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SCO-267, a GPR40 full agonist, improves glycemic and body weight control in rat models of diabetes and obesity

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Running title page

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c) Number of text pages: 36

Number of references: 40

Number of tables: 3

Numbers of figures: 5

Word count of the abstract: 250

Word count of the Introduction: 498

Word count of the Discussion: 1122

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d) Nonstandard abbreviations are defined in the text

GPCR, G-protein-coupled receptor; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent

insulinotropic peptide; PYY, peptide YY; DIO, diet-induced obese; STZ, streptozotocin; CHO, Chinese

hamster ovary; AUC, area under the curve

e) Recommended section: Endocrine and Diabetes

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Abstract

GPR40/FFA1 receptor is a G-protein-coupled receptor expressed in the pancreatic islets and enteroendocrine cells. Here, we report the pharmacological profiles of SCO-267, a novel full agonist of GPR40. Ca^{2+} signaling and insulin and glucagon-like peptide-1 (GLP-1) secretion were evaluated in GPR40-expressing CHO, MIN6, and GLUTag cells. Hormone secretions and effects on fasting glucose were tested in rats. Single or repeated dosing effects were evaluated in N-STZ-1.5 rats, diet-induced obese (DIO) rats, and GPR40-knockout (*Ffar1*^{-/-}) mice. Treatment with SCO-267 activated Gq signaling in both high- and low-*FFAR1*-expressing CHO cells, stimulated insulin secretion in MIN6 cells, and induced GLP-1 release in GLUTag cells. When administered to normal rats, SCO-267 increased insulin, glucagon, GLP-1, glucose-dependent insulintropic peptide, and peptide YY (PYY) secretions under non-fasting conditions. These results show the full agonistic property of SCO-267 against GPR40. Hypoglycemia was not induced in SCO-267-treated rats during the fasting condition. In diabetic N-STZ-1.5 rats, SCO-267 was highly effective in improving glucose tolerance in single and 2-week dosing studies. DIO rats treated with SCO-267 for 2 weeks showed elevated plasma GLP-1 and PYY levels, reduced food intake, and decreased body weight. In wild-type mice, SCO-267 induced GLP-1 secretion, food intake inhibition, and body weight reduction; however, these effects were abolished in *Ffar1*^{-/-} mice, indicating a GPR40-dependent mechanism. In conclusion, SCO-267 stimulated islet and gut hormone secretion, improved glycemic control in diabetic rats, and decreased body weight in obese rats. These data suggest the therapeutic potential of SCO-267 for the treatment of diabetes and obesity.

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Introduction

GPR40/FFA1 receptor, a G-protein-coupled receptor (GPCR), couples predominantly with the Gq/11 protein, promoting phospholipase C-dependent hydrolysis of phosphatidylinositol 4,5-bisphosphate into diacylglycerol and inositol 1,4,5-triphosphate (Ghislain and Poitout, 2017). This in turn increases intracellular Ca^{2+} concentrations (Li et al., 2018). Medium-to-long chain fatty acids activate intracellular Ca^{2+} responses in GPR40-expressing cells, demonstrating that they are natural GPR40 ligands (Briscoe et al., 2003; Itoh et al., 2003; Kotarsky et al., 2003). GPR40 is expressed in pancreatic beta-cells and intestinal endocrine cells, and its activation stimulates insulin and incretin secretion (Mancini and Poitout, 2013; Pais et al., 2016). As insulin and incretin are pivotal for glycemic control (Nauck and Meier, 2018), GPR40 activation is considered a novel option for treating diabetes (Eleazu et al., 2018).

To date, several synthetic compounds targeting GPR40 have been developed, and structure–activity relationships of these agonists have been investigated (Defossa and Wagner, 2014). Importantly, fasiglifam (Negoro et al., 2010; Tsujihata et al., 2011; Ito et al., 2013), a GPR40 agonist, has been shown to significantly improve glycemic control in patients with type 2 diabetes (Burant et al., 2012; Kaku et al., 2013; Kaku et al., 2016); however, fasiglifam testing was voluntarily terminated in phase 3 clinical trials because of possible adverse effects on the liver (Wolenski et al., 2017). Proof of concept of a GPR40 agonist to treat diabetes has attracted considerable attention owing to its potential as a valuable drug target. Concurrently, fasiglifam was demonstrated as a GPR40 partial agonist based on Ca^{2+} mobilization in GPR40-expressing cells (Yabuki et al., 2013). Additional studies identified a novel series of GPR40 agonists with different profiles from partial

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agonists. A seminal study demonstrated that AM-1638 has full agonistic activity towards GPR40, and was more effective in stimulating insulin secretion and improving glucose-lowering efficacy *in vivo* than partial agonists (Brown et al., 2012; Luo et al., 2012).

Importantly, GPR40 full agonists can activate the enteroendocrine system while stimulating insulin secretion (Luo et al., 2012). Such mechanisms seem highly valuable to developing a novel strategy for treating diabetes. Indeed, GLP-1 signaling activation has been proven to have beneficial effects, including improved long-term glucose control and decreased incidence of cardiovascular death, in patients with type 2 diabetes mellitus (Nauck et al., 2017). As GPR40 partial agonists improve glycemic control in patients with diabetes, GPR40 full agonists may provide superior efficacy and additional benefits in patients with metabolic diseases. To date, there has been no report on the clinical efficacy of GPR40 full agonist. Thus, preclinical efficacy studies including fasiglifam as a comparator would provide important information to develop novel GPR40 full agonists.

Here, we report the pharmacological profiles and efficacy of SCO-267, a novel GPR40 full agonist (Aida et al., 2015). Its *in-vitro* efficacy was evaluated using cell models, and its effect on islet, gut hormones and glycemic control was evaluated in rat models. SCO-267's contribution to body weight reduction was evaluated in obese rats and GPR40 knockout mice. Finally, we compared some key effects induced by SCO-267 to fasiglifam.

Materials and Methods

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Materials

All reagents were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), Sigma-Aldrich (Tokyo, Japan), or Cayman Chemical (Michigan, USA) unless otherwise indicated. SCO-267, fasiglifam, and AM-1638 were obtained from SCOHIA PHARMA, Inc. and Takeda Pharmaceutical Company Limited. Glimepiride was purchased from FUJIFILM Wako. For *in-vitro* studies, compounds were dissolved in dimethyl sulfoxide, and for *in-vivo* studies, compounds were suspended in 0.5% methylcellulose (MC) solution (FUJIFILM Wako). The dose of each compound was expressed as the free base form.

FFAR1-expressing CHO cell assay

Chinese hamster ovary (CHO) dhfr- cells (Clones #104 and #2) stably expressing human *FFAR1* (Yabuki et al., 2013) were cultured with minimum essential medium- α (FUJIFILM Wako) containing 10% fetal bovine serum (FBS), 10 mmol/L HEPES (Thermo Fisher Scientific, Waltham, MA, USA), 100 IU/mL penicillin, and 100 μ g/mL streptomycin in 5% CO₂ at 37°C. The cells (1×10^4 cells/well) were seeded in 384-well (black-walled clear-bottom) culture plates and incubated overnight in 5% CO₂ at 37°C. After removing the medium, the cells were incubated in 30 μ L of loading buffer (Hank's Balanced Salt Solution, Thermo Fisher Scientific) containing 20 mmol/L HEPES, 0.1% fatty acid-free bovine serum albumin (BSA), 0.08% Pluronic F127 (#CSK-01F, Dojindo, Kumamoto, Japan), 2.5 mmol/L Probenecid (#CSK-03F, Dojindo), and 2.5 μ g/mL Fluo4 (#F311, Dojindo) for 60 min in 5% CO₂ at 37°C. Test compounds of various

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concentrations were added to the cells and the increase in intracellular Ca^{2+} concentration was monitored for 180 s using the FLIPR Tetra system (Molecular Devices, Tokyo, Japan).

MIN6 cell assay

The pancreatic beta-cell line, MIN6, displays features of glucose metabolism and glucose-induced insulin secretion similar to those of normal islets (Miyazaki et al., 1990). MIN6 cells were seeded at 5×10^4 cells/well in 96-well plates and cultured as described previously (Miyazaki et al., 1990). After discarding the medium, the cells were pre-incubated for 2 h at 37°C with 100 μL of Krebs-Ringer Bicarbonate–HEPES (KRBH) buffer (116 mmol/L NaCl, 4.7 mmol/L KCl, 1.17 mmol/L KH_2PO_4 , 1.17 mmol/L MgSO_4 , 25 mmol/L NaHCO_3 , 2.52 mmol/L CaCl_2 , and 24 mmol/L HEPES) containing 0.2% fatty acid-free BSA and 1 mmol/L glucose. After discarding the buffer, KRBH buffer containing 1 or 16 mmol/L glucose, 0.2% fatty acid-free BSA and test materials at indicated concentrations was added and the plate was further incubated for 2 h at 37°C. After incubation, supernatants were collected from all wells and the insulin concentrations were determined.

