

# DS-8500a, an Orally Available G Protein-Coupled Receptor 119 Agonist, Upregulates Glucagon-Like Peptide-1 and Enhances Glucose-Dependent Insulin Secretion and Improves Glucose Homeostasis in Type 2 Diabetic Rats<sup>§</sup>

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Received June 19, 2018; accepted September 13, 2018

## ABSTRACT

G protein-coupled receptor 119 (GPR119) has been shown to be highly expressed in small intestinal L-cells and pancreatic  $\beta$ -cells and mediates intracellular cAMP concentration, glucagon-like peptide (GLP-1) secretion, and glucose-stimulated insulin secretion (GSIS). This study examined the pharmacological effects of 4-(5-((1R)-1-[4-(cyclopropylcarbonyl) phenoxy]propyl)-1,2,4-oxadiazol-3-yl)-2-fluoro-N-[(2R)-1-hydroxypropan-2-yl]benzamide (DS-8500a), a novel, orally available, selective GPR119 agonist. In in vitro studies, DS-8500a increased intracellular cAMP in a concentration-dependent manner in human, rat, and mouse GPR119-expressing Chinese hamster ovary (CHO)-K1 cells, with EC<sub>50</sub> values of 51.5, 98.4, and 108.1 nmol/l, respectively. DS-8500a had no effect on intracellular cAMP in pcDNA3.1/CHO-K1 cells. In in vivo studies, DS-8500a augmented plasma GLP-1 concentration in Zucker fatty (ZF) rats, and enhanced

GSIS and did not change plasma glucose concentration in fasted Sprague-Dawley (SD) rats. A single dose of DS-8500a showed dose-dependent glucose-lowering effects at oral glucose tolerance test (OGTT) in ZF rats. In a repeat-dosing study, DS-8500a had statistically significant glucose-lowering effects at OGTT performed at the 1st day and after 2 weeks of treatment in neonatal streptozotocin-treated (nSTZ) rats, and the efficacy levels of DS-8500a in each test were greater than those of GSK1292263 or MBX-2982, which had been clinically tested previously as GPR119 agonists. Through pharmacokinetics and pharmacodynamics assessment, the high intrinsic activity of DS-8500a was suggested to be one of the reasons for the greater glucose lowering effect in the nSTZ rats. DS-8500a is a useful compound among GPR119 agonists that can maximize the potential benefit of GPR119 in type 2 diabetes.

## Introduction

The rapid increase in the number of patients with type 2 diabetes mellitus (T2DM) worldwide has become a serious challenge for global public health. At present, there are several classes of oral antidiabetic agents available for the treatment of T2DM: sulfonylureas, short-acting insulin secretagogues (meglitinides), dipeptidyl peptidase 4 (DPP-4) inhibitors to increase insulin secretion, thiazolidinediones to improve insulin resistance, biguanides (metformin) to reduce hepatic glucose production, and sodium glucose transporter 2 inhibitors to increase urinary glucose excretion. Injectable glucagon-like peptide-1 (GLP-1) analogs that slow gastric emptying, reduce appetite, and improve insulin secretion are also available. However, given that pancreatic  $\beta$ -cell

dysfunction is a major contributor to the progression of T2DM, a new drug with a mechanism that helps improve insulin secretion from pancreatic  $\beta$ -cells in a mechanism distinct from currently available drugs may hold great promise as a new addition to conventional antidiabetic therapy. As a novel target for antidiabetic drugs, G protein-coupled receptors GPR119 or GPR40 have been noticed by many pharmaceutical companies, because it is possible to synthesize a small-molecule, orally available agonist for each receptor. Both GPRs have similar characteristics, for instance, their tissue distribution (Edfalk et al., 2008; Odori et al., 2013) and their agonists' functions (Shapiro et al., 2005; Chu et al., 2007). On the other hand, there are two significant points of difference. One is the chemical structures of their agonists. GPR119 recognizes 2-monoacylglycerol as the endogenous ligands, whereas GPR40 recognizes medium or long-chain fatty acid (Itoh et al., 2003; Hansen et al., 2011; Ekberg et al., 2016). Like the endogenous ligands, the basic structures of synthetic agonists are also different. GPR40 agonists need the

This work was funded by Daiichi Sankyo Co., Ltd. All authors are employees of Daiichi Sankyo Co., Ltd.

<https://doi.org/10.1124/jpet.118.250019>.

<sup>§</sup> This article has supplemental material available at [jpet.aspetjournals.org](http://jpet.aspetjournals.org).

**ABBREVIATIONS:** AUC, area under the curve; CHO, Chinese hamster ovary cell; DPP-4, dipeptidyl peptidase 4; DS-8500a, 4-(5-((1R)-1-[4-(cyclopropylcarbonyl) phenoxy]propyl)-1,2,4-oxadiazol-3-yl)-2-fluoro-N-[(2R)-1-hydroxypropan-2-yl]benzamide; GLP-1, glucagon-like peptide; GPR, G protein-coupled receptor; GSIS, glucose-stimulated insulin secretion; MC, methylcellulose; nSTZ, streptozotocin-treated; OGTT, oral glucose tolerance test; PG, plasma glucose; T2DM, type 2 diabetes mellitus; ZF, Zucker fatty.

presence of a pharmacophoric carboxylic acid group in their structure (Rodrigues et al., 2018); on the other hand, clinical GPR119 agonists, including DS-8500a, do not have carboxylic acid group in their structure (Ritter et al., 2016). Another difference is the species of G protein-coupled receptor ( $G\alpha$  and  $Gq$  couple with GPR119 and GPR40, respectively) (Shapiro et al., 2005; Soga et al., 2005).

In this report, we focus on the GPR119 agonist class. GPR119 is a class A, rhodopsin-like G protein-coupled receptor first found by searches in human genome databases (Fredriksson et al., 2003). Unlike the GLP-1R, which is a class B G protein-coupled receptor, synthesis of orally active, small-molecule agonists for GPR119 is relatively easy. Considering that injectable peptide agonists of the GLP-1 receptor have significant antidiabetic effects, similar incretin-like effects could be achieved orally by GPR119 agonist, because GLP-1 receptor is also  $G_s$ -coupled and increases cAMP concentration in pancreatic  $\beta$ -cells, and the concentration of plasma GLP-1 itself is upregulated by GPR119 agonist treatment. The identification and optimization of orally available GPR119 agonists has been performed in many pharmaceutical companies, and there are numerous clinical candidates in the GPR119 agonist class (Jones et al., 2009; Ritter et al., 2016). However, there are some differences in pharmacological properties among GPR119 agonist class members. For example, in silico and in vitro studies suggest that synthetic GPR119 agonists differ in their energetically accessible conformation and relative intrinsic activity (McClure et al., 2011). In addition, an in vivo study suggested some GPR119 agonists risk losing efficacy during short term, repeat-dosing studies (Kang, 2013). For example, GSK1292263 did not reduce area-under-the-curve (AUC) glucose (0–24 hours) when administered alone or co-dosed with sitagliptin or metformin on day 14 in a phase 2 trial (Nunez et al., 2014), and NBI104 (30 mg/kg) showed loss of efficacy following 4 days of once-daily dosing in Zucker diabetic fatty rats (Barnes et al., 2010). Therefore, to choose a GPR119 agonist for further development in the clinical stage, two points are critical: One is to confirm the basic pharmacological profile of the compound by estimating the tissue distribution of GPR119, and the other is to obtain differentiation data from other GPR119 agonists that had negative results or risks during clinical development.

DS-8500a is an orally available selective GPR119 agonist synthesized in Daiichi Sankyo, Co., Ltd. Here, the multiple pharmacological effects of DS-8500a were assessed on the basis of tissue distribution of GPR119, augmentation of plasma GLP-1, glucose-stimulated insulin secretion (GSIS), and hypoglycemia risk. Furthermore, the risk of loss of efficacy during repeat dosing of DS-8500a was assessed by comparison with GSK1292263 and MBX-2982, which had already been studied in clinical stages.

## Materials and Methods

### Test Compounds

DS-8500a (4-(5-((1R)-1-[4-(cyclopropylcarbonyl)phenoxy]propyl)-1,2,4-oxadiazol-3-yl)-2-fluoro-*N*-[(2R)-1-hydroxypropan-2-yl]benzamide), GSK1292263 ((5-((1-[3-(1-methylethyl)-1,2,4-oxadiazol-5-yl]-4-piperidinyl)methyl)oxy]-2-[4-(methylsulfonyl)phenyl]pyridine)), and MBX-2982 (5-ethyl-2-[4-[4-(4-tetrazol-1-yl-phenoxy)methyl]-thiazol-2-yl]-piperidin-1-yl)-pyrimidine) were synthesized at Medicinal Chemistry Research Laboratories, Daiichi Sankyo Co., Ltd. (Tokyo, Japan). The chemical structure of DS-8500a is shown (Fig. 1). The test compounds were stored in a refrigerator at 4°C and protected from light. glimepiride

was purchased from MilliporeSigma Corporation (St. Louis, MO). Nateglinide and glibenclamide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

### In Vitro cAMP Assay

Human GPR119 cDNA was amplified by using PCR primers as follows:

Forward primer: 5'-ggggacaagttgtacaaaaaagcaggcttcaccATGG-AATCATCTTTCTCATTGGAGTG-3', (inserts a BamHI site at the 5' end)

Reverse primer: 5'-ggggaccactttgtacaagaaagctgggtcTTAGCA-TCAACTCTGAGCTGGAG-3'.

