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- (54) Benævnelse: **Farmaceutisk sammensætning med (1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol og et insulin-sekretionsfremkaldende stof**
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Description

Technical Field

5 **[0001]** The present invention relates to pharmaceutical compositions useful in the treatment of diabetes mellitus, diseases associated with diabetes mellitus, or complication of diabetes mellitus. More particularly, the present invention relates to pharmaceutical compositions comprising a combination of

10 (A) (1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt; and
(B) an insulin secretagogue.

Background Art

15 **[0002]** Diabetes mellitus is a group of diseases mainly characterized by chronic hyperglycemia due to insufficiency of insulin action and is accompanied by various metabolic abnormalities. The primary treatment of diabetes mellitus consists of exercise and diet therapy; if these methods are incapable of adequate glycemic control, drug therapy with oral anti-diabetic agents is conducted. However, depending on the condition of a diabetic patient, treatment with a single drug sometimes encounters difficulty in achieving good glycemic control, and what is more, the development of side effects
20 prevents the drug from being used in an adequate dose or over an adequate period of time. According to one literature report, a biguanide solely used on patients with type 2 diabetes gave only about 25% probability for the possibility of lowering the value of glycated hemoglobin (HbA1c) to 7% or less (see NPL 1). It is also known that if a sulfonylurea is used alone, side effects such as the development of hypoglycemia and weight gain are not the sole problems but that its action of improving hyperglycemia becomes inadequate in patients whose ability to secrete insulin have been deter-
25 riorated by failure of beta-cells with the progress of diabetic condition. It is also known that if an insulin sensitizer is used alone, there occurs the problem of occasional development of such side effects as weight gain, edema, heart failure, and hepatopathy. It is also known that if insulin is used alone, there occur the problems of hypoglycemia and weight gain. It is also known that if an α -glucosidase inhibitor is used alone, there occurs the problem of occasional development of abdominal symptoms as side effects. In addition, when a GLP-1 mimetic is used alone, nausea and vomiting are
30 known as therapeutic problems. Furthermore, it is known that patients with type 2 diabetes, when they are exposed to a hyperglycemic condition for a prolonged period of time, will suffer a deterioration in the function of beta-cells; however, most antidiabetic agents are incapable of adequately suppressing the deterioration in the function of beta-cells even if the blood glucose levels are lowered.

[0003] As a means of solving the aforementioned problems involved in the treatment with single drugs, a combination
35 therapy is under review that uses a combination of antidiabetic agents that act by different mechanisms. However, few combinations are capable of diminishing the aforementioned problems with the use of single drugs. For example, it is known that the risk for the development of hypoglycemia, a recognized side effect of sulfonylureas, is further increased if they are used in combination with other antidiabetic agents. In addition, the weight gain recognized from sulfonylureas and insulin sensitizers cannot usually be controlled by using them in combination with other antidiabetic agents. Hence,
40 a novel combination of plural pharmaceutical drugs is in demand that can achieve a good glycemic control and which yet causes no appreciable side effects.

[0004] Glucose in the blood is filtered in the glomeruli of each kidney and then reabsorbed as mediated by sodium-dependent glucose cotransporters (SGLTs) located at the beginnings of proximal tubules. SGLT2 inhibitors typified by
45 a 1-thio-D-glucitol compound (see PLT 1) inhibit the activity of SGLT2 having low glucose affinity but high glucose transport capacity, so they exhibit a blood glucose reducing action in various animal models by promoting the excretion of urinary glucose.

[0005] It was recently reported that side effects of insulin sensitizers could be reduced by combining them with SGLT2 inhibitors (see PLT 2) but there has been no report of a pharmaceutical composition that combines an SGLT2 inhibitor
50 with an insulin sensitizer in order to provide an enhanced blood glucose lowering action. There is also literature teaching the combined use of an SGLT2 inhibitor and a dipeptidyl peptidase IV inhibitor (see PLT 3 and 4). However, no report has been made of pharmaceutical compositions of the type contemplated by the present invention that comprise combinations of SGLT2 inhibitors with at least one member of the group consisting of biguanides, insulin secretagogues, insulin sensitizers, insulins, dipeptidyl peptidase IV inhibitors, α -glucosidase inhibitors, and GLP-1 mimetics.

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Citation List

Patent Literature

5 [0006]

PLT 1: Official Gazette of International Publication WO 2006/073197 A1

PLT 2: Patent Official Gazette of EP 1381361 B1

PLT 3: Official Gazette of International Publication WO 2009/022007 A1

10 PLT 4: Official Gazette of International Publication WO 2009/022010 A1

Non Patent Literature

15 [0007] NPL 1: Tonyobyougaku (Kiso to Rinshou) [Diabetology (Fundamentals and Clinical Applications)], pp.949-954, 2007, Nishimura Shoten

Summary of Invention

20 [0008] An object of the present invention is to provide a novel pharmaceutical composition comprising a combination of plural drugs that has superior efficacy in preventing or treating diabetes mellitus, diseases associated with diabetes mellitus, or complications of diabetes mellitus and which yet causes no appreciable side effects.

