesenteric lymph node cells, inguinal lymph node cells, and splenocytes)

The mouse which had received the drug administration for 21 days was euthanized

by cervical dislocation, and then mesenteric lymph node, inguinal lymph node, and spleen were isolated. Each isolated tissue was grinded on a PBS-added plate with a bottom of syringe, and the grinded tissue was put into a 50 mL tube on which a cell strainer was set. The grinded tissue in the tube was centrifuged at room temperature at 500 g for 5 minutes, and the obtained cell pellet was suspended in 10 % FBS-containing RPMI-1640 medium to be used in the experiment shown below.

(Evaluation of the rate of IL-17A-producing CD4-positive T cells)

Each cell suspension prepared above was seeded in a 96-well round-bottom plate, and stimulated with PMA/ionomycin in the presence of Breferdin A. After 4-hour incubation, the cell surface was stained with BV510-labeled anti-CD4 antibody, the cell was fixed and permeabilized with BD Cytofix/Cytoperm, and then IL-17A in the cell was stained with APC-labeled anti-IL-17A antibody. The stained cells were analyzed with a flow cytometer, and thereby the rate of IL-17A-pisitive cells in CD4-positive T cells was evaluated.

[0031] (Result)

Each rate of IL-17A-pisitive cells in CD4-positive T cells which was obtained by stimulating mesenteric lymph node cells, inguinal lymph node cells, or splenocytes with PMA/ionomycin is shown in Figure 1. According to the obtained result, IL-17A-producing cell decreased in the test substance administration group for all the tissues, which suggests that the test substance can have an action reducing IL-17A-producing cell.

[0032] Example 2. Effect on IL-17A-producing cell in kidney of mouse model for SLE

The test substance was administered to a mouse model for SLE, and the effect of the test substance on IL-17A-producing cell in kidney was evaluated.

(Preparation of imiquimod-induced SLE model)

An imiquimod-containing drug, BESELNA CREAM 5 % (MOCHIDA PHARMACEUTICAL CO., LTD.) (0.03 mL, containing 1.5 mg of imiquimod) was applied on the inside of the right auricle of a BALB/c mouse with a small brush under inhalation anesthesia of isoflurane three times a week. The application of imiquimod was continuously carried out from the beginning of the test to the terminal of the test.

(Preparation of test substance)

The test substance was weighed and suspended in 5 % aqueous gum arabic to adjust the concentration to 2 mg/mL. The prepared suspension was stored at 4°C in shade.

[0033] (Drug administration)

From the 14th day after the imiquimod-application was started, the test substance was orally administered to the mouse in a dose of 20 mg/kg once a day for 42 days. The setting of each group was shown in Table 2.

Table 2

	Group	Dose	n
1	Untreated group (without imiquimod-application)	-	6
2	Solvent control group	-	6
3	Test substance administration group	20 mg/kg/day	5

Note: In the test substance administration group, one mouse happened to an accident to be excluded during the drug administration, thus the number of the test mice became 5 mice.

[0034] (Dissection and preprocessing)

The mouse which had received the drug administration for 42 days was euthanized by cervical dislocation, and then a pair of the kidneys was isolated. One of the isolated kidneys was quickly frozen with liquid nitrogen for gene expression analysis, and the other one was stored on a PBS-added plate until the test was started, which is used for the evaluation of IL-17A-producing cell.

(Preparation of immune cells from kidney)

The kidney for evaluating IL-17A-producing cell was transferred per each group onto a plate on which 10 % FBS-containing RPMI-1640 medium was added, and cleaved with scissors into 1 to 2-mm cubes. Collagenase D was added to the plate to adjust the concentration to 5 mg/mL. The sample was incubated at 37°C under 5 % CO_2 for 40 minutes, and transferred into a 50 mL tube equipped with 70 μm cell strainer. The kidney blocks were grinded with a bottom of syringe on the cell strainer, and the cell strainer was washed with PBS. The obtained cell suspension was centrifuged at room temperature at 500 g for 10 minutes. The supernatant was removed, PBS was added to the residue to suspend it. The same volume of 80 % Percoll solution as the PBS used for suspending the cell was added to the suspension to prepare 40 % Percoll cell-suspension, which was piled up in a 50 mL tube which 80 % Percoll solution was already added to. The sample in the tube was centrifuged at room temperature at 1500 g for 30 minutes, and the layer between 80 % Percoll and 40 % Percoll was collected, which was diluted with PBS. The diluted sample was centrifuged at room temperature at 500 g for 10 minutes, the supernatant was removed, the residue was suspended in 10 % FBS-containing RPMI-1640 medium, and the suspension was centrifuged at 4°C at 500 g for 5 minutes. The supernatant was removed, and the residue was suspended in 10 % FBS-containing RPMI-1640 medium, which was used in the following test.