Mouse GPR119 cDNA was amplified by using primers as follows:

Forward primer: 5'-ggggacaagttgtacaaaaaagcaggcttcaccATGG-AGTCATCCTTCTCATTGGAGTG-3',

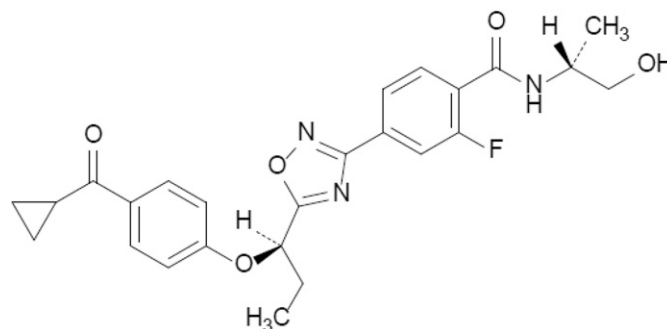
Reverse primer: 5'-ggggaccactttgtacaagaaagctgggtcTTAGCA-TCGAGCTCCGGATGGCTG-3'.

Amplified human and mouse cDNA were cloned in the pcDNA3.1 through pDONR/Zeo (Gateway system; Invitrogen/Thermo Fisher Scientific, Waltham, MA). Rat GPR119 cDNA was amplified by using primers as follows:

Forward primer: 5'-ATGGAGTCATCTTTCTCATTGGAGTGA-3',

Reverse primer: 5'-TTATCCCCCTGCATGTCCTCAGAGGAT-3',

and cloned in the pcDNA3.1 TOPO TA expression kit (Invitrogen/Thermo Fisher Scientific). The Chinese hamster ovary cell (CHO-K1 cell; Summit Pharmaceuticals International Corporation, Tokyo, Japan) was used as the host cells, and human, rat, and mouse GPR119-expressing CHO-K1 stable cell lines were generated by cotransfection of pcDNA3.1-expression vector. The CHO-K1 cells expressing human, rat, and mouse GPR119-pcDNA3.1 are abbreviated as hGPR119/CHO-K1, rGPR119/CHO-K1, and mGPR119/CHO-K1, respectively. For the calculation of  $EC_{50}$  values, cAMP-Screen Direct System (Invitrogen/Thermo Fisher Scientific) was used for the calculation. Human, rat and mouse GPR119/CHO-K1 cells were seeded in a precoated microplate at  $2 \times 10^4$  cells/well and cultured overnight under 5%  $CO_2$  at 37°C. A standard curve was generated by the four-parameter fitting method using relative light units (RLU) and nominal cAMP (pmol/well) of standard solutions, and the amount of cAMP in the cells was determined on the basis of the standard curve. Test compound (DS-8500a) was diluted with 0.5% bovine serum albumin/5 mmol/l IBMX/Ham (F12) to prepare 0, 0.0256, 0.128, 0.64, 3.2, 16, 80, 400, 2000, and 10,000 nmol/l of loading solution. The  $EC_{50}$  values and 95% confidence intervals were obtained from a logistic curve that was generated by the four-parameter method using the amount of cAMP produced by h/r/m GPR119/CHO-K1 cells treated with loading solutions at each concentration of test compound (0, 0.0256, 0.128, 0.64, 3.2, 16, 80, 400, 2000, and 10,000 nmol/l).



**Fig. 1.** Chemical structure of DS-8500a (4-(5-((1R)-1-[4-(cyclopropylcarbonyl)phenoxy]propyl)-1,2,4-oxadiazol-3-yl)-2-fluoro-*N*-[(2R)-1-hydroxypropan-2-yl]benzamide).

## Animals

All procedures involving animal use were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd. All rats were fed regular chow FR-2 purchased from Funabashi Farm Co., Ltd. (Chiba, Japan) and tap water ad libitum with controlled temperature ( $23 \pm 2^\circ\text{C}$ ), humidity ( $50\% \pm 10\%$ ), and lighting (lights on from 7:00 AM to 7:00 PM). Male Zucker fatty (ZF) (Crlj:ZUC-*Lepr<sup>fa</sup>* [Zucker fatty]) rats (Charles River Laboratories Japan, Inc., Yokohama, Japan) were used in the experiments for the plasma GLP-1 concentration and single-dose oral glucose tolerance test (OGTT). Male Sprague-Dawley (SD) (Crl:CD [Sprague Dawley]) rats (Charles River Laboratories Japan, Inc.) were used in the experiments for GSIS, hypoglycemia risk assessment, and the treatment of STZ for the repeat-dosing study. 0.5% Methylcellulose (MC; Wako Pure Chemical Industries, Ltd.) was used as an oral vehicle in all animal studies.

## Effect on Plasma GLP-1 Concentration

At 11 weeks of age, male ZF rats were fasted overnight and orally given vehicle (0.5% MC) or DS-8500a (3 mg/kg). Thirty minutes later, the glucose (–) and glucose (+) group animals received a distilled water or a glucose solution (1 g/kg), respectively. Blood collection was performed from the tail vein 35 minutes before (defined as –30-minute value), 5 minutes before (defined as 0-minute value), and 10, 30, 60, 120, and 180 minutes after the administration of distilled water or glucose. The blood was transferred into the blood collection tube containing aprotinin (50 KIU/tube; Wako Pure Chemical Industries, Ltd.) and DPP-4 inhibitor (5  $\mu\text{L}$ ; MilliporeSigma Corporation, Burlington, MA). Plasma was separated by centrifugation at 11,000 rpm for 5 minutes at room temperature. Plasma total GLP-1 concentration was measured with ELISA kits (MilliporeSigma Corporation).

The delta plasma total GLP-1 concentration ( $\Delta\text{GLP-1}$ ) was calculated using the following equation:

$$\Delta\text{GLP-1} = C_x - C_{-30} (C_x: \text{the plasma concentration of GLP-1}$$

$$\text{X min after the administration of distilled water or glucose}) \quad (1)$$

The delta AUC of plasma total GLP-1 concentration (pM·min) from –30 to 180 minutes after the administration of distilled water was calculated using the following equation:

$$\Delta\text{AUC} = \text{AUC} - C_{-30} \times \{180 - (-30)\} \quad (2)$$

## GSIS

At 8 weeks of age, male SD rats were fasted overnight and orally given vehicle (0.5% MC), DS-8500a (0.1, 1, 3 and 10 mg/kg), or glimepiride (10 mg/kg). Thirty minutes later, all animals received a 50% glucose solution intravenously (glucose load: 0.5 g/kg). Blood collection was performed from the tail vein 35 minutes before, 5 minutes before, and 5 minutes after the glucose load. The plasma insulin concentration was measured using ELISA kits (Morinaga Institute of Biologic Science, Inc., Yokohama, Japan). The values which were obtained 35 minutes before the glucose load were defined as the prevalue. The delta value of plasma insulin concentration was calculated using the following equations:

$$\Delta\text{INS}_{\text{glc-}} (\text{glucose stimulation-independent insulin secretion}) = \text{INS}_{-5} - \text{INS}_{\text{pre}} \quad (3)$$

$$\Delta\text{INS}_{\text{glc+}} (\text{glucose stimulation-dependent insulin secretion}) = \text{INS}_5 - \text{INS}_{-5} \quad (4)$$

## Hypoglycemia Risk Assessment

At 8 weeks of age, male SD rats were fasted overnight and orally given vehicle (0.5% MC), DS-8500a (10 mg/kg), nateglinide (50 mg/kg), glimepiride (10 mg/kg), or glibenclamide (10 mg/kg). Blood was

collected from the tail vein within 1 hour prior to the administration of DS-8500a or comparators (defined as prevalue, used as 0-hour value) and at 1, 2, 4, 6, and 24 hours after the administration of DS-8500a or comparators. The plasma glucose (PG) was measured using an automatic glucose analyzer (GA05; A&T Corporation, Yokohama, Japan). The delta value of PG ( $\Delta\text{PG}$ ) was calculated using the following equation:

$$\Delta\text{PG}_x = \text{PG}_x - \text{PG}_{\text{pre}} (\Delta\text{PG}_x: \text{delta value of PG} \times \text{h after } [\text{PG}_x] \text{ and before } [\text{PG}_{\text{pre}}] \text{ the administration of DS-8500a or comparators, } [\text{PG}_{\text{pre}}] = [\text{PG}_0]) \quad (5)$$