[0009] Another object of the present invention is to provide a pharmaceutical composition for use in preventing or treating diabetes mellitus, diseases associated with diabetes mellitus, or complications of diabetes mellitus using the pharmaceutical composition.

25 [0010] The present inventors found that when a specific 1-thio-D-glucitol compound having an SGLT2 inhibiting action or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt was administered in combination with an insulin secretagogue, a marked blood glucose lowering action, suppression of the failure of pancreatic β -cells, and alleviation of side effects were caused; the present invention has been accomplished on the basis of this finding.

[0011] Thus, the present invention provides the following:

30 (1) A pharmaceutical composition comprising a combination of

(A) (1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt; and

35 (B) an insulin secretagogue.

(2) The pharmaceutical composition according to (2), wherein the insulin secretagogue is glipizide, glibenclamide, or glimepiride.

40 (3) The pharmaceutical composition according to (2), wherein the insulin secretagogue is glipizide or glimepiride.

(4) The pharmaceutical composition according to (2), wherein the insulin secretagogue is glipizide.

45 (6) A combination of:

(A) (1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt; and

(B) an insulin secretagogue,

50 for use in a method of preventing or treating diabetes mellitus, diseases associated with diabetes mellitus, or complications of diabetes mellitus, wherein (A) and (B) are administered to a patient in need either simultaneously or separately.

(7) The combination for use according to (6), wherein the insulin secretagogue is glipizide, glibenclamide, and glimepiride.

55 (8) The combination for use according to (7), wherein the insulin secretagogue is glipizide or glimepiride.

(9) The combination for use according to (7), wherein the insulin secretagogue is glipizide.

(10) The combination for use according to (7), wherein the insulin secretagogue is glimepiride.

(11) The combination for use according to any one of (6) to (10), wherein diabetes mellitus is type 2 diabetes.

(12) The combination for use according to any one of (6) to (11), wherein the complication of diabetes mellitus is diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, cerebrovascular disorder, ischemic cardiac disease, or peripheral arterial disease.

Advantageous Effects of Invention

[0012] The pharmaceutical composition of the present invention comprising plural pharmaceutical drugs showed superior glycated hemoglobin (GHb) value lowering action and plasma glucose level lowering action over the individual drugs. In addition, the pharmaceutical composition of the present invention comprising plural pharmaceutical drugs suppressed the lowering of plasma insulin levels that might have occurred with the progress of diabetic condition. Furthermore, the pharmaceutical composition of the present invention comprising plural pharmaceutical drugs alleviated the side effects of the individual drugs (i.e., weight gain and hypoglycemia).

Description of Embodiments

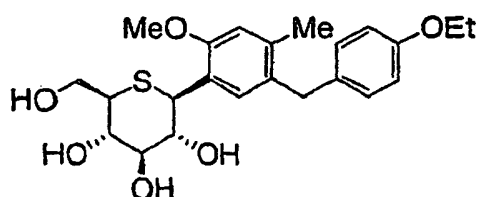
[0013] The "pharmaceutically acceptable salt" may be exemplified by: salts with inorganic acids such as sulfuric acid, hydrochloric acid, hydrobromic acid, phosphoric acid, and nitric acid; salts with organic acids such as acetic acid, oxalic acid, lactic acid, tartaric acid, fumaric acid, maleic acid, citric acid, benzenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, benzoic acid, camphorsulfonic acid, ethanesulfonic acid, glucoheptonic acid, gluconic acid, glutamic acid, glycolic acid, malic acid, malonic acid, mandelic acid, galactaric acid, and naphthalene-2-sulfonic acid; salts with one or more metal ions such as lithium ion, sodium ion, potassium ion, calcium ion, magnesium ion, zinc ion, and aluminum ion; and salts with amines such as ammonia, arginine, lysine, piperazine, choline, diethylamine, 4-phenylcyclohexylamine, 2-aminoethanol, and benzathine.

[0014] The compound A of the present invention can exist as various solvates. They may also be hydrates in view of their applicability as pharmaceutical drugs.

[0015] The methods of producing the 1-thio-D-glucitol compounds of formula (I) which are used in the present invention, as well as the pharmaceutically acceptable salts thereof and the hydrates of the compounds or salts which are also used in the present invention are disclosed in the official gazette of International Publication WO 2006/073197 A1.

[0016] The following 1-thio-D-glucitol compound is used because it exhibits a superior SGLT2 inhibiting activity:

(1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (formula (A))



(A)

[0017] The (1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol is preferably a hydrate.

[0018] The present invention is directed to a pharmaceutical composition comprising the 1-thio-D-glucitol compound of formula (A) or a pharmaceutically acceptable salt thereof or a hydrate of the compound or salt and an insulin secretagogue. This pharmaceutical composition is preferably a medicine against diabetes mellitus, diseases associated with diabetes mellitus, or complications of diabetes mellitus.