GLUTag cell assay

GLUTag cells are stable, immortalized, and relatively differentiated murine enteroendocrine cells (Lee et al., 1992) and in this study, these cells were seeded at 1×10^4 cells/well in 96-well poly-L-lysine-coated plates. Cells were cultured in Dulbecco's modified Eagle's medium with 10% FBS, 100 IU/mL penicillin, 100

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μg/mL streptomycin, and 25 mmol/L glucose. The culture medium was replaced with medium containing 10% FBS, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 5.5 mmol/L glucose prior to overnight incubation. After discarding the medium, KRBH buffer containing 10 mmol/L glucose, 0.2% fatty acid-free BSA and the test materials at indicated concentrations was added and the plate was further incubated for 2 h at 37°C. The supernatants were collected from all wells and the GLP-1 concentrations were determined.

Plasma protein binding

Plasma samples of rats, dogs, and humans were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan), Kitayama Labes Co. Ltd. (Nagano, Japan), and Cosmo Bio (Tokyo, Japan), respectively. *In-vitro* plasma protein binding of SCO-267 in rats, dogs, and humans was evaluated by using LC/MS/MS following ultracentrifugation.

Animals

All animals were housed in a room with controlled temperature (23°C), humidity (55%), and lighting (lights on between 7:00 am and 7:00 pm). All animals were allowed free access to standard laboratory chow diet (CE-2, CLEA Japan, Inc.) and tap water. The care and use of animals and the experimental protocols were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company, Ltd. and SCOHIA PHARMA, Inc. All experiments were performed according to the guidelines and regulations of Takeda Pharmaceutical Company, Ltd., and Shonan Health Innovation Park. For animal studies, 0.5%

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methylcellulose was used as vehicle. All blood samples used in the present study were obtained via the tail vein of animals.

Sprague-Dawley rat study

Male Sprague-Dawley (SD) rats were obtained from CLEA Japan, Inc (Tokyo, Japan). For the hormone secretion experiment in the non-fasted state, 6-week-old rats were randomized into groups (n= 6) based on body weight. To evaluate the compound's effect on fasting plasma glucose levels, 8-week-old rats were fasted overnight. Rats were randomized into groups (n= 6) based on body weight and plasma glucose. The test materials were orally administered at the indicated doses. Blood samples were collected at indicated time points, and plasma parameters were determined. A fasting plasma glucose range below 70 mg/dL was considered hypoglycemic (American Diabetes, 2018). A pharmacokinetic study was performed using non-fasted 6-week-old male SD rats (n=3) obtained from Charles River Laboratories Japan Inc.

N-STZ-1.5 rat study

Male N-STZ-1.5 Wistar Kyoto rats (N-STZ-1.5 rats), which are diabetic, were developed via subcutaneous administration of 120 mg/kg streptozotocin (STZ) to Wistar Kyoto rats (RABICS, LTD. Kanagawa, Japan) at 1.5 days after birth. N-STZ rats were reported to display defects in insulin secretion and action which, in many ways, resemble those observed in human diabetic patients (Portha et al., 2003). To evaluate the efficacy of single dosing, 25-week-old N-STZ-1.5 rats were subjected to overnight fasting and were randomized into

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groups (n = 6) based on fasting plasma glucose, triglyceride levels and body weight. The test materials were orally administered 60 min before oral glucose loading (1.5 g/kg). The effect of an oral dose of AM-1638 on glucose tolerance and its plasma concentration were compared to SCO-267 in a similarly designed experiment using male N-STZ-1.5 rats (32- and 18-week-old for glucose tolerance test (n=6) and pharmacokinetic study(n=3), respectively). Then, to evaluate the effects of repeated-dosing, 27-week-old N-STZ-1.5 rats (baseline body weight, 373 g) were randomized into groups (n = 6) based on glycosylated hemoglobin level, plasma glucose concentration, body weight, and food intake. Each group was treated with the indicated test materials once daily for 2 weeks. Following the 2-week treatment period, the rats were fasted overnight, and the test materials were orally administered at indicated doses 60 min before an oral glucose load (1.5 g/kg). Blood samples were collected at indicated time points and plasma parameters were determined. Following the oral glucose load test, a pharmacokinetic study was performed with the same non-fasted rats. A pharmacokinetic study of 0.3 mg/kg SCO-267 and 3 mg/kg fasiglifam was conducted under the same experimental conditions in 20-week-old male N-STZ-1.5 rats (n=6).

Diet-induced obesity (DIO) rat study

Male F344 rats aged 29 weeks were obtained from CLEA Japan, Inc. and were fed a high-fat diet (D12451M, 45 kcal%, Research Diets, Inc., New Brunswick, NJ, USA) to induce obesity. At 49 weeks, the DIO rats (baseline body weight, 487 g) were randomized into groups (n = 6) based on body fat mass, daily food intake, and body weight. Each group was treated with the indicated test materials once daily for 2 weeks. Blood

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samples were collected at indicated time points and plasma parameters were determined. Body compositions were determined after 2 weeks of treatment. GLP-1 and peptide YY (PYY) levels were measured 16 h after the 15th dosing and a pharmacokinetic study was performed with non-fasted DIO rats after the 20th drug dosing.

***Ffar1*^{-/-} mouse study**

Male *Ffar1*^{-/-} and their wild-type (WT) littermates were obtained from RABICS, LTD. (Matsuda-Nagasumi et al., 2013). *Ffar1*^{-/-} and WT mice were fed a high-fat diet (D12451, Research Diets, Inc.) from the age of 21 weeks. At 29 weeks of age, mice from each strain were randomized into groups based on food intake and body weight (baseline body weight, 40 g for WT and 41 g for *Ffar1*^{-/-}, n = 6). The test materials were orally administered once daily. After 3 days of dosing, food intake and body weight changes were calculated. GLP-1 levels were measured 1 h after the 4th dosing of the test materials. A pharmacokinetic study was performed using 10-week-old male nonfasted C57BL/6J mice (n=3) obtained from CLEA Japan.

Measurements

Plasma metabolic parameters were measured with an Autoanalyzer 7180 (Hitachi, Tokyo, Japan). Insulin levels were measured using a radioimmunoassay kit (RI-13K, Merck Millipore, Burlington, MA, USA), ELISA kit (MS301, Takara, Shiga, Japan), or alphaLISA (PerkinElmer, Waltham, MA, USA). Plasma total GIP levels were determined using an ELISA kit (EZRMGIP-55K, Merck Millipore). Plasma glucagon was

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measured using a radioimmunoassay kit (GL-32K, Merck Millipore), or ELISA kit (10-1271-01, Mercodia, Uppsala, Sweden). Plasma total GLP-1 was determined by using an ELISA established by Takeda Pharmaceutical Company Limited or a commercially available ELISA kit (299-75501, FUJIFILM Wako). Plasma total PYY levels were determined using ELISA (291-73501, FUJIFILM Wako) or Rat/Mouse PYY RIA (RMPYY-68HK, Merck Millipore). Body composition was quantified by magnetic resonance imaging to directly measure total body fat mass and total body lean mass of rats that were not anaesthetized, at indicated ages (EchoMRI-900, Hitachi).

ELISA for GLP-1

Anti-GLP-1 mAbs (GLIT2-863.35-7 and GLIT4-1448.7) were generated from Balb/c mice immunized with GLP-1-BSA. These mAb pairs were screened for specificity to total GLP-1 in plasma and serum. Conventional sandwich ELISAs were established in a 96-well plate using those mAb pairs. GLP-1 (7-36) amide (PEPTIDE INSTITUTE, INC., Osaka, Japan) was used as a reference standard for the assay. The detection limit of this assay was 0.3-300 pmol/L; intra-assay and inter-assay variations were $\leq 15\%$.

Statistical analysis

Statistical significance was first analyzed using Bartlett's test for homogeneity of variances, followed by the Williams' test ($P > 0.05$) and Shirley-Williams test ($P \leq 0.05$) for dose-dependent studies, and Dunnett's test ($P > 0.05$) and Steel test ($P \leq 0.05$) for multiple comparisons. Alternatively, statistical significance was

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analyzed using the F test for homogeneity of variances, followed by Student's *t*-test ($P > 0.2$) or the Aspin–Welch test ($P \leq 0.2$). The Williams' and Shirley–Williams tests were conducted using a one-tailed significance level of 2.5% (0.025). Other tests were conducted using a two-tailed significance level of 5% (0.05). The half maximal (50%) effective concentration (EC_{50}) values and maximum effect (E_{max}) were calculated using the 4-parameter logistic equation in Prism 7 software (GraphPad Software, San Diego, CA, USA). All data are presented as mean \pm standard deviation (S.D.) (for *in-vivo* experiments) or mean \pm standard error of the mean (S.E.M.) (for *in-vitro* experiments).