The areas under the curve (AUCs) (mg·h/dl) of  $\Delta\text{PG}$  from 0 to 2 hours ( $\Delta\text{PG-AUC}_{(0-2)}$ ), from 0 to 6 hours ( $\Delta\text{PG-AUC}_{(0-6)}$ ), and from 0 to 24 hours ( $\Delta\text{PG-AUC}_{(0-24)}$ ) after the administration of DS-8500a or comparators were calculated using the following equations:

$$\Delta\text{PG-AUC}_{(0-2)} = \{(\Delta\text{PG}_0 + \Delta\text{PG}_1) \times (1-0)\}/2 + \{(\Delta\text{PG}_1 + \Delta\text{PG}_2) \times (2-1)\}/2 \quad (6)$$

$$\Delta\text{PG-AUC}_{(0-6)} = \{(\Delta\text{PG}_0 + \Delta\text{PG}_1) \times (1-0)\}/2 + \{(\Delta\text{PG}_1 + \Delta\text{PG}_2) \times (2-1)\}/2 + \{(\Delta\text{PG}_2 + \Delta\text{PG}_4) \times (4-2)\}/2 + \{(\Delta\text{PG}_4 + \Delta\text{PG}_6) \times (6-4)\}/2 \quad (7)$$

$$\Delta\text{PG-AUC}_{(0-24)} = \{(\Delta\text{PG}_0 + \Delta\text{PG}_1) \times (1-0)\}/2 + \{(\Delta\text{PG}_1 + \Delta\text{PG}_2) \times (2-1)\}/2 + \{(\Delta\text{PG}_2 + \Delta\text{PG}_4) \times (4-2)\}/2 + \{(\Delta\text{PG}_4 + \Delta\text{PG}_6) \times (6-4)\}/2 + \{(\Delta\text{PG}_6 + \Delta\text{PG}_{24}) \times (24-6)\}/2 \quad (8)$$

## OGTT in Zucker Fatty Rats

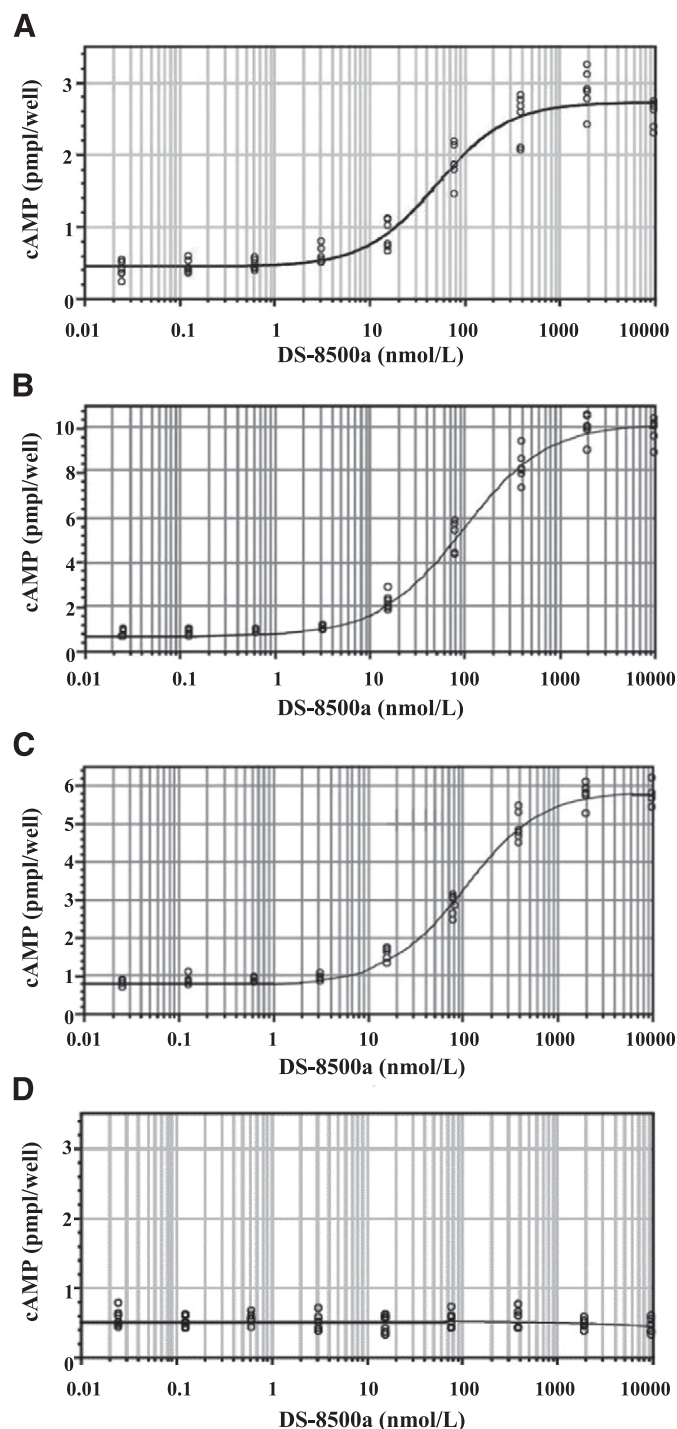
At 11 weeks of age, male ZF rats were fasted overnight and orally given vehicle (0.5% MC) or DS-8500a (1, 3 and 10 mg/kg). Thirty minutes later, all animals received a 50% glucose solution (Otsuka Pharmaceutical Factory, Inc., www.otsuka.com) orally (glucose load: 2.5 g/kg). Blood collection was performed as described above within 1 hour prior to the administration of vehicle or DS-8500a (defined as prevalue), 5 minutes before (defined as 0-hour value) and 0.5, 1, 1.5, 2, and 3 hours after the glucose load. The AUC of plasma glucose level (mg·h/dl) from 0 to 3 hours after the glucose load was calculated using following equation:

$$\text{AUC} = \{(C_0 + C_{0.5} \times (0.5-0))\}/2 + \{(C_{0.5} + C_1 \times (1-0.5))\}/2 + \{(C_1 + C_{1.5} \times (1.5-1))\}/2 + \{(C_{1.5} + C_2 \times (2-1.5))\}/2 + \{(C_2 + C_3 \times (3-2))\}/2 (C_x: \text{plasma glucose level} \times \text{h after the glucose load}) \quad (10)$$

## Repeat-Dosing Study in Neonatal STZ Rats

Male neonatal STZ-induced SD rats (7 weeks of age) were orally given vehicle (0.5% MC), DS-8500a (30 mg/kg), GSK1292263 (30 mg/kg), or MBX-2982 (30 mg/kg) once a day for 2 weeks. At day 0 and day14, to perform OGTT, the rats were fasted overnight and orally given vehicle or compounds. Thirty minutes later, all animals received a 50% glucose solution (Otsuka Pharmaceutical Factory, Inc.) orally (glucose load: 2.0 g/kg). Blood was collected from tail vein within 30 minutes prior to the compound administration (defined as –30 minutes), and 5 minutes before (defined as –5 minutes), 30, 60, 120, and 180 minutes after the glucose load. The AUC of plasma glucose ( $\text{AUC}_{\text{PG}}$ ) levels from –5 to 180 minutes after the glucose load were calculated using the following equation:

$$\text{AUC}_{\text{PG}} = \{(C_{-5} + C_{30}) \times (30 - (-5))\}/2 + \{(C_{30} + C_{60}) \times (60 - 30)\}/2 + \{(C_{60} + C_{120}) \times (120 - 60)\}/2 + \{(C_{120} + C_{180}) \times (180 - 120)\}/2 (C_x: \text{plasma glucose level} \times \text{min after the glucose load}) \quad (11)$$



**Fig. 2.** DS-8500a showed potent agonist activities in human, rat, and mouse GPR119-expressing CHO-K1 cells. Effects of DS-8500a on cAMP production in human (A), rat (B), and mouse (C) GPR119/CHO-K1 cells and in pcDNA3.1/CHO-K1 cells (D). DS-8500a at the final concentrations of 0, 0.0256, 0.128, 0.64, 3.2, 16, 80, 400, 2000, and 10,000 nmol/l ( $N = 6$  wells) was added to the CHO-K1 cells expressing human, rat, and mouse GPR119, or CHO-K1 cell transfected with pcDNA3.1 expression vector (pcDNA3.1/CHO-K1 cells). After a 1-hour incubation, intracellular cAMP was measured and  $EC_{50}$  was obtained from a logistic curve that was generated by the four-parameter method.

For the pharmacokinetics and pharmacodynamics assessment in streptozotocin-treated (nSTZ) rats, an additional in vitro study using rat GPR119-expressing CHO-K1 cells was performed. The study protocol was the same as described above (in vitro cAMP assay) except

for the culture medium. Fetal bovine serum was used in this experiment instead of the Ham12.

**Statistical Analysis.** Data are expressed as mean  $\pm$  S.E.M. Statistical analyses between two groups were performed by Student's *t* test. For multiple comparisons, the Dunnett's test was performed. A *P* value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SAS System Release 8.2 or 9.2 (SAS Institute Inc., Tokyo, Japan).