[0019] The composition may be used in a method of preventing or treating diabetes mellitus, diseases associated with diabetes mellitus, or complications of diabetes mellitus, which comprises administering a patient in need the 1-thio-D-glucitol compound of formula (A) or a pharmaceutically acceptable salt thereof or a hydrate of the compound or salt and an insulin secretagogue, either simultaneously or separately.

[0020] Insulin secretagogues are drugs having the property to promote insulin secretion from pancreatic β -cells. Exemplary insulin secretagogues are sulfonylureas (glipizide, glibenclamide, glimepiride, gliclazide, acetohexamide, tolbutamide, etc.).

utamide, glycopyramide, chlorpropamide, and tolazamide) and glinides (nateglinide, mitiglinide, and repaglinide). Among these, glipizide, glibenclamide and glimepiride which are sulfonylureas are particularly preferred from the viewpoint of blood glucose lowering action. These insulin secretagogues are known substances and commercial product may typically be used.

[0021] "Diabetes mellitus" encompasses type 1 diabetes, type 2 diabetes, and other types of diabetes mellitus due to specific causes. The diseases targeted by the pharmaceutical drugs of the present invention are preferably type 1 diabetes and type 2 diabetes.

[0022] "Diseases associated with diabetes mellitus" include obesity, hyperinsulinemia, dysglycemia (glucose metabolism disorder), hyperlipidemia, hypercholesteolemia, hypertriglyceridemia, dyslipidemia (lipid metabolism disorder), hypertension, congestive heart failure, edema, hyperuricemia, and gout.

"Complications of diabetes mellitus" are classified into acute and chronic ones.

"Acute complications" include hyperglycemic (e.g., ketoacidosis), hyperglycemic hyperosmolar syndrome, lactic acidosis, hypoglycemia, and infectious diseases (e.g., skin, soft tissue, biliary, respiratory, and urinary-tract infections).

"chronic complications" include microangiopathy (diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy), as well as macroangiopathy (cerebrovascular disorder, ischemic heart disease, and peripheral arterial disease).

[0023] "Treatment" means administering the pharmaceutical compositions of the present invention to patients who have already manifested diseases such as diabetes mellitus, diseases associated with diabetes mellitus, or complications of diabetes mellitus. Acts of this treatment cover symptomatic therapy intended to mitigate the symptoms derived from the above-mentioned diseases. Also included are treatments for partial or complete recovery from disease, as well as treatments that stop or retard the progress of disease.

[0024] "Prevention" means a practice by which patients who have a risk for manifesting diseases such as diabetes mellitus, diseases associated with diabetes mellitus, or complications of diabetes mellitus are administered the pharmaceutical compositions of the present invention before the disease manifests itself.

[0025] The pharmaceutical composition of the present invention can be such that the active ingredients described above are formulated as a single preparation (combination preparation) or they are separately formulated as two or more preparations. These preparations may be tablets, granules, powders, capsules, emulsions, suspensions or syrups or alternatively they may be injections in such forms as a sterile solution or a liquid suspension, all being obtained by commonly employed means. If the active ingredients are separately formulated as two or more preparations, the respective preparations may be administered either simultaneously or at given time intervals spaced apart. The two or more preparations may be administered at different frequencies in a day. The pharmaceutical composition of the present invention may be administered either systemically or topically by an oral or parenteral route. If the active ingredients are separately formulated as two or more preparations, the respective preparations may be administered by different routes.

[0026] If the pharmaceutical composition of the present invention is formulated as two different preparations, they are highly likely to be administered either simultaneously or at a very short interval, so a document such as a package insert or a sales brochure that accompanies a commercial product preferably states to the effect that the two preparations should be administered in combination. Another preferred embodiment is a kit of two preparations, one comprising

(A) (1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol or a pharmaceutically acceptable salt thereof or a hydrate of the compound or the salt; and the other comprising

(B) an insulin secretagogue.

[0027] The dosage of the pharmaceutical composition of the present invention varies with the target, method, etc. of administration. Take, for example, the case of oral administration; a patient with diabetes mellitus is preferably administered the following daily doses:

(1) 0.1-50 mg, preferably 0.5-25 mg, of the 1-thio-D-glucitol compound A;

(3) 0.5-2000 mg of insulin secretagogues;

(3-1) 0.5-100 mg, preferably 1-10 mg, of glimepiride;

(3-2) 0.5-100 mg, preferably 1-10 mg, of glibenclamide;

(3-3) 10-2000 mg, preferably 100-1000 mg, of acetohexamide;

(3-4) 100-2000 mg, preferably 300-2000 mg, of tolbutamide;

(3-5) 50-2000 mg, preferably 100-500 mg, of glycopyramide;

(3-6) 10-1000 mg, preferably 50-500 mg, of chlorpropamide;