Results

SCO-267 is a GPR40 full agonist that stimulates insulin and GLP-1 secretions in cells

We identified SCO-267 as a new GPR40 agonist (Fig. 1A), and compound profiles were determined by *in-vitro* experiments. We used CHO cells expressing high (clone #104) or low (clone #2) levels of human *FFAR1* to evaluate the agonistic activity of SCO-267 on Ca^{2+} response. SCO-267 showed EC_{50} values of 1.3 nmol/L (clone #104) and 12 nmol/L (clone #2), and E_{max} values (% γ -linolenic acid, an endogenous ligand[γ -LA]) of 125% (clone #104) and 201% (clone #2) (Fig. 1B, C, Table 1). AM-1638 (Brown et al., 2012), a well-known GPR40 full agonist, had EC_{50} values of 7.1 nmol/L (clone #104) and 150 nmol/L (clone #2), and E_{max} values (% γ -LA) of 110% (clone #104) and 182% (clone #2) (Fig. 1B and C, Table 1). The insulinotropic effect of SCO-267 was examined in mouse insulinoma MIN6 cells. SCO-267 effectively stimulated insulin secretion under the high-glucose condition, unlike fasiglifam ($EC_{50} = 0.85$ nmol/L for

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SCO-267 and $EC_{50} = 530$ nmol/L for fasiglifam; E_{max} [% fasiglifam], 142% for SCO-267) (Fig. 1D). When tested in mouse enteroendocrine GLUTag cells, SCO-267 stimulated GLP-1 secretion by more than 4-fold ($EC_{50} = 8.5$ nmol/L) the level observed upon treatment with dimethyl sulfoxide. SCO-267 was also more effective than 10 μ mol/L fasiglifam (Fig. 1E). These data indicate that SCO-267 has full agonistic activity against GPR40. SCO-267 showed 99.6–99.7% plasma protein binding ability in rats, dogs, and humans (Table 2).

SCO-267 stimulates secretion of islet and enteroendocrine hormones and does not induce hypoglycemia in normal rats

In the non-fasting condition, SCO-267 (0.3–10 mg/kg) administration stimulated the secretion of insulin, glucagon, GLP-1, GIP, and PYY (Fig. 2A-E). Fasiglifam (10 mg/kg) was not effective in inducing these hormonal changes (Fig. 2A-E). Administration of glimepiride, a sulfonylurea class antidiabetic drug (Korytkowski, 2004), at 10 mg/kg induced overt hypoglycemia, with plasma glucose concentrations below 70 mg/dL, in fasting rats. Although SCO-267 slightly decreased fasting plasma glucose levels, it did not induce hypoglycemia in normoglycemic rats (Fig. 2F). Pharmacokinetics analysis showed that a 0.3 mg/kg oral dose of SCO-267 resulted in a C_{max} of 5.34 ng/ml, T_{max} of 4.0 h, area under the curve (AUC)_{0-24h} of 59.4 ng·h/mL, and bioavailability (BA) of 24.8% in normal rats (Supplemental Figure 1).

SCO-267 improves glucose tolerance and its efficacy is durable in diabetic rats

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A single oral administration of SCO-267 (0.1, 0.3, 1 and 3 mg/kg) increased plasma insulin and GLP-1 levels and improved glucose tolerance after an oral glucose load in N-STZ-1.5 rats in a dose-dependent manner (Fig. 3A-C). SCO-267 (0.3 mg/kg) and fasiglifam (3 mg/kg) had C_{\max} values of 22.7 ng/mL and 6.17 μ g/mL, respectively, in N-STZ-1.5 rats. The efficacy of SCO-267 was also compared to AM-1638 (Luo et al., 2012), a well-studied GPR40 full agonist, in N-STZ-1.5 rats. As shown in Supplemental Figure 2, a lower plasma exposure of SCO-267 was more effective in enhancing insulin secretion and improving glucose tolerance compared to those by AM-1638 in N-STZ-1.5 rats. To test the efficacy and durability, SCO-267 (1 mg/kg) was repeatedly administered to N-STZ-1.5 rats for 2 weeks. Consistent with the single dosing efficacy, SCO-267 increased insulin secretion and improved glucose tolerance in N-STZ-1.5 rats after 2 weeks of repeated dosing (Fig. 3D, E). At the end of the study, SCO-267 (1 mg/kg) had a C_{\max} of 139 ng/mL and AUC_{0-24h} of 626 ng·h/mL, and fasiglifam (10 mg/kg) had a C_{\max} of 39.8 μ g/mL and AUC_{0-24h} of 255 μ g·h/mL in N-STZ-1.5 rats (Supplemental Figure 3). Food intake and body weight were unaltered in SCO-267-administered N-STZ-1.5 rats (Supplemental Figure 4).

SCO-267 elevates circulating gut hormone and decreases body weight in DIO rats

The chronic effect of SCO-267 (0.3-3 mg/kg) was evaluated in obese rats that were fed a high-fat diet. After chronic dosing, GLP-1 and PYY levels were elevated after 16 h of SCO-267 dosing (Fig. 4A, B). During the experimental period, food intake levels were lower and total food intake was decreased in SCO-267-treated rats (Fig. 4C, D). Consistent with the reduction in food intake, body weight and fat mass were decreased in

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SCO-267-treated rats (Fig. 4E, F). The lean mass of SCO-267-treated DIO rats did not change. Plasma levels of glucose, triglycerides, alanine aminotransferase, and aspartate aminotransferase remained the same, whereas total cholesterol was reduced in SCO-267-treated DIO rats (Table 3). Pharmacokinetic analysis confirmed that sustained exposure to SCO-267 was effective in inducing these efficacies in DIO rats (Supplemental Figure 5).

SCO-267 induces GLP-1 secretion and food intake inhibition in a GPR40-dependent manner

Based on the pharmacokinetic data of the relatively short half-life of SCO-267 in mice (Supplemental Figure 6), we used 30 mg/kg as the oral SCO-267 dose in a mouse study. In WT mice, SCO-267 stimulated GLP-1 secretion, inhibited food intake, and reduced body weight (Fig. 5A-C). In contrast, SCO-267 did not induce GLP-1 secretion, food intake inhibition, and body weight reduction in *Ffar1*^{-/-} mice (Fig. 5A-C).

Discussion

In this study, we demonstrated that SCO-267, a new GPR40 agonist, was highly effective in activating GPR40 and improving glucose control *in vivo*. The *in-vitro* experiments confirmed the stimulatory effect of SCO-267 on Ca²⁺ signaling, insulin release, and GLP-1 secretion in *FFAR1*-expressing CHO, MIN6, and GLUTag cells, respectively. When tested in normal rats, SCO-267 stimulated the secretion of hormones from the islet and gut. These results demonstrate the full agonistic property of SCO-267 against GPR40. Hypoglycemia was not induced in rats during the fasting condition. Studies in N-STZ-1.5 rats with diabetes confirmed the efficacy of

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SCO-267 in improving glucose control, and the durability of this effect. SCO-267 was also effective in decreasing body weight in DIO rats, which may have been via a GPR40-dependent mechanism. The data suggest that SCO-267 has potential as a novel therapeutic agent for treating diabetes and obesity.

In a single dosing study of N-STZ-1.5 rats, 0.3 mg/kg SCO-267 (C_{\max} , 22.7 ng/mL) had a glucose-lowering efficacy comparable to that of 3 mg/kg fasiglifam (C_{\max} , 6.17 μ g/mL). This indicated that a substantially lower compound exposure is sufficient for inducing the efficacy of SCO-267. In a repeated-dosing study of N-STZ-1.5 rats, 1 mg/kg SCO-267 (C_{\max} , 139 ng/mL; AUC_{0-24h} , 626 ng·h/mL) was more effective in improving glucose tolerance than 10 mg/kg fasiglifam (C_{\max} , 39.8 μ g/mL; AUC_{0-24h} , 255 μ g·h/mL). Plasma protein binding of SCO-267 was similar across species (99.6–99.7%), and fasiglifam showed similar plasma protein binding across species (>99.4% for fasiglifam (Kogame et al., 2018)). With the clinically effective exposure of 50 mg fasiglifam (C_{\max} , 5.3 μ g/mL; AUC_{0-24h} , 100.3 μ g·h/mL (Leifke et al., 2012)), SCO-267 may be effective in improving glucose control in patients with type 2 diabetes. Additionally, the lower plasma exposure to SCO-267 may be a safety advantage compared to exposure to fasiglifam, which was voluntarily terminated in phase 3 due to possible adverse effects on the liver.

SCO-267 robustly stimulated insulin secretion in dysfunctional beta-cells in N-STZ-1.5 rats. In addition, SCO-267 stimulated the secretion of GLP-1 and GIP, both of which are incretin hormones having insulinotropic action (Nauck and Meier, 2018). Taken together with the direct insulinotropic effect on beta-cells expressing *Ffar1*, increased secretion of GLP-1 and GIP may have contributed to the enhanced insulin secretion observed in SCO-267-administered rats.