## Results

### $EC_{50}$ Values of DS-8500a in Human, Rat, and Mouse GPR119-Expressing CHO-K1 Cells

The chemical structure of DS-8500a is shown in Fig. 1. DS-8500a increased intracellular cAMP in human, rat, and mouse GPR119/CHO-K1 cells in a concentration-dependent manner (Fig. 2, A–C), and their 50% effective concentrations were 51.5, 98.4, and 108.1 nmol/l, respectively (Table 1). DS-8500a had no effect on intracellular cAMP in pcDNA3.1/CHO-K1 cells (Fig. 2D). In addition, in vitro pharmacological activities of DS-8500a on a total of 67 receptors, channels, and transporters were evaluated by radioligand binding assays (Supplemental Table 1). DS-8500a at 10  $\mu$ mol/l inhibited the binding of the radioligands to melatonin MT1 binding site ( $IC_{50}$ : 12.1  $\mu$ mol/l), and it had no effect on the other receptors, channels, or transporters. These results indicated that DS-8500a had potent and selective agonistic effects on human, rat, and mouse GPR119.

**Effect on Plasma GLP-1 Concentration.** The time course of plasma total GLP-1 concentration in Zucker fatty rats treated with or without glucose load after the treatment of DS-8500a was monitored (Fig. 3A). Under without glucose [glucose (–)] condition, the  $\Delta$ GLP-1 in the DS-8500a/glucose (–) group at 0, 10, 30, 60, 120, and 180 minutes was statistically significantly higher than that of the control/glucose (–) group (Fig. 3B). In addition, the  $\Delta$ AUC of GLP-1 in the DS-8500a/glucose (–) group was statistically significantly higher than that of the control/glucose (–) group (Fig. 3C). Under glucose (+) condition, the AUC of GLP-1 in the DS-8500a/glucose (+) group was statistically significantly higher than that of the control/glucose (+) group (Fig. 3D). As a consequence, plasma total GLP-1 concentrations were significantly upregulated by treatment with DS-8500a and this effect was observed in both glucose (–) and (+) conditions.

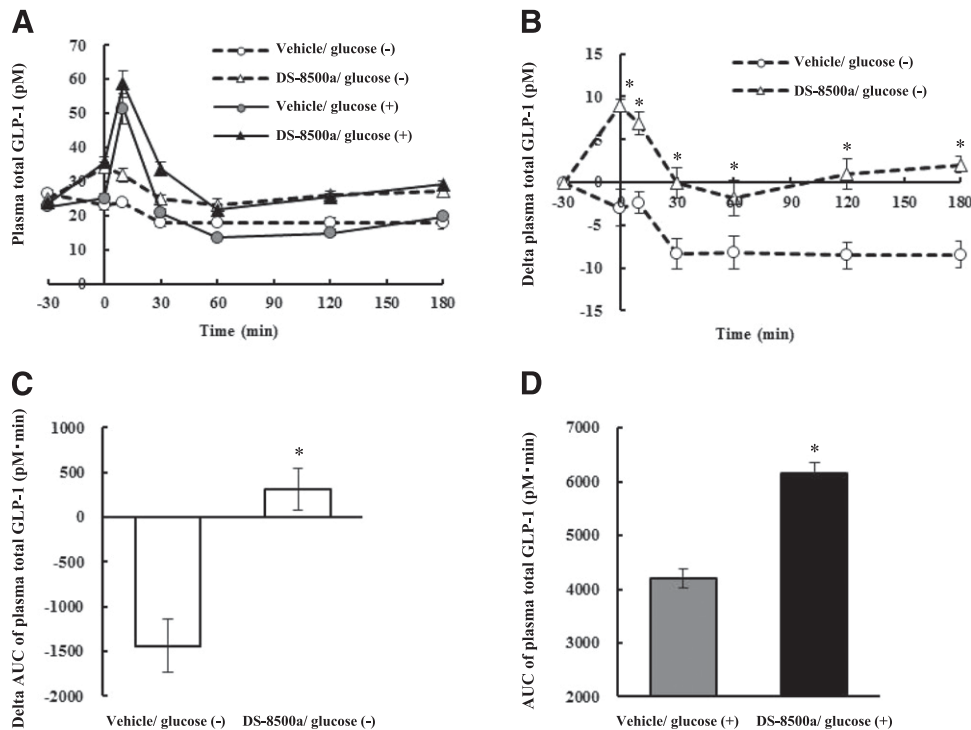
**GSIS.** The change in plasma insulin concentration after DS-8500a or glimepiride treatments in SD rats was monitored (Fig. 4A). Before the glucose load, the  $\Delta$ INS<sub>glc</sub> in the glimepiride-administered group of 10 mg/kg was statistically significantly higher than that of the control group. On

**TABLE 1**

$EC_{50}$  values of DS-8500a on cAMP production in human, rat, and mouse GPR119/CHO-K1 cells and in pcDNA3.1/CHO-K1 cells

Each  $EC_{50}$  value was obtained from a logistic curve that was generated by the four-parameter method. Effects of DS-8500a on cAMP elevation in human, rat, and mouse GPR119 transfected CHO-K1 cells.

Cell	$EC_{50}$ nmol/l	95% Confidence Interval	
		Lower nmol/l	Upper nmol/l
hGPR119/CHO-K1	51.5	37.8	65.2
rGPR119/CHO-K1	98.4	82.9	113.9
mGPR119/CHO-K1	108.1	92.9	123.4



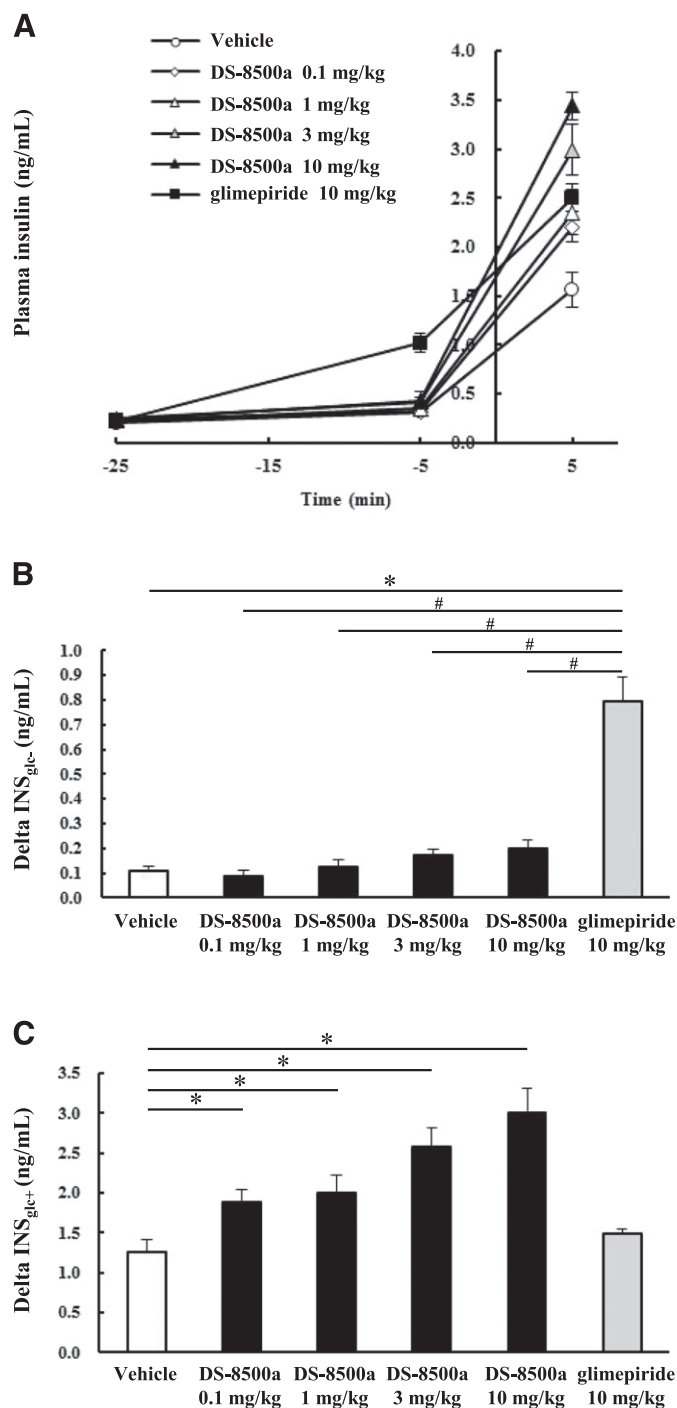
**Fig. 3.** Effects of DS-8500a on plasma GLP-1 concentration. DS-8500a was administered orally at the dosage of 3 mg/kg to overnight-fasted ZF rats. 0.5% MC was administered to the control groups. Thirty minutes after the administration of DS-8500a or 0.5% MC, glucose was administered orally at a dosage of 1 g/kg to the glucose (+) groups and distilled water was administered to the glucose (–) groups. The plasma total GLP-1 concentration was measured 30 minutes before and 0, 10, 30, 60, 120, and 180 minutes after the administration of glucose or distilled water. Data are expressed as the mean  $\pm$  S.E.M. [ $N = 6$  and 18 in the glucose (–) group and glucose (+) group, respectively]. (A) Time course of plasma total GLP-1 concentration in Zucker fatty rats treated with or without glucose load. (B) Delta plasma total GLP-1 concentration without glucose load. The  $\Delta$ plasma total GLP-1 concentration at each time point except for –30 minutes in the DS-8500a/glucose (–) group was compared with that of the control/glucose (–) group by a Student's  $t$  test.  $*P < 0.05$  vs. control group. (C) Delta AUC of plasma total GLP-1 concentration without glucose load. The  $\Delta$ AUC of plasma total GLP-1 concentration (from –30 to 180 minutes) was calculated by the  $\Delta$ plasma total GLP-1 concentration. The  $\Delta$ AUC of plasma total GLP-1 concentration in the DS-8500a/glucose (–) group was compared with that of the control/glucose (–) group by a Student's  $t$  test.  $*P < 0.05$  vs. the control group. (D) AUC of plasma total GLP-1 concentration with glucose load. The AUC of plasma total GLP-1 concentration (from –30 to 180 minutes) was calculated. The AUC of plasma total GLP-1 concentration in the DS-8500a/glucose (+) group was compared with that of the control/glucose (+) group by a Student's  $t$  test.  $*P < 0.05$  vs. the control group.