(3-7) 0.5-2000 mg, preferably 2-40 mg, of glipizide;

(3-8) 10-2000 mg, preferably 50-500 mg, of tolazamide;

(3-9) 10-500 mg, preferably 30-200 mg, of gliclazide

[0028] The preparations described above are preferably oral preparations such as tablets, granules, powders, cap-

sules, emulsions, suspensions, and syrups. Specifically, the active ingredients described above may be mixed, either simultaneously or separately, with excipients such as mannitol and lactose and, after granulation, filled into capsules either directly or after being mixed with other additives for oral administration, which are specifically exemplified by excipients (e.g., sugar-based or sugar alcoholic excipients such as glucose, sucrose, mannitol, lactose, xylitol, sorbitol, maltitol, and pullulan; cellulosic excipients such as microcrystalline cellulose; starch-based excipients such as corn starch; inorganic excipients such as anhydrous dibasic calcium phosphate), binders (e.g., cellulosic binders such as methylcellulose, hydroxypropyl cellulose, and Hydroxypropyl methylcellulose), disintegrants (e.g., cellulosic disintegrants such as croscarmellose calcium, low-substitution hydroxypropyl cellulose, and croscarmellose sodium, or starch-based disintegrants such as partially gelatinized starch and carboxymethyl starch sodium), fluidizers (e.g., inorganic fluidizers such as light silicic anhydride), or lubricants (e.g., stearic acid, magnesium stearate, calcium stearate, talc, and sodium stearyl fumarate); alternatively, the granulation may be pelletized into tablets.

[0029] The proportion at which the 1-thio-D-glucitol compound of the present invention or a pharmaceutically acceptable salt thereof or a hydrate of the compound or salt is combined with at least one member of the group of insulin secretagogues, varies with the target, method, etc. of administration. Take, for example, the case of administering the pharmaceutical composition of the present invention to a human; if one part by mass of the 1-thio-D-glucitol compound is combined with 0.01-1000 parts by mass of at least one member of the group of insulin secretagogues, it is possible to obtain a superior blood glucose lowering action than is achieved by administering the individual drugs. It is particularly preferred to combine one part by mass of the 1-thio-D-glucitol compound in combination with 0.1-100 parts by mass of at least one member of the group of insulin secretagogues. This enables satisfactory efficacy to be obtained by using the respective drugs in smaller amounts than when they are administered individually. As a further advantage, the pharmaceutical composition of the present invention has fewer side effects because it does not cause hypoglycemia or weight gain that might result from excessive insulin secretion.

[0030] Patients who are to be treated by the pharmaceutical composition of the present invention are those who are unable to have good glycemic control in spite of continued diet and exercise and who therefore need be subjected to drug therapy; preferred patients are those who are unable to have good glycemic control in spite of being administered with a single oral antidiabetic drug and who therefore need to take another drug that acts by a different mechanism.

[0031] The pharmaceutical composition of the present invention can typically be produced according to the following formulas.

(Preparation 1) Tablet (Reference Example not belonging to the invention)

[0032] Tablets with a diameter of 13 mm were prepared; each tablet contained:

(1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol (hereinafter referred to as compound A)	5 mg
Metformin hydrochloride	500 mg
Microcrystalline cellulose	70 mg
Hydroxypropyl cellulose	25 mg
Carboxymethyl starch sodium	30 mg
Magnesium stearate	3 mg

(Preparation 2) Tablet (Reference Example not belonging to the invention)

[0033] Tablets with a diameter of 7 mm were prepared; each tablet contained:

Compound A	5 mg
Pioglitazone hydrochloride	16.53 mg
Microcrystalline cellulose	48 mg
Lactose	50 mg
Hydroxypropyl cellulose	5 mg
Low-substitution hydroxypropyl cellulose	14 mg
Magnesium stearate	1 mg.

(Preparation 3) Tablet

[0034] Tablets with a diameter of 7 mm were prepared; each tablet contained:

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Compound A	5 mg
Glimepiride	4 mg
Microcrystalline cellulose	61 mg
Lactose	50 mg
Hydroxypropyl cellulose	5 mg
Low-substitution hydroxypropyl	14 mg
Magnesium stearate	1 mg.

(Preparation 4) Tablet (Reference Example not belonging to the invention)

[0035] Tablets with a diameter of 8 mm were prepared; each tablet contained:

Compound A	5 mg
Sitagliptin	50 mg
Microcrystalline cellulose	45 mg
Mannitol	69 mg
Hydroxypropyl cellulose	10 mg
Low-substitution hydroxypropyl cellulose	20 mg
Magnesium stearate	1 mg.

Examples

[0036] On the following pages, the present invention is described in greater detail by means of examples.