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We compared the efficacy of SCO-267 to AM-1638, a well-studied GPR40 full agonist. *In vitro* experiment showed that Ca^{2+} influx activity of SCO-267 was 5.5-12.5-fold more potent compared to AM-1638 in human *FFAR1*-expressing CHO cells. When each compound was orally dosed at the same dosage levels, SCO-267 was more effective in improving glucose tolerance compared to AM-1638 in N-STZ-1.5 rats. These results indicate that SCO-267 is a potent GPR40 full agonist. Considering the lower plasma exposure of SCO-267 in inducing efficacy and good pharmacokinetic profiles, SCO-267 is likely a good candidate for testing in clinical trials.

When a GPCR is exposed to its agonist over a period of minutes to hours or days, the response is significantly reduced and is associated with decreased receptor expression at the plasma membrane. This process is referred to as downregulation (Zhang et al., 2014; Rajagopal and Shenoy, 2018). Some GPCRs undergo receptor desensitization after repeated stimulation, thus decreasing and, in some cases, abolishing the biological response to a drug (Zhang et al., 2014; Rajagopal and Shenoy, 2018). In diabetes and obesity, sustained efficacy is important for a drug targeting GPCR to induce therapeutic benefits. In the present study, 2 weeks of repeated dosing of SCO-267 resulted in sustained glucose lowering, and the efficacy was much better than that of fasiglifam in N-STZ-1.5 rats. This suggests that the efficacy of SCO-267 is strong and durable. Although the agonism of fasiglifam is partial, it has shown effectiveness in decreasing HbA1c levels in a 52-week study of patients with type 2 diabetes (Kaku et al., 2016). Therefore, SCO-267 may induce similar durability and better efficacy in humans.

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Food intake was diminished, and body weight was lowered in SCO-267-treated DIO rats. Plasma GLP-1 and PYY levels remained high 16 h after the final dose was administered to DIO rats. Considering the elevated levels of GLP-1 and PYY, both of which are physiological hormones regulating satiety and body weight (Moran, 2006; Troke et al., 2014), these hormones may have contributed to the body weight reduction observed in SCO-267-administered DIO rats. In addition, sustained plasma exposure of SCO-267 was confirmed in DIO rats with reduced body weight, suggesting that the efficacy is durable. SCO-267-induced GLP-1 secretion and food intake inhibition were observed in WT mice and were abolished in *Ffar1*^{-/-} mice. This suggests that the above processes are controlled by GPR40-dependent mechanisms. Although a further study is essential to elucidate the mechanism by which GPR40 agonism regulates food intake and body weight, this property of SCO-267 may be beneficial in patients who are overweight or obese and have type 2 diabetes.

SCO-267 stimulated glucagon secretion in rats, which is consistent with a previous study using a GPR40 full agonist (Pachanski et al., 2017). This suggests that GPR40 full agonists exhibit a direct targeted effect on glucagon secretion. Glucagon has pleiotropic physiological effects in the body as evidenced by the high expression of its receptors in the liver and low expression in the kidneys, adipose tissue, lymphoblasts, spleen, pancreas, brain, adrenal glands, and gastrointestinal tract (Svoboda et al., 1994). In addition to its pivotal role in hepatic glucose metabolism, glucagon regulates hepatic fat metabolism promoting lipid oxidation and lowering lipid synthesis (Seghieri et al., 2018). It is well established that non-alcoholic fatty liver disease is common among individuals with type 2 diabetes and increases the risk of diabetes and diabetic complications (Hazlehurst et al., 2016). Therefore, the activation of liver glucagon signaling which decreases hepatic lipid

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may improve hepatocyte function. Furthermore, a recent study suggested that glucagon receptor signaling is essential in the control of murine hepatocyte survival (Sinclair et al., 2008). Glucagon signaling activation has also been suggested to exert anti-apoptotic actions in the liver (Sinclair et al., 2008). Therefore, glucagon signaling activation may be beneficial in treating liver diseases such as non-alcoholic fatty liver disease and more advanced non-alcoholic steatohepatitis. In fact, the addition of glucagon agonism to GLP-1R agonism improved lipid metabolism and hepatic steatosis when compared to GLP-1R agonism alone in rodents (Day et al., 2009). However, the contribution of SCO-267-induced glucagon stimulation to liver health requires further studies.

In conclusion, the GPR40 full agonist, SCO-267, stimulated insulin, glucagon, GLP-1, GIP, and PYY secretion. Furthermore, SCO-267 effectively improved glucose control and exerted strong efficacy in rats with diabetes. In addition, body weight loss was observed in obese rats. Thus, SCO-267 is effective in improving diabetes and obesity in rats and may induce similar favorable effects in patients with diabetes and obesity.

Acknowledgments

MIN6 murine pancreatic β -cell cells and GLUTag intestinal L-cells were kindly provided by Jun-ichi Miyazaki (Osaka University) and Daniel J. Drucker (University of Toronto), respectively. We thank Ikumi Chisaki for measuring pharmacokinetics parameters, Seigo Izumo for valuable discussions and helpful suggestions. We gratefully acknowledge Tomofumi Kurokawa, Yukio Toyoda, Yoshitaka Yasuhara, Naoyoshi Noguchi, Toshitake Kobayashi, Toshikazu Ando and Masahiro Ide for support.

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Conflicts of interest

H.U., R.I., H.M., H.O., Ya.M., T.Y., Y.T., K.T., N.N. are/were employees of Takeda Pharmaceutical

Company Ltd. S.A., M.O., A.K., M.W., Y.Y. T.M. and Yu.M. are employees of SCOHIA PHARMA, Inc.

Authorship Contributions

Participated in research design: All authors.

Conducted experiments: Ueno, Ito, Abe, Ookawara, Miyashita, Ogino, Kobayashi, Moritoh

Contributed new reagents or analytic tools: Miyamoto, Yoshihara, Maekawa

Performed data analysis: All authors.

Wrote or contributed to the writing of the manuscript: Ueno, Nishigaki and Moritoh.

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Footnotes

The present study was conducted with financial support from Takeda Pharmaceutical Company Ltd and SCOHIA PHARMA Inc.

Legends for figures

Figure 1. Effects of SCO-267 on Ca^{2+} concentration in *FFAR1*-expressing CHO cells, insulin secretion in MIN6 cells and GLP-1 secretion in GLUTag cells.

Chemical structure of SCO-267 (A). Intracellular Ca^{2+} responses against SCO-267 and other agonists in CHO cells expressing high (B, clone #104) and low (C, clone #2) levels of human *FFAR1* (mean \pm S.E.M. of quadruplicate wells, similar results were obtained in an independent study). Concentration-dependent insulin secretion response of SCO-267 in MIN6 cells with 16 mmol/L glucose condition (mean \pm S.E.M. of triplicate wells) (D). Stimulation of GLP-1 secretion in GLUTag cells with 10 mmol/L glucose condition (mean \pm S.E.M. and individual data of triplicate wells) (E). SCO-267 activated an intracellular Ca^{2+} response in CHO cells expressing high and low levels of human *FFAR1*, showing full agonistic potency for GPR40. SCO-267

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stimulated insulin from MIN6 β cells and GLP-1 secretion from GLUTag enteroendocrine cells. Fasi, fasiglifam

Figure 2. Effect of a single dosing of SCO-267 on hormone secretion and fasting glucose in normal rats.

Plasma levels of insulin (A), glucagon (B), GLP-1 (C), GIP (D), and PYY (E) in SCO-267-administered non-fasting normal rats. (F) Effects on fasting plasma glucose levels in overnight-fasted normal rats. SCO-267 increased insulin, glucagon, GLP-1, GIP, and PYY levels in non-fasting normal rats. When administered to fasted normal rats, SCO-267 did not induce hypoglycemia, which was defined as plasma glucose levels <70 mg/dL in this experiment. * and # $P < 0.025$ vs. vehicle by one-tailed Williams' test and Shirley–Williams test, respectively. Values are presented as mean \pm S.D. (n = 6). Fasi, fasiglifam

Figure 3. Effects of single and repeated administration of SCO-267 on hormone secretion and glucose control in diabetic N-STZ-1.5 rats.

Plasma insulin (A), GLP-1 (B), and glucose levels (C) in a single-dosing study with N-STZ-1.5 rats. Plasma levels of insulin (D) and glucose (E) after 2 weeks of repeated-dosing with SCO-267 in N-STZ-1.5 rats. In a single dosing study, SCO-267 increased insulin levels, stimulated GLP-1 secretion, and improved glucose tolerance. Enhanced insulin secretion and improved glucose tolerance were maintained after the 2-week repeated administration of SCO-267. * and # $P < 0.025$ vs. vehicle by one-tailed Williams' test and one-tailed

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Shirley–Williams test, respectively. † $P < 0.05$ vs vehicle by Student's *t*-test. § and ¶ $P < 0.05$ vs. vehicle by Dunnett's test and Steel test, respectively. Values are presented as mean \pm S.D. (n = 6). Fasi, fasiglifam

Figure 4. Effect of SCO-267 on gut hormone secretion and body weight control in DIO-rats.