the other hand, the  $\Delta\text{INS}_{\text{glc-}}$  in the DS-8500a-administered groups did not show significant differences compared with the control group (Fig. 4B). In addition, the  $\Delta\text{INS}_{\text{glc-}}$  in the DS-8500a-administered groups were statistically significantly lower than that of the glimepiride-administered group (Fig. 4B). On the other hand, after the glucose load, the GSIS ( $\Delta\text{INS}_{\text{glc+}}$ ) in the DS-8500a-administered groups of 0.1, 1, 3, and 10 mg/kg was statistically significantly higher than that of the control group, and these effects were dose-dependent in the range of 0.1–10 mg/kg (Fig. 4C).

**Hypoglycemia Risk Assessment.** The hypoglycemic risk of DS-8500a, nateglinide, glimepiride, and glibenclamide was assessed in fasted SD rats. The time course of plasma glucose after the treatment with these insulin secretagogues was monitored (Fig. 5A). The  $\Delta\text{PG-AUC}_{(0-2)}$  in the DS-8500a-administered group did not show significant difference compared with that of the control group (Fig. 5B). On the other hand, the  $\Delta\text{PG-AUC}_{(0-2)}$  values in the nateglinide-, glimepiride-, and glibenclamide-administered groups were significantly lower than that of the control group (Fig. 5B). In addition, the  $\Delta\text{PG-AUC}_{(0-2)}$  in the DS-8500a-administered group was significantly higher than that of the nateglinide-, glimepiride- and glibenclamide-administered groups (Fig. 5B). The  $\Delta\text{PG-AUC}_{(0-6)}$  in the DS-8500a-administered group did not show significant difference compared with that of the control

group (Fig. 5C). The  $\Delta\text{PG-AUC}_{(0-6)}$  in the nateglinide-administered group did not show significant difference compared with that of the control group, but in the glimepiride- and glibenclamide-administered groups,  $\Delta\text{PG-AUC}_{(0-6)}$  were significantly lower than that of the control group (Fig. 5C). The  $\Delta\text{PG-AUC}_{(0-6)}$  in the DS-8500a-administered group was significantly higher than that of the nateglinide-, glimepiride- and glibenclamide-administered groups (Fig. 5C). The  $\Delta\text{PG-AUC}_{(0-24)}$  in the DS-8500a-administered group did not show significant difference compared with that of the control group (Fig. 5D). The  $\Delta\text{PG-AUC}_{(0-24)}$  in the nateglinide-administered group did not show significant difference compared with that of the control group, but in the glimepiride- and glibenclamide-administered groups,  $\Delta\text{PG-AUC}_{(0-24)}$  values were significantly lower than that of the control group (Fig. 5D). The  $\Delta\text{PG-AUC}_{(0-24)}$  in the DS-8500a-administered group did not show significant difference compared with that of the nateglinide-administered group, but was significantly higher than those of the glimepiride and glibenclamide-administered groups.

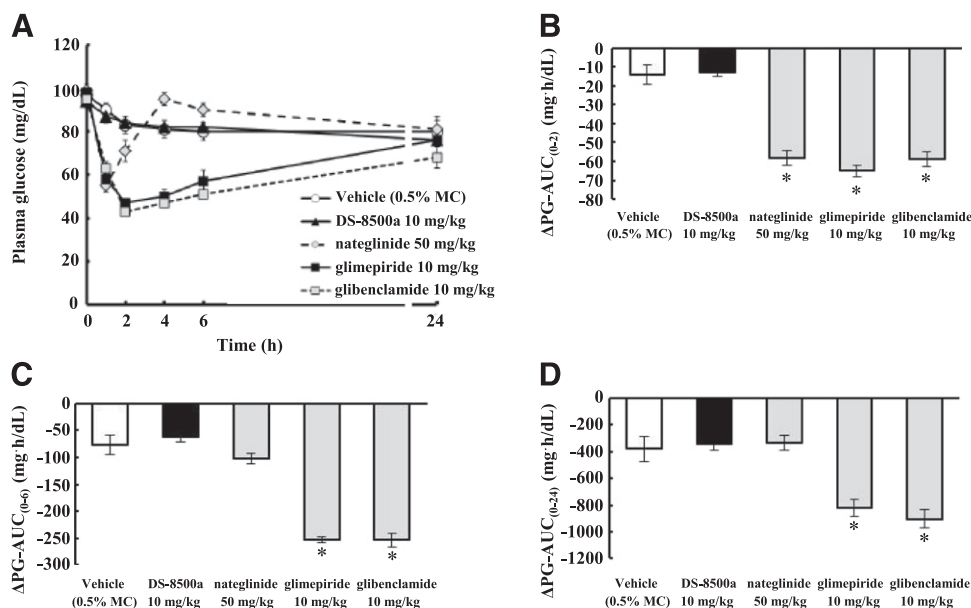
**OGTT in Zucker Fatty Rats.** The single oral administration of DS-8500a at dosages of 1, 3, and 10 mg/kg before glucose load showed statistically significant lowering in the AUC of plasma glucose level (from 0 to 3 hours after glucose load) in a dose-dependent manner compared with the control (Fig. 6).



**Fig. 4.** Effects of DS-8500a and glimepiride on the change in plasma insulin concentration before and after glucose load in SD rats. DS-8500a was administered orally at the dosage of 0.1, 1, 3 and 10 mg/kg, and glimepiride was administered at the dosage of 10 mg/kg to overnight-fasted SD rats. 0.5% MC was administered to the control group. Thirty minutes after the administration of DS-8500a, glimepiride, or 0.5% MC, glucose was loaded intravenously at a dosage of 0.5 g/kg to all the groups. The plasma insulin concentration was measured 35 minutes before (shown as the prevalue ( $INS_{pre}$ )) and 5 minutes before ( $INS_{-5}$ ) and after ( $INS_5$ ) the glucose load. Data are expressed as the mean  $\pm$  S.E.M. ( $N = 12, 11, 9, 11, 11$ , and  $6$  in the control group, the DS-8500a-administered groups of 0.1, 1, 3, and 10 mg/kg and the glimepiride-administered group, respectively.) (A) Plasma insulin concentration before and after glucose load. The  $\Delta INS_{glc-}$  was calculated by subtracting  $INS_{pre}$  from  $INS_{-5}$ . The  $\Delta INS_{glc-}$  in the glimepiride-administered group was compared with that of the control group by Student's  $t$  test. \* $P < 0.05$  vs. the control group. The

**Repeated Dose in Neonatal STZ Rats.** The neonatal STZ-induced rats showed a statistically significant impaired glucose tolerance compared with control rats (Fig. 7B) and the severity of glucose intolerance was slightly increased by aging because the  $AUC_{PG}$  in vehicle group during OGTT performed at day 14 was numerically increased from that of the  $AUC_{PG}$  at day 0 (Fig. 7, D and B). During OGTT performed at day 0, a single treatment of DS-8500a showed statistically significant glucose lowering compared with vehicle group. The  $AUC_{PG}$  in the single treatment of the GSK1292263 or MBX2982 groups was numerically lower than that of the vehicle group, but their effects were modest (not statistically significant vs. vehicle). During OGTT performed at day 14, the  $AUC_{PG}$  of DS-8500a group was statistically significantly lower than that of the vehicle group as well as the test performed at day 0, and no statistically significant effects on the  $AUC_{PG}$  were observed in the GSK1292263- or MBX2982-treated groups compared vehicle group. To assess the reason why only DS-8500a had statistically significant glucose-lowering effects during OGTTs compared with vehicle group, plasma insulin concentration was measured (Supplemental Fig. 1; Supplemental Table 2). At 30 minutes after glucose administration, modest increases in plasma insulin concentration were observed in DS-8500a treated groups compared with that of the GSK1292263- or MBX-2982-treated groups during OGTTs performed on days 0 and 14 (Supplemental Table 2). This modest augmentation of plasma insulin concentration was supposed to contribute the statistically significant glucose-lowering effects in the DS-8500a-treated group compared with vehicle group. In terms of future study for the contribution of insulin on the glucose-lowering effect during OGTT, a time point to collect blood sample immediately after glucose load (for example, 15 minutes after glucose load) will be needed, because the peak value of plasma insulin concentration was achieved within 30 minutes after oral glucose load in neonatal STZ-induced rats (Cancelas et al., 2006). In addition, plasma concentrations of each compound were measured and the fold values against their  $EC_{50}$  values on rat GPR119 were compared among three compounds (Supplemental Table 3). The plasma concentration of each of three compounds at the point of glucose entry during OGTT was considered sufficient to exert a pharmacological effect, because the plasma concentrations were beyond their  $EC_{50}$  values on rat GPR119 (Supplemental Table 3). Since MBX-2982 had greater plasma exposure levels compared with those of DS-8500a (Supplemental Table 3), the intrinsic activity levels of MBX-2982 and DS-8500a for the intracellular cAMP production in rat GPR119-expressing CHO-K1 cells were estimated. There was 30% difference in the intrinsic activity between MBX-2982 and DS-8500a (76% and 106%, respectively, Supplemental Fig. 2). All three compounds had no effect on plasma fasting glucose level after 2-weeks of treatment (Supplemental Table 4).