Test Example 1 (Reference Example not belonging to the invention)

<Test item>

[0037] Effects of chronic combination treatment of compound A and metformin hydrochloride in diabetic mice

<Test method>

[0038] Eight male db/db mice/group (11-week old; CLEA JAPAN, Inc.) were orally administered with repeated doses of compound A (3 mg/kg, once daily) and a biguanide, metformin hydrochloride (50 mg/kg or 150 mg/kg, twice daily; SIGMA-ALDRICH JAPAN K.K.), either alone or in combination, for 27 days. Eight db/m mice (11-week old; CLEA JAPAN, Inc.) were used as nondiabetic normal controls.

[0039] Before and 27th day after the start of repeated administration, blood was taken from the tail vein of each mouse and centrifuged to collect hematocyte fractions. After hemolysis of the hematocyte fractions, glycated hemoglobin (GHb) values were measured by affinity column chromatography with an automated glycated hemoglobin analyzer (TOSOH CORPORATION). Starting at the 27th day of repeated administration, mice were fasted for 16 hours, thereafter, blood was taken from the tail vein of each mouse and centrifuged to collect plasma. Plasma glucose levels were measured by the mutarotase-GOD method with a glucose assay kit (Glucose C2 Test Wako; Wako Pure Chemical Industries, Ltd.) Further, before and 27th day after the start of repeated administration, blood was taken from the tail vein of each mouse under non-fasting conditions and centrifuged to collect plasma. Plasma insulin levels were measured by ELISA with an insulin assay kit (Levis: Mouse Insulin ELISA KIT (H-Type); SHIBAYAGI Co., Ltd.)

<Result 1>

[0040] The changes in GHb are expressed as the mean \pm standard error in Table 1. The combination treatment of compound A (3 mg/kg) and metformin hydrochloride (300 mg/kg) markedly decreased the change in GHb, compared with each drug alone. A test by two-way ANOVA showed a significant interaction effect of compound A (3 mg/kg) and metformin hydrochloride (300 mg/kg) on the changes in GHb.

Table 1

Group	Change in GHb (%)
Normal control	-0.05 ± 0.10
Diabetic control	1.20 ± 0.32
Metformin hydrochloride, 100 mg/kg	2.08 ± 0.33
Metformin hydrochloride, 300 mg/kg	1.70 ± 0.23
Compound A, 3 mg/kg	0.14 ± 0.39
Compound A (3 mg/kg) + metformin hydrochloride (100 mg/kg)	0.63 ± 0.27
Compound A (3 mg/kg) + metformin hydrochloride (300 mg/kg)	-0.54 ± 0.15*
Change in GHb (%) = GHb (%) after repeated administration - GHb (%) before start of repeated administration	
*: P<0.05 for interaction effect of compound A (3 mg/kg) and metformin hydrochloride (300 mg/kg) (in two-way ANOVA)	

<Result 2>

[0041] Fasting plasma glucose levels are expressed as the mean \pm standard error in Table 2. The Student's t-test was used to detect the difference between two groups. The combination treatment of compound A (3 mg/kg) and metformin hydrochloride (300 mg/kg) markedly decreased the fasting plasma glucose level, compared with each drug alone.

Table 2

Group	Fasting plasma glucose level (mg/dL)
Normal control	76.2 \pm 2.2
Diabetic control	416.2 \pm 24.4
Metformin hydrochloride, 100 mg/kg	378.3 \pm 33.4
Metformin hydrochloride, 300 mg/kg	254.0 \pm 24.7
Compound A, 3 mg/kg	275.0 \pm 28.7
Compound A (3 mg/kg) + metformin hydrochloride (100 mg/kg)	226.8 \pm 18.3 §§
Compound A (3 mg/kg) + metformin hydrochloride (300 mg/kg)	171.6 \pm 13.5 ††, #
§§: P<0.01 vs. metformin hydrochloride (100 mg/kg) group ††: P<0.01 vs. compound A (3 mg/kg) group #: P<0.05 vs. metformin hydrochloride (300 mg/kg) group.	

<Result 3>

[0042] The changes in non-fasting plasma insulin (IRI) levels are expressed as the mean \pm standard error in Table 3. The Student's t-test was used to detect the difference between two groups. The combination treatment of compound A (3 mg/kg) and metformin hydrochloride (300 mg/kg) markedly inhibited the decrease in plasma IRI levels, compared with each drug alone.

Table 3

Group	Change in plasma IRI level (ng/mL)
Normal control	-1.23 ± 0.70
Diabetic control	-9.15 ± 1.05
Metformin hydrochloride, 100 mg/kg	-9.81 ± 0.91
Metformin hydrochloride, 300 mg/kg	-7.36 ± 1.29
Compound A, 3 mg/kg	-5.01 ± 2.11
Compound A (3 mg/kg) + metformin hydrochloride (100 mg/kg)	-5.07 ± 1.50 §
Compound A (3 mg/kg) + metformin hydrochloride (300 mg/kg)	1.88 ± 1.87 †, ##
Change in plasma IRI level (ng/mL) = plasma IRI level (ng/mL) after repeated administration - plasma IRI level (ng/mL) before start of repeated administration	

§: P<0.05 vs. metformin hydrochloride (100 mg/kg) group
†: P<0.05 vs. compound A (3 mg/kg) group
##: P<0.01 vs. metformin hydrochloride (300 mg/kg) group.