Plasma levels of GLP-1 (A), PYY (B), daily food intake (C), total food intake (D), body weight change (E), and fat mass change (F) in DIO-rats. SCO-267 increased plasma GLP-1 and PYY after 2 weeks of treatment. Food intake was inhibited, and body weight was decreased by SCO-267 treatment. Fat mass was decreased in SCO-267-administered rats. * $P < 0.025$ vs. vehicle by one-tailed Williams' test. Values are presented as mean \pm S.D. (n = 6)

Figure 5. Effects of SCO-267 on GLP-1, food intake, and body weight in *Ffar1*^{-/-} mice.

GLP-1 levels (A), food intake (B), and body weight change (C). WT and *Ffar1*^{-/-} mice were treated with either vehicle or SCO-267 (30 mg/kg) for 3–4 days. SCO-267 increased plasma GLP-1, decreased total food intake, and decreased body weight in WT mice, whereas these effects were abolished in *Ffar1*^{-/-} mice. † and ‡ $P < 0.05$ vs. vehicle-treated WT mice by Student's *t*-test and the Aspin–Welch test, respectively. Values are presented as mean \pm S.D. (n = 6).

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Tables

Table 1. Ca²⁺ influx activity in human *FFAR1*-expressing CHO cells.

Test material	CHO cells with high <i>FFAR1</i>		CHO cells with low <i>FFAR1</i>	
	expression (clone #104)		expression (clone #2)	
	EC ₅₀ (nmol/L)	E _{max}	EC ₅₀ (nmol/L)	E _{max}
	[95% CI]	(%γ-linolenic acid)	[95% CI]	(%γ-linolenic acid)
SCO-267	1.3 [0.97–1.7]	125	12 [11–14]	201
AM-1638	7.1 [5.6–9.2]	110	150 [120–180]	182
Fasiglifam	24 [16–37]	100	>1000	22
α-linolenic acid	>10000	110	>10000	93
Linoleic acid	>10000	109	>10000	102
Palmitic acid	>10000	113	>10000	78
γ-linolenic acid	>10000	100	>10000	100

CI, confidence interval

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Table 2. *In-vitro* plasma protein binding ratios of SCO-267 in rats, dogs, and humans.

Matrix	Concentration of compounds	Binding ratio
	($\mu\text{g/mL}$)	(%)
Rat	0.05	99.6
	0.5	99.6
	5	99.6
Dog	0.05	99.6
	0.5	99.7
	5	99.7
Human	0.05	99.7
	0.5	99.7
	5	99.7

Mean of three samples is presented. Matrices shown are pooled plasma samples of 20 male SD rats (8 weeks old), pooled plasma samples of 10 male beagles (10 months old), and pooled plasma samples of 10 male and 10 female humans.

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Table 3. Plasma metabolic parameters in SCO-267-treated DIO rats.

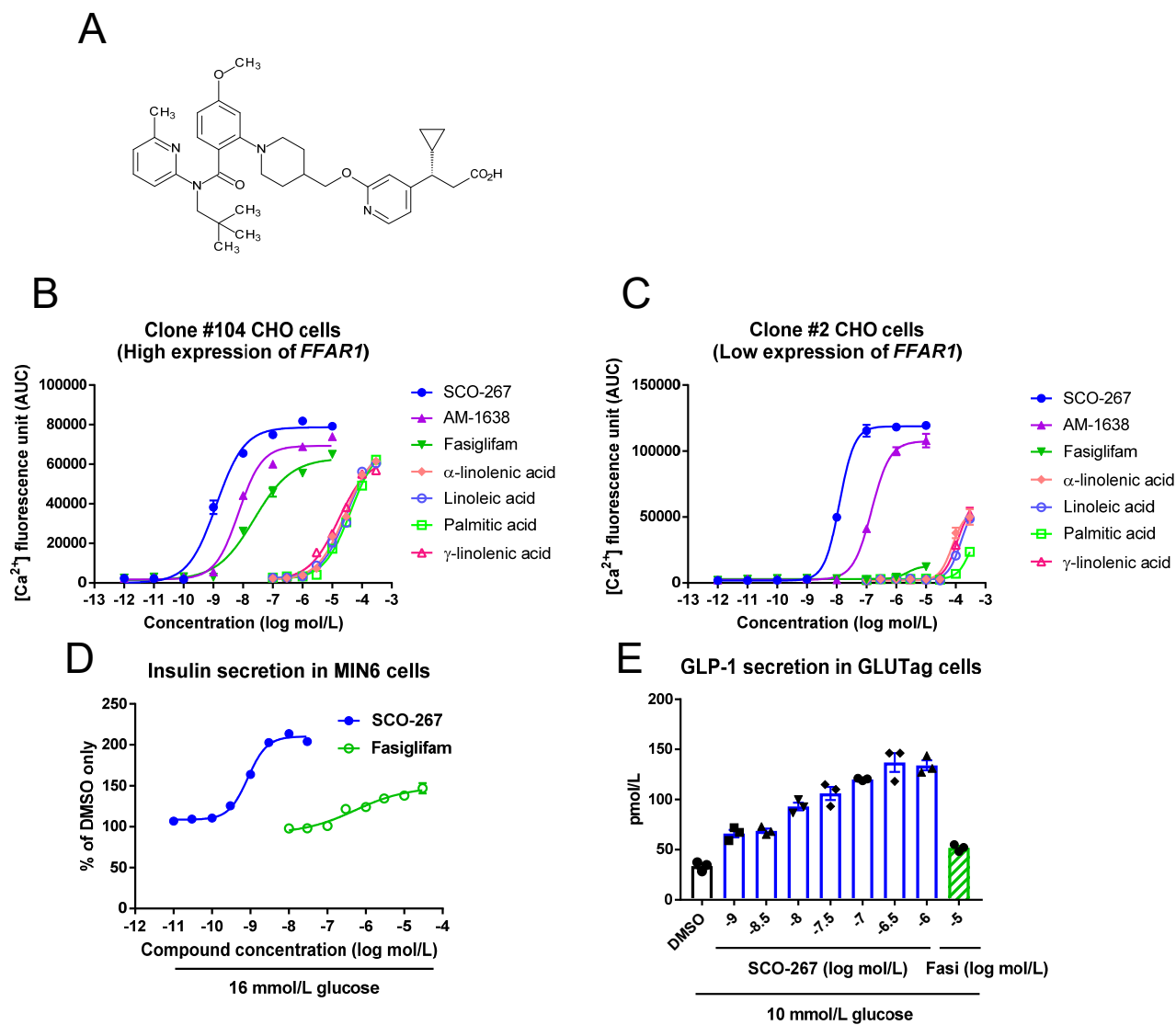
Dosage	Glucose	Triglyceride	Total cholesterol	Alanine aminotransferase	Aspartate aminotransferase
	(mg/dL)	(mg/dL)	(mg/dL)	(U/L)	(U/L)
Vehicle	119 ± 10	296 ± 98	117 ± 17	42 ± 8	74 ± 29
SCO-267 (0.3 mg/kg)	120 ± 2	271 ± 63	109 ± 8	48 ± 15	61 ± 13
SCO-267 (1 mg/kg)	116 ± 14	242 ± 75	100 ± 12 *	45 ± 10	64 ± 10
SCO-267 (3 mg/kg)	118 ± 9	261 ± 106	102 ± 10 *	47 ± 8	62 ± 8

* $P < 0.025$ vs. vehicle by one-tailed Williams' test. n = 6, mean ± S.D.