[0035] (Evaluation of the rate of IL-17A-producing T cells)

The cell-suspension of each administration group was seeded on a 96-well round-bottom plate, which was stimulated with PMA/ionomycin in the presence of Breferdin A. After 4-hour cultivation, the cell surface was stained with FITC-labeled anti-TCRβ

antibody, PerCP/Cy5.5-labeled anti-CD8α antibody, BV421-labeled anti-CD3 antibody, BV510-labeled anti-CD4 antibody, the cell was fixed and permeabilized with BD Cytofix/Cytoperm, and then IL-17A in the cell was stained with APC-labeled anti-IL-17A antibody. The stained cells were analyzed with a flow cytometer, and thereby the rate of IL-17A-pisitive cells in CD4-positive T cells ($CD3^+TCR\beta^+CD4^+CD8\alpha^-$), CD4-negative CD8-negative T cells ($CD3^+TCR\beta^+CD4^-CD8\alpha^-$), or TCRβ chain-negative T cells ($CD3^+TCR\beta^-$) was evaluated.

(Evaluation of IL-17A gene expression)

The cryopreserved kidney was homogenized in ISOGEN, and RNA was extracted. The extracted RNA was reversetranscribed to prepare cDNA. By using the obtained cDNA as a template, the expression analyses of Actb gene and Il17a gene were done. The sample was treated in each individual, and the analytical results were showed with Fold change wherein Actb gene is endogenous control and the average of the untreated group is a standard control.

[0036] (Result)

Each rate of IL-17A-pisitive cells in CD4-positive T cells ($CD3^+TCR\beta^+CD4^+CD8\alpha^-$), CD4-negative CD8-negative T cells ($CD3^+TCR\beta^+CD4^-CD8\alpha^-$), TCRβ chain-negative T cells ($CD3^+TCR\beta^-$) which were obtained by stimulating the immune cells prepared from kidney with PMA/ionomycin is shown in Figure 2, and the analytical result of IL-17A gene expression in kidney is shown in Figure 3.

According to the analytical result of the IL-17A-producing cell rate in each cell strain, the data showed that IL-17A-producing cell in kidney in the test substance administration group decreased, which was also shown in the analytical result of IL-17A gene expression, and showed that the test substance has an action for decreasing IL-17A-producing cell in kidney of a SLE model.

[0037] Example 3. Effect on kidney function of mouse model for SLE

The test substance is administered to a mouse model for SLE, and the effect of the test substance on kidney function is evaluated.

(Preparation of test substance)

The test substance is weighed and suspended in 5 % aqueous gum arabic to adjust the concentration to 0.5, 1, and 2 mg/mL. The prepared suspension is stored at 4°C in shade.

(Drug administration)

According to Table 3, the test substance is orally administered to the mouse once a day. The administration of the substance is continued until the terminal of the test.

Table 3

	Group	Dose	n
1	Untreated group (without imiquimod-application)	-	3
2	Solvent control group	-	10
3	Test substance (0.5) administration group	5 mg/kg/day	10
4	Test substance (1) administration group	10 mg/kg/day	10
5	Test substance (2) administration group	20 mg/kg/day	10

[0038] (Preparation of imiquimod-induced SLE model)

From the 29th day after the drug-administration is started, an imiquimod-containing drug, BESELNA CREAM 5 % (MOCHIDA PHARMACEUTICAL CO., LTD.) (0.03 mL, containing 1.5 mg of imiquimod) is applied on the inside of the right auricle of a BALB/c mouse with a small brush under inhalation anesthesia of isoflurane three times a week. The application of imiquimod is continuously carried out to the terminal of the test.