$\Delta INS_{glc-}$  in each DS-8500a-administered group was compared with that of the glimepiride-administered group by a Dunnett's test. # $P < 0.05$  vs. the glimepiride-administered group. (C) Glucose-stimulated insulin secretion after glucose load. The  $\Delta INS_{glc+}$  was calculated by subtracting  $INS_{-5}$  from  $INS_5$ . The  $\Delta INS_{glc+}$  in each DS-8500a-administered group was compared with that of the control group by the closed testing procedure using a Student's  $t$  test. \* $P < 0.05$  vs. the control group.



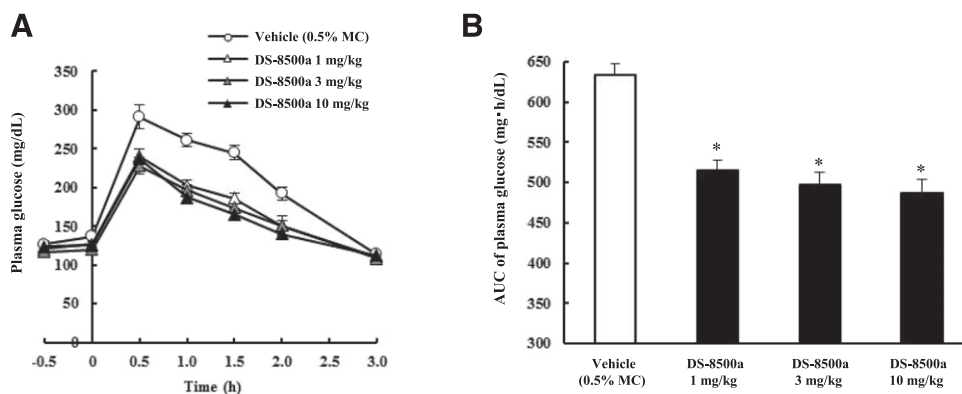
**Fig. 5.** Hypoglycemia risk assessment of DS-8500a comparing nateglinide, glimepiride, and glibenclamide. Data are expressed as the mean  $\pm$  S.E.M. ( $N = 8$  in each group). (A) Plasma glucose levels after the administration of DS-8500a, nateglinide, glimepiride, and glibenclamide in SD rats. The delta value of plasma glucose ( $\Delta$ PG) was calculated by subtracting the PG prior to the administration in each rat. The areas under the curve (AUCs) of  $\Delta$ PG from 0 to 2 hours ( $\Delta$ PG-AUC<sub>(0-2)</sub>) (B), from 0 to 6 hours ( $\Delta$ PG-AUC<sub>(0-6)</sub>) (C), and from 0 to 24 hours ( $\Delta$ PG-AUC<sub>(0-24)</sub>) (D) were calculated. The  $\Delta$ PG-AUC<sub>(0-2)</sub>,  $\Delta$ PG-AUC<sub>(0-6)</sub> and  $\Delta$ PG-AUC<sub>(0-24)</sub> in the DS-8500a, nateglinide, glimepiride, and glibenclamide-administered groups were compared with that of the control group by an unpaired Student's  $t$  test. \* $P < 0.05$  vs. the control group.

## Discussion

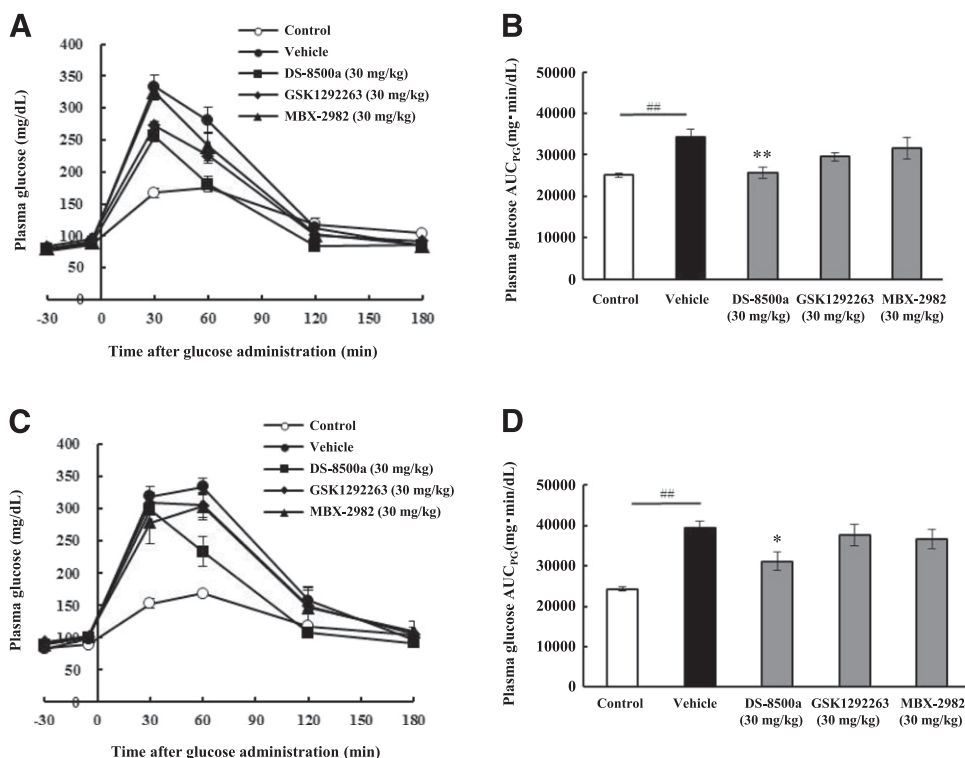
GPR119 agonist has been noticed as one of the next generation of therapeutics for diabetes because it is closely related to glucose homeostasis via the secretion of GLP-1 and insulin by regulating intracellular cAMP concentration in intestinal L-cells and pancreatic  $\beta$ -cells, respectively. From an effectiveness point of view, when we select a compound to be developed clinically, it is important to confirm each pharmacological effect of the compound predicted by the distribution of GPR119 and the compound's putative mechanism of action. In this report, single-dose studies of DS-8500a were performed to confirm these effects. In addition, a repeat-dose study in diabetic rats was performed comparing with other clinically tested GPR119 agonists.

The effect on the small intestinal L-cells and the upregulation of plasma GLP-1 concentration is one of the physiologically important actions of the GPR119 agonist class in the control of glucose homeostasis in patients with T2DM (Lauffer et al., 2008). The on-target mechanism for the upregulation of

plasma GLP-1 level via GPR119 is supported by the defect in glucose-triggered upregulation of plasma GLP-1[7–36]amide levels in GPR119 knockout mice (Lan et al., 2009). To date, the upregulation of plasma GLP-1 concentration by GPR119 agonist has been confirmed in enteroendocrine L-cell lines and in mice species (Chu et al., 2008; Lan et al., 2012). Here, we found that DS-8500a had an additive effect on the plasma GLP-1 levels under a glucose (+) condition and decreased plasma glucose excursion at OGTT in ZF rats. This is the first investigation of the contribution of GLP-1 to control of glucose homeostasis in ZF rats, one of the standard type 2 diabetic animal models. On the other hand, the upregulation of plasma GLP-1 concentration by DS-8500a was also observed under a glucose (–) condition. This observation indicates that DS-8500a can enhance GLP-1 secretion in glucose-independent manner. Unlike GLP-1 secretion, the enhancement of insulin secretion induced by DS-8500a was strictly glucose-dependent. As with DS-8500a, rolipram, a selective PDE4 inhibitor, increased intracellular cAMP level in glucagon gene-simian virus-40 large T-antigen (GLUTag)



**Fig. 6.** Plasma glucose level during OGTT in ZF rats administered with DS-8500a. Data are expressed as the mean  $\pm$  S.E.M. ( $N = 10$  in each group) (A) Plasma glucose level during OGTT. (B) The AUC of plasma glucose level after the glucose load. The AUCs in the groups of 1, 3 and 10 mg/kg of DS-8500a were  $515 \pm 13$  mg·h/dL,  $497 \pm 16$  mg·h/dL and  $486 \pm 18$  mg·h/dL and which were statistically significantly lower than that of the control group ( $633 \pm 15$  mg·h/dL,  $P < 0.0001$ ), and dose-dependent lowering was shown in the range of 1 mg/kg to 10 mg/kg (Spearman's rank correlation coefficient =  $-0.6762$ ,  $P < 0.0001$ ). The AUC of plasma glucose level in each DS-8500a-administered group was compared to that of in the control group by the closed testing procedure using an unpaired Student's  $t$ -test. \* $P < 0.05$  vs. the control group.