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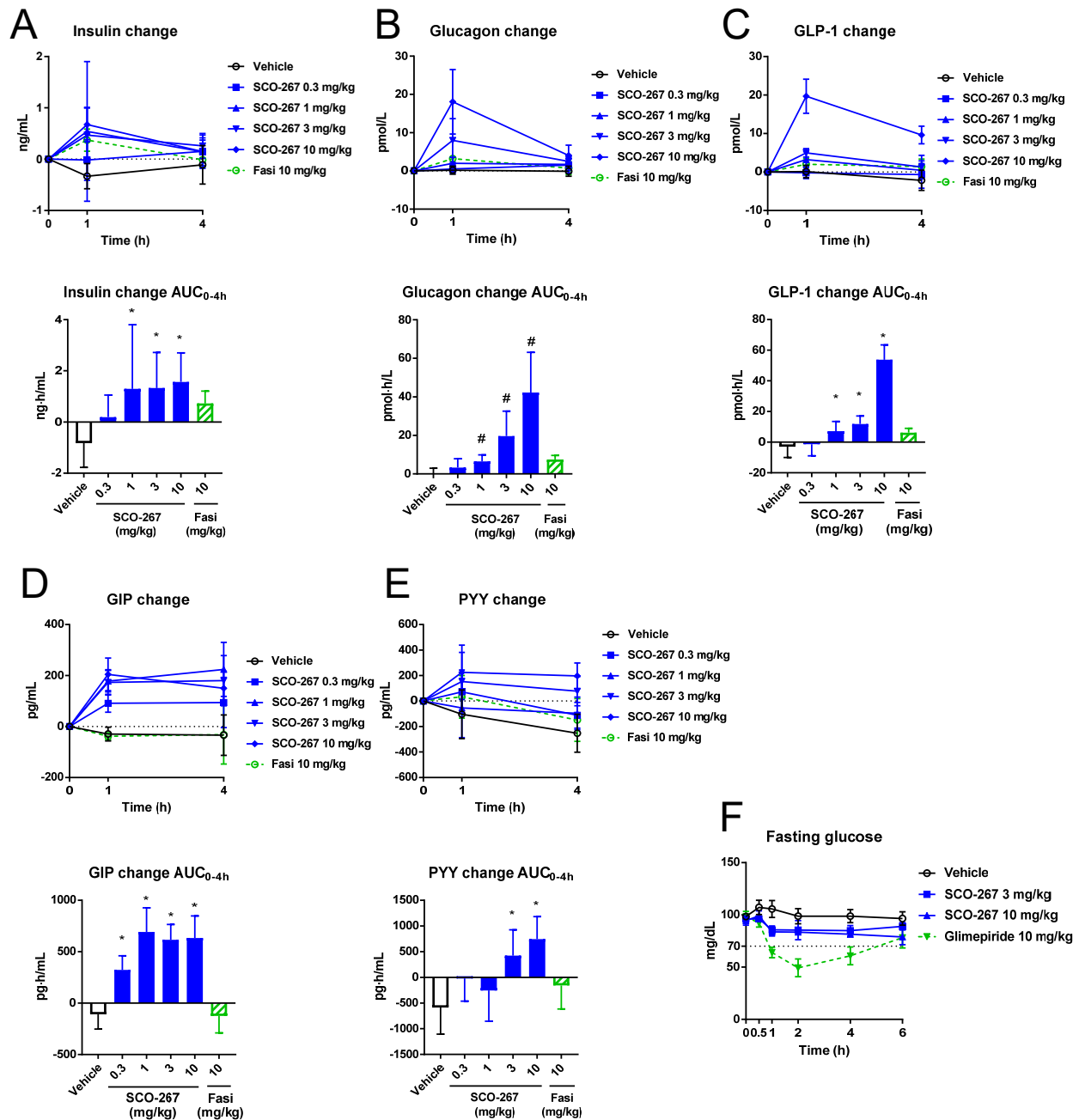
Figures

Figure 1



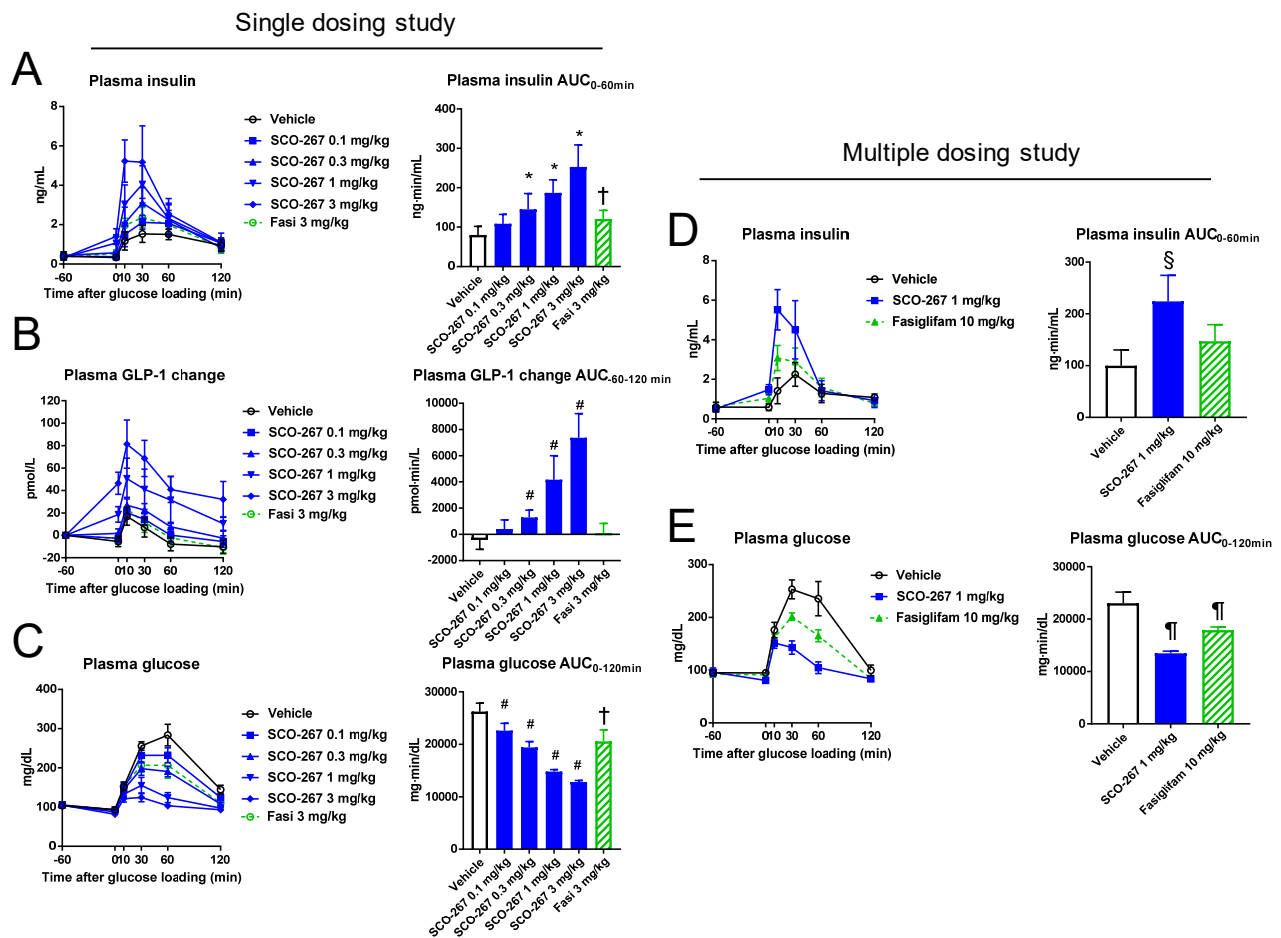
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Figure 2



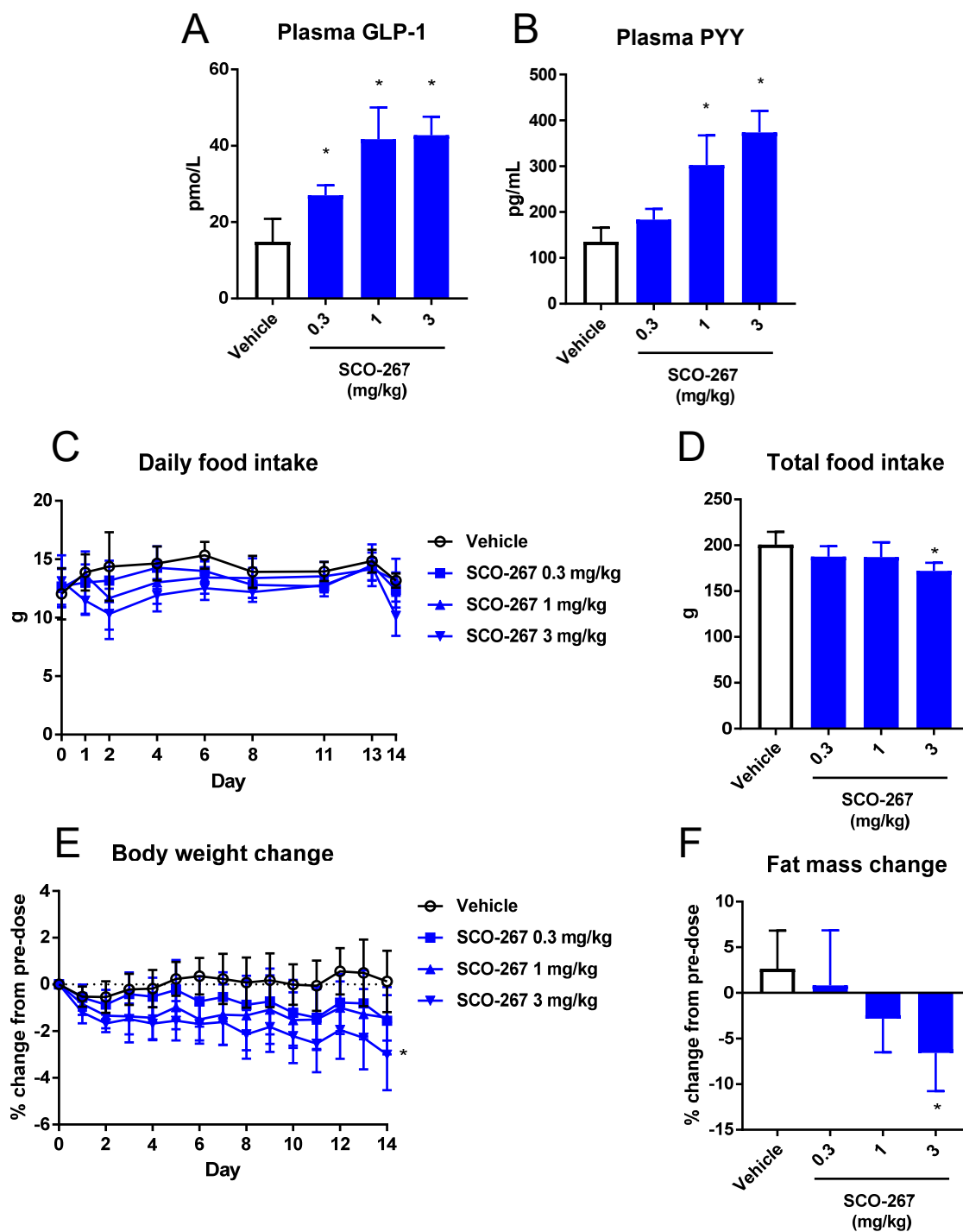
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Figure 3