(Evaluation of kidney function)

The urine excreted for one night is collected with a metabolism cage on the day before the drug-administration is started, on the day before the imiquimod-application is started, and on the 14th, 28th, 42nd, and 56th days after the imiquimod-application is started. The collected urine is centrifuged at 4°C at 3000 g for 10 minutes, and then the concentration of albumin and the concentration of creatinine are measured. Based on the obtained results, the ratio of albumin/creatinine is calculated to use for analysis.

[0039] Example 4. Effect on mouse model for rheumatoid arthritis

The test substance is administered to a mouse model for rheumatoid arthritis, and the effect of the test substance on pathological condition is evaluated.

(Preparation of test substance)

The test substance is weighed and suspended in 5 % aqueous gum arabic to adjust the concentration to 0.5, 1, and 2 mg/mL. The prepared suspension is stored at 4°C in shade.

(Drug administration)

According to Table 4, the test substance is orally administered to the mouse once a day. The administration of the substance is continued until the terminal of the test.

Table 4			
	Group	Dose	n
1	Untreated group (without immunizing collagen)	-	3
2	Solvent control group	-	10
3	Test substance (0.5) administration group	5 mg/kg/day	10
4	Test substance (1) administration group	10 mg/kg/day	10
5	Test substance (2) administration group	20 mg/kg/day	10

[0040] (Preparation of rheumatoid arthritis model and evaluation of pathological condition)

From the 29th day after the drug-administration is started, type II collagen is immunized to make type II collagen-induced arthritis. The method for inducing arthritis and the method for evaluating its pathological condition may refer to Depis, et al. (Arthritis Rheum. 2012 Oct; 64(10): 3189-98).

[0041] Example 5. Effect on mouse model for scleroderma

The test substance is administered to a mouse model for scleroderma, and the effect of the test substance on pathological condition is evaluated.

(Preparation of test substance)

The test substance is weighed and suspended in 5 % aqueous gum arabic to adjust the concentration to 0.5, 1, and 2 mg/mL. The prepared suspension is stored at 4°C in shade.

(Drug administration)

According to Table 5, the test substance is orally administered to the mouse once a day. The administration of the substance is continued until the terminal of the test.

Table 5			
	Group	Dose	n
1	Untreated group (without administering bleomycin)	-	3
2	Solvent control group	-	10
3	Test substance (0.5) administration group	5 mg/kg/day	10
4	Test substance (1) administration group	10 mg/kg/day	10
5	Test substance (2) administration group	20 mg/kg/day	10

[0042] (Preparation of scleroderma model and evaluation of pathological condition)

From the 29th day after the drug-administration is started, bleomycin is subcutaneously administered to the mouse to induce scleroderma-like symptom. The method for inducing scleroderma-like symptom and the method for evaluating its pathological

condition may refer to Park, et al. (Front Immunol. 2018 Jul 10; 9: 1611).

[0043] Example 6. Effect on mouse model for multiple sclerosis

The test substance is administered to a mouse model for multiple sclerosis, and the effect of the test substance on pathological condition is evaluated.

(Preparation of test substance)

The test substance is weighed and suspended in 5 % aqueous gum arabic to adjust the concentration to 0.5, 1, and 2 mg/mL. The prepared suspension is stored at 4°C in shade.

(Drug administration)

According to Table 6, the test substance is orally administered to the mouse once a day. The administration of the substance is continued until the terminal of the test.

Table 6

	Group	Dose	n
1	Untreated group (without EAE-induction)	-	3
2	Solvent control group	-	10
3	Test substance (0.5) administration group	5 mg/kg/day	10
4	Test substance (1) administration group	10 mg/kg/day	10
5	Test substance (2) administration group	20 mg/kg/day	10

[0044] (Preparation of mouse model for multiple sclerosis and evaluation of pathological condition)

From the 29th day after the drug-administration is started, the peptide derived from myelin protein is immunized to induce experimental autoimmune encephalomyelitis (EAE) which is known as a mouse model for multiple sclerosis. The method for inducing EAE and the method for evaluating its pathological condition may refer to Chiba, et al. (Int Immunopharmacol. 2011 Mar; 11(3): 366-72).