**Fig. 7.** Change in plasma glucose levels and the AUC during OGTT performed at days 0 (A and B) and 14 (C and D). DS-8500a, GSK1292263 and MBX-2982 were orally administered to nSTZ rats 30 minute before 2 g/kg of glucose load. The time of the glucose load was set as 0 minutes and plasma glucose levels at -30, -5, 30, 60, 120, and 180 minutes were measured. Data are expressed as the mean  $\pm$  S.E.M. ( $N = 6$  in each group). The difference of AUC<sub>PG</sub> between the control group and the vehicle group was compared using a Student's *t* test, and the difference of DS-8500a, GSK1292263, and MBX-2982 groups were compared with the vehicle group by a Dunnett's test.  $^{*}P < 0.01$  vs. control.  $^{*}P < 0.05$  vs. vehicle.  $^{**}P < 0.01$  vs. vehicle.

cells derived from large intestinal L-cells in mice, and induced GLP-1 secretion in a glucose-independent manner (Ong et al., 2009). In addition, it stimulated insulin secretion in glucose-dependent manner in  $\beta$ -cell-derived BRIN-BD11 cells (Ahmad et al., 2000). These results indicate that the increase of intracellular cAMP levels in L-cells and  $\beta$ -cells leads to "glucose-independent" GLP-1 secretion and "glucose-dependent" insulin secretion, respectively. The intracellular metabolism of glucose and production of ATP may be involved in the potentiation of "glucose-dependent" insulin secretion in pancreatic  $\beta$ -cells (Seino et al., 2009); however, the molecular mechanism for the difference in glucose dependency of both cell types needs further investigation.

The mode of GSIS observed in DS-8500a was clearly distinguished from glimepiride, one of K-ATP channel-closing sulfonylureas. These results suggest that DS-8500a could present a low risk of hypoglycemia, an adverse effect common to sulfonylureas and meglitinides in the clinic. To understand the risk of hypoglycemia more precisely, especially in fasted state, DS-8500a, nateglinide, glimepiride, and glibenclamide were assessed in fasted SD rats. A single oral administration of nateglinide, glimepiride, or glibenclamide around the therapeutic dose (Ladrière et al., 1997; de Souza et al., 2001) statistically significantly lowered the fasting plasma glucose compared with vehicle. In contrast, the administration of DS-8500a did not show a statistically significant mean difference in the fasting plasma glucose compared with vehicle. These results suggest that DS-8500a would have a lower risk of hypoglycemia compared with nateglinide, glimepiride, and glibenclamide, which do have a risk of hypoglycemia in clinical use.

In the repeat-dosing study, among the three compounds, only DS-8500a had statistically significant glucose-lowering effects during OGTTs performed at day 0 and day 14 in neonatal STZ rats. Unexpectedly, during the repeat doses,

there was no obvious loss of efficacy by any of the three GPR119 agonists tested here. These observations seem to differ from the clinical results showing that a single dose of GSK1292263 (25–800 mg) had a tendency to reduce glucose incremental AUC during OGTT, although there was no reduction in weighted-mean glucose AUC after 13 or 14 days of dosing (Nunez et al., 2014). Even though the loss of efficacy in the short-term repeat-dosing study among clinically tested GPR119 agonists suggests one reason for discontinuance (Kang, 2013), our results here suggest that the efficacy level of the glucose-lowering effect should be considered as a critical risk factor rather than the occurrence of loss of efficacy. Two rationales support this hypothesis: 1) The study protocol to assess the risk of loss of efficacy, which consisted of the two sets of OGTT (day 0 and day 14) during a repeat-dosing study, appears to have been sufficient, because it was known that glucose-lowering effects of sulfonylurea, the representative insulin secretagogue, were diminished in preclinical studies after 6–27 days of treatment (Asakawa et al., 2009; Someya et al., 2009); and 2) the dual pathways of GPR119 agonist could be integrated into the enhancement of GSIS, which is a key element for the reduction of glucose excursion at OGTT. One of the possible reasons to consider for the weak in vivo efficacy levels of GSK1292263 and MBX-2982 compared with DS-8500a are the piperidine rings within the chemical structure of some GPR119 agonists, which are attributable to the antagonist conformer (McClure et al., 2011). Both GSK1292263 and MBX-2982 include a piperidine ring (Ritter et al., 2016), whereas DS-8500a does not have this moiety. Actually in rat GPR119-expressing CHO-K1 cells, DS-8500a had a 30% greater intrinsic activity level for intracellular cAMP production compared with MBX-2982 (Supplemental Fig. 2). Considering that MBX-2982 had greater plasma exposure levels compared with DS-8500a,

the difference in intrinsic activity level is supposed to have some impact on glucose-lowering in nSTZ rats. Considering that the impact of antagonist conformation on loss of efficacy is not fully understood, the low glucose-lowering effect attributable to the low level of intrinsic activity could be one of the critical risk factors with the GPR119 agonist class rather than the risk of loss of efficacy (Kang, 2013).

In terms of future studies, it will be of interest to determine whether chronic exposure to DS-8500a in vivo prevents the decline in functional  $\beta$ -cell mass, including islet graft in transplantation therapy, and whether chronic combination with DPP-4 inhibitor and/or metformin leads to benefits in sustained blood glucose control in type 2 diabetic patients.

In conclusion, the basic profiles of DS-8500a were highly validated in rats, including the augmentation of plasma GLP-1 concentration, the enhancement of GSIS, the risks of hypoglycemia, and loss of efficacy for the glucose-lowering effects. Further investigation for the target disease or target patients will be needed to explore a clinically meaningful application for DS-8500a.

#### Acknowledgments

We thank LSI Medience Corporation (Tokyo, Japan) for their expertly conducted experiments.

#### Authorship Contributions

*Participated in research design:* Matsumoto, Yoshitomi, Ishimoto.

*Conducted experiments:* Matsumoto, Yoshitomi, Ishimoto, Tanaka, Takahashi, Watanabe, Chiba.

*Performed data analysis:* Matsumoto, Yoshitomi, Ishimoto, Takahashi, Tanaka, Watanabe, Chiba.

*Wrote or contributed to the writing of the manuscript:* Matsumoto.

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DS-8500a, an orally available G protein-coupled receptor 119 agonist, upregulates glucagon-like peptide-1 and enhances glucose-dependent insulin secretion and improves glucose homeostasis in type 2 diabetic rats

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## Supplemental Data

Supplemental Table S1 In vitro pharmacological activities of DS-8500a on receptors, channels, and transporters