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Test Example 2

<Test item>

[0043] Effects of the combination treatment of compound A and glipizide in diabetic mice

<Test method>

[0044] After 3 weeks a high-fat diet (D12492 of Research Diets, Inc.) feeding, ICR mice (CHARLES RIVER LABORATORIES JAPAN, INC.) were intraperitoneally administered streptozocin (SIGMA-ALDRICH JAPAN K.K.; hereinafter abbreviated as STZ) to induce diabetes. Under non-fasting conditions, the high-fat diet loaded mice with STZ-induced diabetes mellitus (15-week old) in groups each consisting of 11-12 mice were orally administered single doses of compound A (1 mg/kg) and an insulin secretagogue, glipizide (10 mg/kg; SIGMA-ALDRICH JAPAN K.K.), either alone or in combination. Blood was taken from the tail vein of each mouse at specified time intervals and plasma glucose levels were measured by GOD colorimetry with a self-testing glucose measuring instrument (Medisafe Mini GR-102; TERUMO CORPORATION, Japan).

<Results>

[0045] The areas under the plasma glucose levels curve for a period up to 8 hours after drug administration (Δ plasma glucose AUC) are expressed as the mean \pm standard error in Table 4. The Welch t-test was used to detect the difference between two groups. The combination treatment of compound A and glipizide markedly decreased Δ plasma glucose AUC, compared with each drug alone.

Table 4

Group	Δ plasma glucose AUC (mg/dL x hr)
Diabetic control	104.7 \pm 137.4
Compound A, 1 mg/kg	-310.3 \pm 157.2
Glipizide, 10 mg/kg	-222.3 \pm 103.2
Compound A (1 mg/kg) + glipizide (10 mg/kg)	-989.7 \pm 232.8 †, ##
†: P<0.05 vs. compound A (1 mg/kg) group ##: P<0.01 vs. glipizide (10 mg/kg) group.	

Test Example 3

<Test item>

[0046] Effects of the combination treatment of compound A and glimepiride in diabetic mice and normal mice

<Test method>

[0047] Under non-fasting conditions, 10 female KKAY mice/group (diabetic mice; 4-week old; CLEA Japan, Inc.) and 10 female C57BL mice/group (normal mice; 4-week old; CLEA Japan, Inc.) were orally administered with single doses of compound A (10 mg/kg) and an insulin secretagogue, glimepiride (0.5 mg/kg; SIGMA-ALDRICH JAPAN K.K.), either alone or in combination. Blood was taken from the tail vein of each mouse at specified time intervals and plasma glucose levels were measured by the GDH electrode method with a self-testing glucose measuring instrument (Glutest Neo Super; SANWA KAGAKU KENKYUSHO CO., LTD.)

<Results>

[0048] The areas under the plasma glucose levels curve for a period up to 3 hours after drug administration (Δ plasma glucose AUC) are expressed as the mean \pm standard error in Table 5 and 6. The two-way ANOVA was used to detect main effects and interaction effect of compound A and glimepiride. In KKAY mice (diabetic mice), the combination

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treatment of compound A and glimepiride markedly decreased Δ plasma glucose AUC, compared with each drug alone. On the other hand, the combination treatment of compound A and glimepiride decreased Δ plasma glucose AUC equally to treatment of glimepiride in normoglycemic C57BL mice (normal mice). The combined compound A with glimepiride exerted no more glucose lowering effect, compared with glimepiride alone. These results suggest that the combination treatment of compound A and glimepiride is expected to reduce the risk of hypoglycemia as a side effect.

Table 5 Effects of the combination treatment of compound A and glimepiride in KKAy mice

Group	Δ plasma glucose AUC (mg/dL x hr)
Diabetic control	0.1 ± 7.3
Compound A, 10 mg/kg	-83.0 ± 14.6
Glimepiride, 0.5 mg/kg	-118.6 ± 20.2
Compound A (10 mg/kg) + glimepiride (0.5 mg/kg)	-151.5 ± 11.6
Main effect of compound A (10 mg/kg): $P < 0.001$ Main effect of glimepiride (0.5 mg/kg): $P < 0.0001$ Interaction effect of compound A (10 mg/kg) and glimepiride (0.5 mg/kg): No significant difference (in two-way ANOVA).	