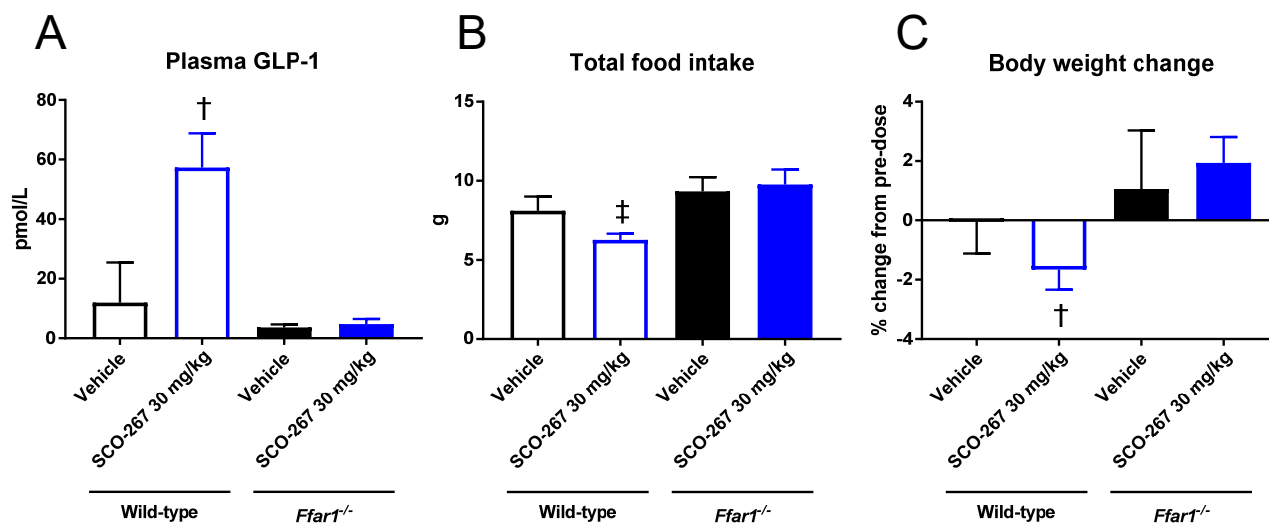
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Figure 4



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Figure 5

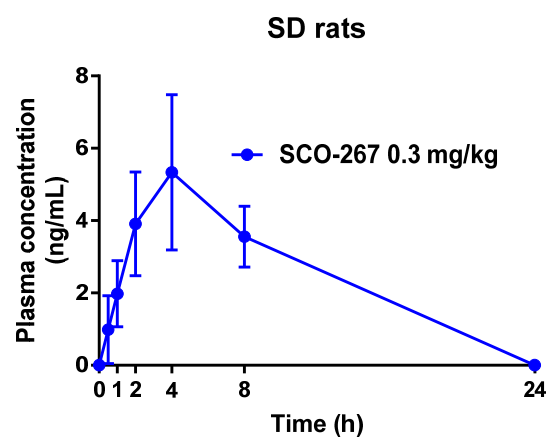


SCO-267, a GPR40 full agonist, improves glycemic and body weight control in rat models of diabetes and obesity

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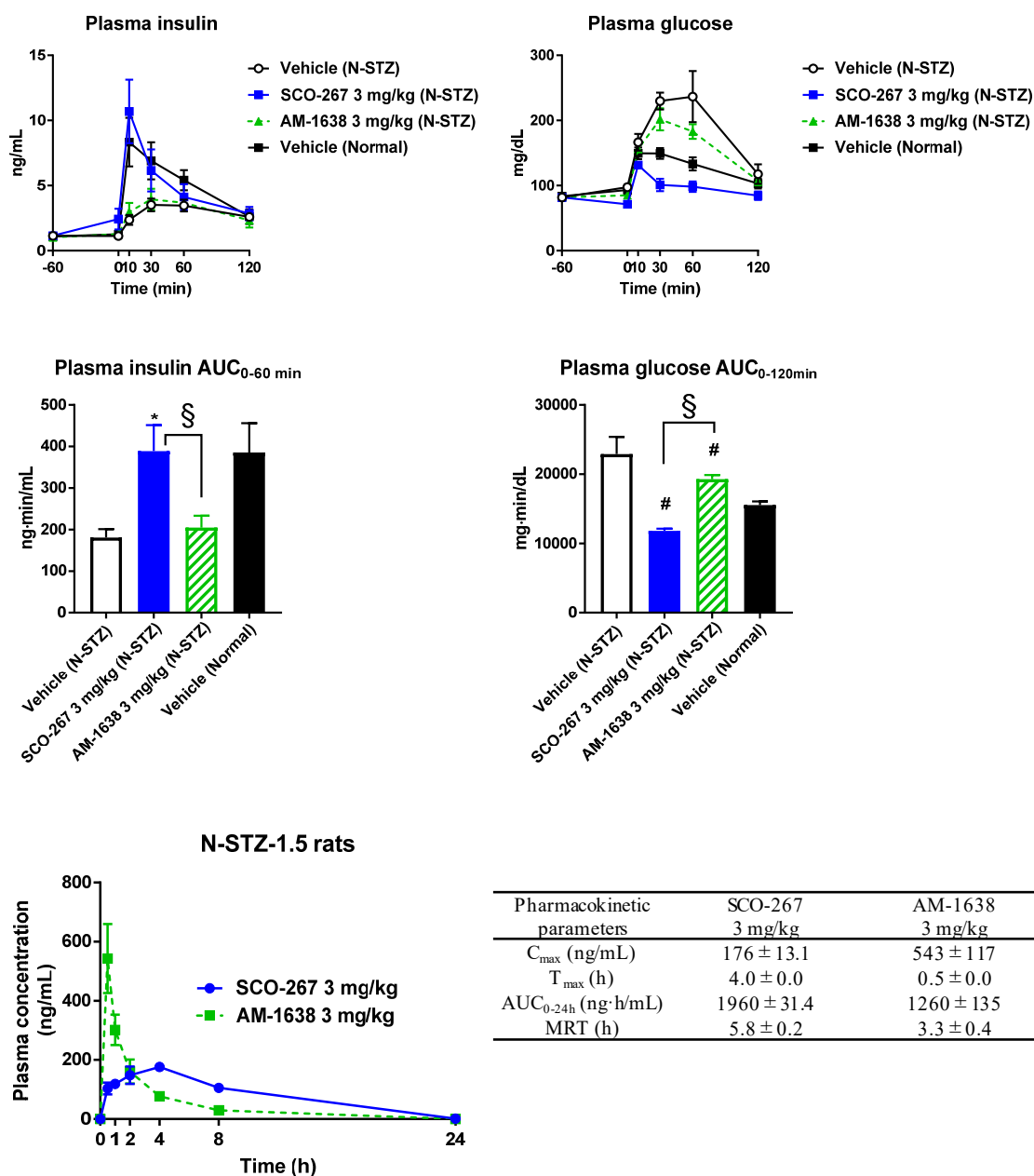
Supplemental Data



Pharmacokinetic parameters	SCO-267	SCO-267
	Oral gavage 0.3 mg/kg	Intravenous 0.1 mg/kg
C_{\max} (ng/mL)	5.34 ± 2.14	122 ± 22.2
T_{\max} (h)	4.0 ± 0.0	-
AUC_{0-24h} (ng·h/mL)	59.4 ± 17.5	79.8 ± 31.3
$T_{1/2}$ (h)	-	4.1 ± 1.4
CL_{tot} (mL/h/kg)	-	1280 ± 333
V_{ss} (mL/kg)	-	4950 ± 2470
BA (%)	24.8 ± 7.3	-