[0045] Example 7. Effect on mouse model for psoriasis

The test substance is administered to a mouse model for psoriasis, and the effect of the test substance on pathological condition is evaluated.

(Test method)

(Preparation of test substance)

The test substance is weighed and suspended in 5 % aqueous gum arabic to adjust the concentration to 0.5, 1, and 2 mg/mL. The prepared suspension is stored at 4°C in shade.

(Drug administration)

According to Table 7, the test substance is orally administered to the mouse once a day. The administration of the substance is continued until the terminal of the test.

Table 7

	Group	Dose	n
1	Untreated group (without imiquimod-application)	-	3
2	Solvent control group	-	10
3	Test substance (0.5) administration group	5 mg/kg/day	10
4	Test substance (1) administration group	10 mg/kg/day	10
5	Test substance (2) administration group	20 mg/kg/day	10

[0046] (Preparation of mouse model for psoriasis and evaluation of pathological condition)

From the 29th day after the drug-administration is started, an imiquimod-containing drug, BESELNA CREAM 5 % (MOCHIDA PHARMACEUTICAL CO., LTD.) is applied on the back and right auricle of the mouse to induce psoriasis-like symptom. The method for inducing psoriasis-like symptom and the method for evaluating its pathological condition may refer to van der Fits, et al. (J Immunol. 2009 May 1; 182(9): 5836-45).

[0047] Example 8. Effect on mouse model for psoriasis

The test substance was administered to a mouse model for psoriasis, and the effect of the test substance on pathological condition and IL-17A-producing cell was evaluated.

(Test method)

(Preparation of test substance)

The test substance is weighed and suspended in 5 % aqueous gum arabic to adjust the concentration to 2 mg/mL. The prepared suspension is stored at 4°C in shade.

(Drug administration)

According to Table 8, the test substance was orally administered to the mouse once a day. The administration of the substance was continued until the terminal of the test.

Table 8

	Group	Dose	n
1	Untreated group (without imiquimod-application)	-	3
2	Solvent control group	-	10
3	Test substance administration group	20 mg/kg/day	10

[0048] (Preparation of mouse model for imiquimod-induced psoriasis)

From the 29th day after the drug-administration is started, an imiquimod-containing drug BESELNA CREAM 5 % (MOCHIDA PHARMACEUTICAL CO., LTD.) (0.03 mL, containing 1.5 mg of imiquimod) was applied on the inside of the right auricle of a BALB/c mouse with a small brush under inhalation anesthesia of isoflurane three times

a week to induce psoriasis-like symptom.

(Measurement of auricle thickness)

After the imiquimod-application was started, the thickness of the auricle was measured with a caliper once a week.

(Dissection and collection)

On the 44th day after the imiquimod-application was started, the mouse was euthanized by cervical spine fracture dislocation, and its right auricle was collected. The collected auricle was quickly frozen with liquid nitrogen, and stored in a freezer at -80°C.

(Evaluation of IL-17A gene expression in auricle)

The frozen auricle was crushed in ISOGEN, and RNA was extracted. The extracted RNA was reversetranscribed to prepare cDNA. Using the obtained cDNA as a template, the expressions of 18S rRNA gene and IL-17A gene were analyzed by real-time PCR. The sample was treated in each individual, and the analytical results were showed with Fold change wherein 18S rRNA gene is endogenous control and the average of the untreated group is a standard control.

[0049] (Result)

The result of the measured auricle thickness is shown in Figure 4, and the evaluation result of the IL-17A gene expression in auricle is shown in Figure 5.

The result of continuously-measuring the thickness of the auricle applied with imiquimod showed that the hyperplasia of auricle was suppressed in the test substance administration group. And, the evaluation result of the IL-17A gene expression in auricle showed that the IL-17A gene expression was lowered in the test substance administration group. Thus, it was shown that the test substance can reduce the IL-17A-producing cell and suppress the hyperplasia of auricle in a model for psoriasis.