Table 6 Effects of the combination treatment of compound A and glimepiride in C57BL mice

Group	Δ plasma glucose AUC (mg/dL x hr)
Normal control	16.3 ± 10.8
Compound A, 10 mg/kg	-4.2 ± 8.5
Glimepiride, 0.5 mg/kg	-104.0 ± 9.3
Compound A (10 mg/kg) + glimepiride (0.5 mg/kg)	-97.1 ± 15.1
Main effect of compound A (10 mg/kg): $P = 0.5516$ Main effect of glimepiride (0.5 mg/kg): $P < 0.0001$ Interaction effect of compound A (10 mg/kg) and glimepiride (0.5 mg/kg): No significant difference (in two-way ANOVA).	

Test Example 4

<Test item>

[0049] Effects of chronic combination treatment of compound A and glimepiride in diabetic mice

<Test method>

[0050] Seven or eight female KKAy mice/group (4-week old; CLEA JAPAN, Inc.) were orally administered with repeated doses of compound A (0.03% mixed diet; ad libitum) and an insulin secretagogue, glimepiride (0.5 mg/kg, once daily; SIGMA-ALDRICH JAPAN K.K.), either alone or in combination, for 8 weeks.

[0051] After 4 weeks and 8 weeks, blood was taken from the tail vein of each mouse at one hour after the drug administration under non-fasting conditions. Plasma glucose levels were measured by the GDH electrode method with a self-testing glucose measuring instrument (Glutest Neo Super; SANWAKAGAKU KENKYUSHO CO., LTD.) In addition, body weights were measured both before and the days at 4th and 8th weeks after the start of repeated administration.

<Results>

[0052] The plasma glucose levels at the 4th and 8th weeks of repeated administration are expressed as the mean \pm

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standard error in Table 7 and 8. The two-way ANOVA was used to detect main effects and interaction effect of compound A and glimepiride. Both at the 4th week and the 8th week of repeated administration, the combination treatment of compound A and glimepiride markedly decreased the plasma glucose level, compared with each drug alone.

[0053] The percent changes in body weights from the values before the start of repeated administration are expressed as the mean \pm standard error in Table 9 and 10. The dunnett's test was used to detect the difference between two groups. Both at the 4th week and the 8th week of repeated administration, the glimepiride-treated mice tended to increase the body weights compared with diabetic control mice. On the other hand, the compound A-treated mice and the combination-treated mice significantly decreased body weights, compared with diabetic control mice.

[0054] These results show that the combination treatment with glimepiride and compound A markedly lowered the plasma glucose level and suppressed the body weight gain induced by glimepiride, suggesting the possibility of mitigating a side effect of glimepiride.

Table 7

Group	Plasma glucose level at 4 wk of administration (mg/dL)
Diabetic control	192.1 \pm 3.5
Mixed diet containing compound A, 0.03%	154.8 \pm 5.9
Glimepiride, 0.5 mg/kg	137.6 \pm 13.3
Mixed diet containing compound A (0.03%) + glimepiride (0.5 mg/kg)	115.6 \pm 16.1
Data represented the plasma glucose level at one hour after drug administration. Main effect of mixed diet containing compound A (0.03%): P<0.05 Main effect of glimepiride (0.5 mg/kg): P<0.001 Interaction effect of mixed diet containing compound A (0.03%) and glimepiride (0.5 mg/kg): No significant difference (in two-way ANOVA).	

Table 8

Group	Plasma glucose level at 8 wk of administration (mg/dL)
Diabetic control	206.6 \pm 9.8
Mixed diet containing compound A, 0.03%	168.6 \pm 2.2
Glimepiride, 0.5 mg/kg	160.4 \pm 8.2
Mixed diet containing compound A (0.03%) + glimepiride (0.5 mg/kg)	123.6 \pm 6.2
Data represented the plasma glucose level at one hour after drug administration. Main effect of mixed diet containing compound A (0.03%): P<0.0001 Main effect of glimepiride (0.5 mg/kg): P<0.0001 Interaction effect of mixed diet containing compound A (0.03%) and glimepiride (0.5 mg/kg): No significant difference (in two-way ANOVA).	

Table 9

Group	Weight change (%) at 4 wk of administration
Diabetic control	178.1 \pm 4.3
Mixed diet containing compound A, 0.03%	138.0 \pm 3.7***
Glimepiride, 0.5 mg/kg	189.9 \pm 4.4
Mixed diet containing compound A (0.03%) + glimepiride (0.5 mg/kg)	144.1 \pm 3.7***
Weight change (%) represented the percent change in body weight from the value before the start of repeated administration. ***: P<0.001 vs. diabetic control group	

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Table 10

Group	Weight change (%) at 8 wk of administration
Diabetic control	218.9 \pm 4.7
Mixed diet containing compound A, 0.03%	166.7 \pm 6.0***
Glimepiride, 0.5 mg/kg	232.5 \pm 6.3
Mixed diet containing compound A (0.03%) + glimepiride (0.5 mg/kg)	166.7 \pm 4.4***
Weight change (%) represented the percent change in body weight from the value before the start of repeated administration. ***: P<0.001 vs. diabetic control group	

Test Example 5

<Test item>

[0055] Effects of the combination treatment of compound A and glibenclamide in diabetic mice