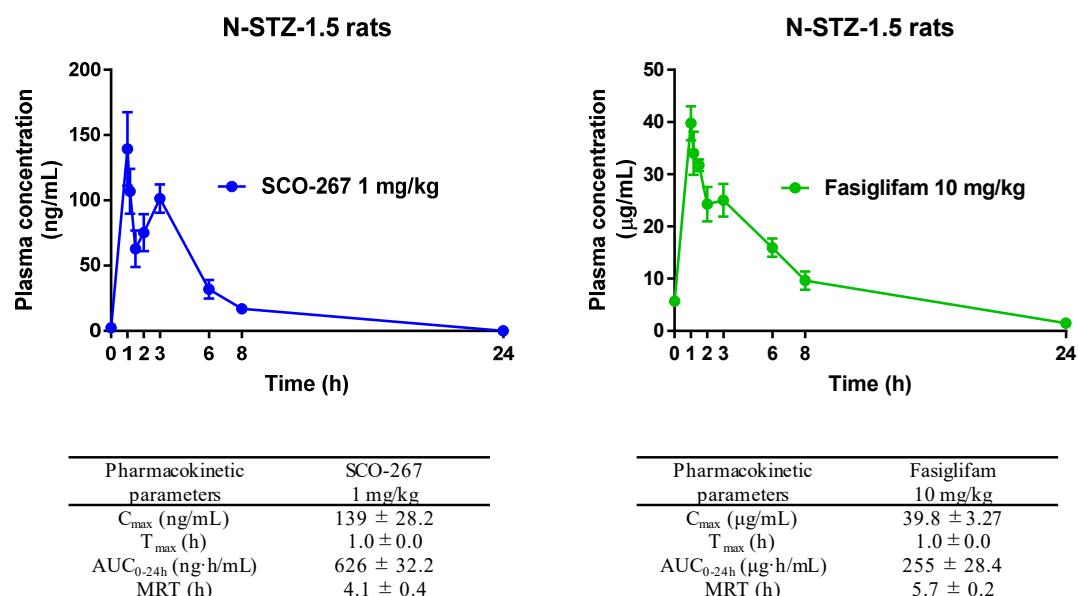
Supplemental Figure 1. Pharmacokinetic analysis in SD rats.

C_{\max} by an intravenous dosing was observed at 5 min. Values are presented as mean \pm S.D. (n = 3). C_{\max} , maximum concentration; T_{\max} , time to reach maximum concentration after dosing; AUC_{0-24h} , the area under the concentration-time curve from the time 0 to 24 hours; $T_{1/2}$, the terminal half-life; CL_{tot} , the total plasma clearance; V_{ss} , volume of distribution at steady state; BA, bioavailability.



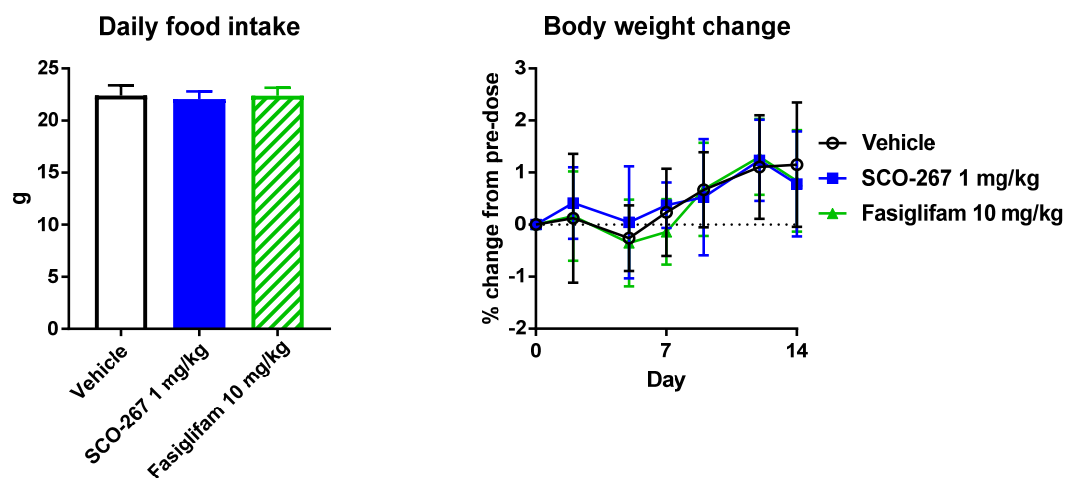
Supplemental Figure 2. Glucose tolerance test and pharmacokinetic analysis of SCO-267 and AM-1638 in N-STZ-1.5 rats.

Lower plasma exposure of SCO-267 effectively improved glucose tolerance than AM-1638 in N-STZ-1.5 rats. * and # $P < 0.05$ vs. vehicle (N-STZ) by Dunnett's test and Steel test, respectively. § $P < 0.05$ by Aspin-Welch test. Normal, Wistar Kyoto rats. Values are presented as mean \pm S.D. (n = 6 for glucose tolerance test and 3 for pharmacokinetic study). C_{max} , maximum concentration: T_{max} , time to reach maximum concentration after dosing: AUC_{0-24h} , the area under the concentration-time curve from the time 0 to 24 hours: MRT, mean residence time.



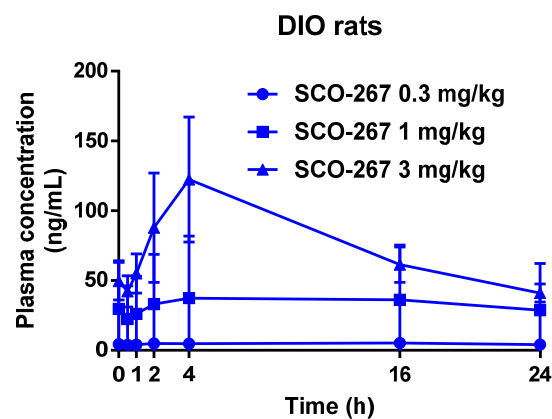
Supplemental Figure 3. Pharmacokinetic analysis in N-STZ-1.5 rats

Values are presented as mean \pm S.D. ($n = 6$). C_{max} , maximum concentration: T_{max} , time to reach maximum concentration after dosing: AUC_{0-24h} , the area under the concentration-time curve from the time 0 to 24 hours: MRT, mean residence time.



Supplemental Figure 4. Daily food intake and body weight change in N-STZ-1.5 rats.

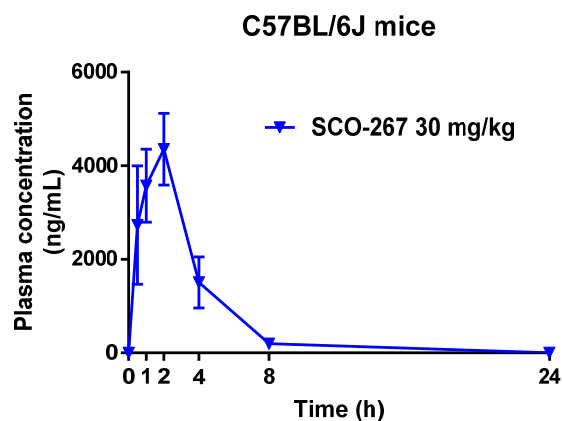
SCO-267 had no significant effect on daily food intake and body weight in N-STZ-1.5 rats. Values are presented as mean \pm S.D. (n = 6).



Pharmacokinetic parameters	SCO-267 0.3 mg/kg	SCO-267 1 mg/kg	SCO-267 3 mg/kg
C_{\max} (ng/mL)	5.50 ± 0.548	39.5 ± 43.5	122 ± 44.9
T_{\max} (h)	10.0 ± 6.9	8.8 ± 6.6	4.0 ± 0.0
AUC_{0-24h} (ng·h/mL)	114 ± 7.99	825 ± 932	1840 ± 419
MRT (h)	11.9 ± 0.7	11.8 ± 0.8	9.7 ± 1.3

Supplemental Figure 5. Pharmacokinetic analysis in DIO F344 rats.

Values are presented as mean \pm S.D. ($n = 6$). C_{\max} , maximum concentration: T_{\max} , time to reach maximum concentration after dosing: AUC_{0-24h} , the area under the concentration-time curve from the time 0 to 24 hours: MRT, mean residence time.



Pharmacokinetic parameters	SCO-267 30 mg/kg
C_{\max} (ng/mL)	4530 ± 634
T_{\max} (h)	1.7 ± 0.6
AUC_{0-24h} (ng·h/mL)	17100 ± 2890
MRT (h)	3.0 ± 0.1

Supplemental Figure 6. Pharmacokinetic analysis in C57BL/6J mice.

Values are presented as mean \pm S.D. ($n = 3$). C_{\max} , maximum concentration; T_{\max} , time to reach maximum. concentration after dosing; AUC_{0-24h} , the area under the concentration-time curve from the time 0 to 24 hours; MRT, mean residence time.