Target receptor	Concentration (μmol/L)	% Inhibition
Adenosine A <sub>1</sub>	10	9
Adenosine A <sub>2A</sub>	10	-3
Adenosine A <sub>3</sub>	10	24
Adrenergic α <sub>1A</sub>	10	13
Adrenergic α <sub>1B</sub>	10	-2
Adrenergic α <sub>1D</sub>	10	2
Adrenergic α <sub>2A</sub>	10	1
Adrenergic β <sub>1</sub>	10	8
Adrenergic β <sub>2</sub>	10	-1
Transporter, Norepinephrine (NET)	10	20
Bradykinin B <sub>1</sub>	10	-9
Bradykinin B <sub>2</sub>	10	-2
Calcium Channel L-Type, Benzothiazepine	10	7
Calcium Channel L-Type, Dihydropyridine	10	23
Calcium Channel N-Type	10	2
Cannabinoid CB <sub>1</sub>	10	5
Dopamine D <sub>1</sub>	10	2
Dopamine D <sub>2S</sub>	10	1
Dopamine D <sub>3</sub>	10	1
Dopamine D <sub>4.2</sub>	10	1
Transporter, Dopamine (DAT)	10	13
Endothelin ET <sub>A</sub>	10	1
Endothelin ET <sub>B</sub>	10	-7
Epidermal Growth Factor (EGF)	10	9
Estrogen ERα	10	5
Transporter, GABA	10	-1
GABA <sub>A</sub> , Muscimol, Central	10	3
GABA <sub>A</sub> , Flunitrazepam, Central	10	1
GABA <sub>B1A</sub>	10	12
Glucocorticoid	10	3
Glutamate, Kainate	10	-3
Glutamate, NMDA, Agonism	10	11
Glutamate, NMDA, Glycine	10	9
Glutamate, NMDA, Phencyclidine	10	1
Histamine H <sub>1</sub>	10	0
Histamine H <sub>2</sub>	10	3
Histamine H <sub>3</sub>	10	-9
Imidazoline I <sub>2</sub> , Central	10	9
Interleukin IL-1	10	-14
Leukotriene, Cysteinyl CysLT <sub>1</sub>	10	17
Melatonin MT <sub>1</sub>	10	70
	100	89
	30	70
	10	44
	3	24
	1	9
Muscarinic M <sub>1</sub>	10	4
Muscarinic M <sub>2</sub>	10	4
Muscarinic M <sub>3</sub>	10	-8
Tachykinin NK <sub>1</sub>	10	7
Neuropeptide YY <sub>1</sub>	10	19
Neuropeptide YY <sub>2</sub>	10	6
Nicotinic Acetylcholine	10	-8
Nicotinic Acetylcholine α1, Bungarotoxin	10	-4
Opiate δ <sub>1</sub> (OP1, DOP)	10	4
Opiate κ (OP2, KOP)	10	-8
Opiate μ (OP3, MOP)	10	2
Phorbol Ester	10	2
Platelet Activating Factor (PAF)	10	-10
Potassium Channel [K <sub>ATP</sub> ]	10	1
Potassium Channel hERG	10	9
Prostanoid EP <sub>4</sub>	10	7
Purinergic P <sub>2X</sub>	10	19
Purinergic P <sub>2Y</sub>	10	1
Rolipram	10	-1
Serotonin (5-Hydroxytryptamine) 5-HT <sub>1A</sub>	10	0
Serotonin (5-Hydroxytryptamine) 5-HT <sub>2B</sub>	10	21
Serotonin (5-Hydroxytryptamine) 5-HT <sub>3</sub>	10	-5
Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	10	5
Sigma σ1	10	-3
Androgen (Testosterone) AR	10	30
Thyroid Hormone	10	12

Supplemental Table S2 Plasma insulin levels at 30 minutes after glucose load during OGTTs performed before (day 0) and after (day 14) repeat dosing of DS-8500a, GSK1292263 and MBX-2982 in nSTZ rats.

Group	Plasma insulin levels at 30 minutes after glucose load (ng/ mL)	
	Day 0 (Before repeat dosing)	Day 14 (After repeat dosing)
Control	2.32 ± 0.52	2.68 ± 0.51
Vehicle	2.07 ± 0.26	2.02 ± 0.23
DS-8500a	3.67 ± 0.71	3.42 ± 0.40
GSK1292263	2.81 ± 0.43	2.62 ± 0.40
MBX-2982	2.73 ± 0.42	2.56 ± 0.32

Supplemental Table 2 Plasma insulin levels at 30 minutes after glucose load during OGTTs performed before (day 0) and after (day 14) repeat dosing of DS-8500a, GSK1292263 and MBX-2982 in nSTZ rats. Data are expressed as the mean ± S.E.M. ( $N = 6$  in each group except for the control group in OGTT performed at day 14 ( $N = 5$ , due to shortage of plasma sample volume for the measurement)).

Supplemental Table S3 Plasma concentrations of DS-8500a, GSK1292263 and MBX-2982 and the fold values against their EC<sub>50</sub> values.

Compound	(a) EC <sub>50</sub> value on rGPR119 CHO-K1 cells (nmol/L)	Day 0		Day 14	
		(b) Plasma concentration at 5 min before glucose load (nmol/L)	Fold (b/a)	(b) Plasma concentration at 5 min before glucose load (nmol/L)	Fold (b/a)
DS-8500a	98.4	2502.7	25	3486.7	35
GSK1292263	75.0	284.7	4	1169.6	16
MBX-2982	24.9	1863.8	75	4592.7	184

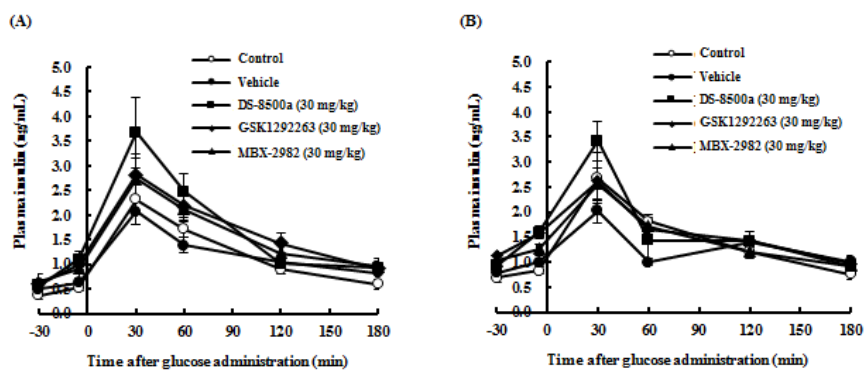
Supplemental Table 2 Plasma concentrations of DS-8500a, GSK1292263 and MBX-2982 and the fold values against their EC<sub>50</sub> values (98.4 nM, 75.0 nM and 24.9 nM in rat GPR119 expressing CHO-K1 cells, respectively) at the point of glucose administration (25 minutes after oral administration of compound) during OGTT performed in nSTZ rats at day 0 and 14. Each plasma sample of the same group (*N* = 6) were pooled and plasma concentration of each compound was measured by LC-MS/MS (Prominence LC-20A system (Shimadzu Corp.) and API 4000 LC-MS/MS System (AB SCIEX)).

Supplemental Table S4 Fasting plasma glucose levels before and 2-weeks after repeat dosing of DS-8500a, GSK1292263 and MBX-2982 in nSTZ rats.

Group	Fasting plasma glucose levels (mg/ dL)	
	Day 0 (Before repeat dosing)	Day 14 (After repeat dosing)
Control	76.5 ± 1.8	83.2 ± 1.4
Vehicle	79.0 ± 3.0	83.0 ± 1.5
DS-8500a	78.7 ± 1.8	88.7 ± 2.3
GSK1292263	83.0 ± 2.6	93.6 ± 3.6
MBX-2982	80.2 ± 1.2	88.9 ± 5.9

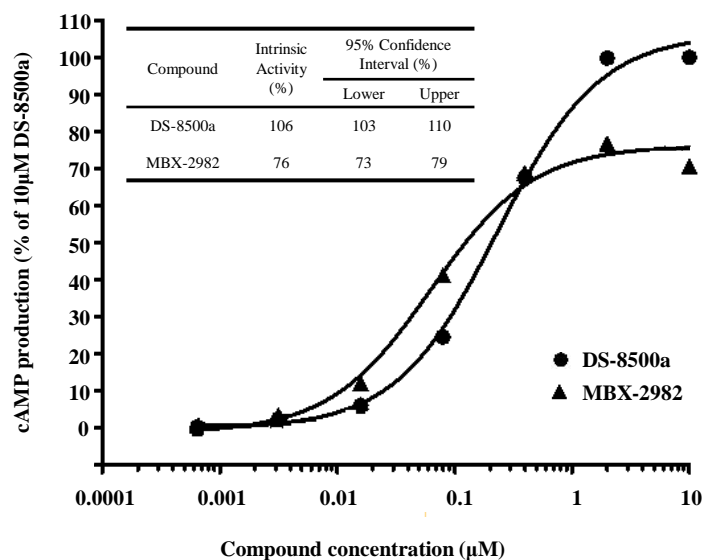
Supplemental Table 3 Fasting plasma glucose levels before and 2-weeks after repeat dosing of DS-8500a, GSK1292263 and MBX-2982 in nSTZ rats. Data are expressed as the mean ± S.E.M. ( $N = 6$  in each group).

Supplemental Figure S1. Change in plasma insulin levels during OGTT performed at day 0 (A) and 14 (B).



Supplemental Figure 1. Change in plasma insulin levels during OGTT performed at day 0 (A) and 14 (B). DS-8500a, GSK1292263 and MBX-2982 were orally administered to nSTZ rats 30 min before 2 g/kg of glucose load. The time of the glucose load was set as 0 min and plasma insulin levels at -30, -5, 30, 60, 120 and 180 min were measured. Data are expressed as the mean  $\pm$  S.E.M. ( $N = 6$  in each group).

Supplemental Figure S2. Intracellular cAMP production levels of DS-8500a and MBX-2982 in rat GPR119 expressing CHO-K1 cells.



Supplemental Figure 2. Intracellular cAMP production levels of DS-8500a and MBX-2982 in rat GPR119 expressing CHO-K1 cells. Assay was developed as shown in Materials and Methods. Fetal bovine serum was used as culture medium instead of the Ham (F12) in this experiment. Compound ( $N = 4$  wells) was added to the rat GPR119 expressing CHO-K1 cells. Mean value was plotted and a logistic curve was generated by the 4-parameter method. Relative intracellular cAMP levels of DS-8500a and MBX-2982 compared to the 10  $\mu$ M of DS-8500a were estimated by Graph Pad Prism Software version 5.04, and their 95% confidence intervals (lower/ upper) were calculated.