<Test method>

[0056] After 3 weeks a high-fat diet (D12492 of Research Diets, Inc.) feeding, ICR mice (CHARLES RIVER LABORATORIES JAPAN, INC.) were intraperitoneally administered with streptozocin (SIGMA-ALDRICH JAPAN K.K.; hereinafter abbreviated as STZ) to induce diabetes. Under non-fasting conditions, the high-fat diet loaded mice with STZ-induced diabetes mellitus (12-week old) in groups each consisting of 10-12 mice were orally administered with single doses of compound A (1 mg/kg) and an insulin secretagogue, glibenclamide (10 mg/kg; SIGMA-ALDRICH JAPAN K.K.), either alone or in combination. Blood was taken from the tail vein of each mouse at specified time intervals and centrifuged to collect plasma. Plasma glucose levels were measured by mutarotase-GOD method with a glucose measuring kit (Glucose C2 Test Wako; Wako Pure Chemical Industries, Ltd.)

<Results>

[0057] The areas under the plasma glucose levels curve for a period up to 8 hours after drug administration (Δ plasma glucose AUC) are expressed as the mean \pm standard error in Table 11. The two-way ANOVA was used to detect main effects and interaction effect of compound A and glibenclamide. The combination treatment of compound A and glibenclamide markedly decreased Δ plasma glucose AUC, compared with each drug alone.

Table 11

Group	Δ plasma glucose AUC (mg/dL x hr)
Diabetic control	407.5 \pm 67.4
Compound A, 1 mg/kg	-130.1 \pm 164.9
Glibenclamide, 10 mg/kg	140.3 \pm 107.2
Compound A (1 mg/kg) + glibenclamide (10 mg/kg)	-550.5 \pm 167.4
Main effect of compound A (1 mg/kg): P<0.0001 Main effect of glibenclamide (10 mg/kg): P<0.05 Interaction effect of compound A (1 mg/kg) and glibenclamide (10 mg/kg): No significant difference (in two-way ANOVA).	

Industrial Applicability

[0058] The present invention can provide superior pharmaceutical compositions for preventing or treating diabetes mellitus that exhibit an effective blood glucose lowering action in many diabetic patients and which yet cause fewer side

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effects. The present invention can also provide pharmaceutical compositions for preventing or treating various diabetic complications that result from hyperglycemia, such as diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, cerebrovascular disorder, ischemic heart disease, and peripheral arterial disease.

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P A T E N T K R A V

1. Farmaceutisk sammensætning omfattende en kombination af
 - (A) (1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol eller et farmaceutisk acceptabelt salt deraf eller et hydrat af forbindelsen eller saltet; og
 - (B) et insulin-sekretionsfremkaldende stof.
2. Farmaceutisk sammensætning ifølge krav 1, hvor det insulin-sekretionsfremkaldende stof er glipizid, glibenclamid eller glimepirid.
3. Farmaceutisk sammensætning ifølge krav 2, hvor det insulin-sekretionsfremkaldende stof er glipizid eller glimepirid.
4. Farmaceutisk sammensætning ifølge krav 2, hvor det insulin-sekretionsfremkaldende stof er glipizid.
5. Farmaceutisk sammensætning ifølge krav 2, hvor det insulin-sekretionsfremkaldende stof er glimepirid.
6. Kombination af:
 - (A) (1S)-1,5-anhydro-1-[5-(4-ethoxybenzyl)-2-methoxy-4-methylphenyl]-1-thio-D-glucitol eller et farmaceutisk acceptabelt salt deraf eller et hydrat af forbindelsen eller saltet; og
 - (B) et insulin-sekretionsfremkaldende stof,til anvendelse i en fremgangsmåde til forebyggelse eller behandling af diabetes mellitus, sygdomme forbundet med diabetes mellitus eller komplikationer fra diabetes mellitus, hvor (A) og (B) er indgivet til en patient med behov enten samtidigt eller separat.
7. kombination til anvendelse ifølge krav 6, hvor det insulin-sekretionsfremkaldende stof er glipizid, glibenclamid, og glimepirid.
8. kombination til anvendelse ifølge krav 7, hvor det insulin-sekretionsfremkaldende stof er glipizid eller glimepirid.
9. kombination til anvendelse ifølge krav 7, hvor det insulin-sekretionsfremkaldende stof er glipizid.
10. kombination til anvendelse ifølge krav 7, hvor det insulin-sekretionsfremkaldende stof er glimepirid.
11. kombination til anvendelse ifølge et hvilket som helst af krav 6 til 10, hvor diabetes mellitus er type 2 diabetes.
12. kombination til anvendelse ifølge et hvilket som helst af krav 6 til 11, hvor komplikationerne fra diabetes mellitus er diabetisk retinopati, diabetisk nefropati, diabetisk neuropati, cerebrovaskulær sygdom, iskæmisk hjertesygdom eller perifer arteriel sygdom.