Regular Article

Identification of 2-[2-(4-*tert*-Butylphenyl)ethyl]-*N*-[4-(3-cyclopentylpropyl)-2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide as an Orally Active MGAT2 Inhibitor

Tsuyoshi Busujima,^{*,a} Hiroaki Tanaka,^a Kanako Iwakiri,^a Yoshihisa Shirasaki,^a Eiji Munetomo,^b Masako Saito,^b Aiko Masuko,^b Kiyokazu Kitano,^b Fusayo Io,^b Koji Kato,^c Shunsuke Kamigaso,^c Akiko Nozoe,^d and Nagaaki Sato^a

^a Chemistry Laboratories, Taisho Pharmaceutical Co., Ltd.; 1–403 Yoshino-cho, Kita-ku, Saitama 331–9530, Japan: ^b Pharmacology Laboratories, Taisho Pharmaceutical Co., Ltd.; 1–403 Yoshino-cho, Kita-ku, Saitama 331–9530, Japan: ^c Drug Safety and Pharmacokinetics Laboratories, Taisho Pharmaceutical Co., Ltd.; 1–403 Yoshino-cho, Kitaku, Saitama 331–9530, Japan: and ^d Pharmaceutical Science Laboratories, Taisho Pharmaceutical Co., Ltd.; 1–403 Yoshino-cho, Kita-ku, Saitama 331–9530, Japan.

Received October 20, 2015; accepted December 15, 2015

We previously reported 2-[2-(4-tert-butylphenyl)ethyl]-N-(4-fluorophenyl)-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide 2 as on orally available monoacylglycerol acyltransferase 2 (MGAT2) inhibitor which exhibited an *in vivo* efficacy at an oral dose of 100 mg/kg in a mouse oral lipid tolerance test. Further optimization of compound 2 to improve the intrinsic potency culminated in the identification of compound 11. Compound 11 showed a >50-fold lower IC₅₀ against human MGAT2 enzyme than 2. Oral administration of 11 at a dose of 3 mg/kg in the oral lipid tolerance test resulted in significant suppression of triglyceride synthesis.

Key words monoacylglycerol acyltransferase 2; fat absorption; oral lipid tolerance test; tetrahydroisoquinoline

A rapid increases of obesity, type 2 diabetes and cardiovascular disease have become a huge issue in the world's industrialized countries. The unmet medical need for the treatment of these diseases has promoted the identification of many new targets, one of which is the inhibition of triacylglycerol synthesis.

Dietary-derived fats are degraded in the gastrointestinal tract and then taken up by small-intestinal epithelial cells. Acyl CoA: monoacylglycerol acyltransferase (MGAT), which catalyzes the synthesis of diacylglycerol from monoacylglycerol and acyl-CoA, plays a crucial role in triglyceride (TG) re-synthesis in the small intestine.¹⁾ After re-synthesis into neutral fats in the cells,²⁾ the neutral fats are packaged into chylomicrons, secreted into the lymphatics, and taken up into body. Therefore, the inhibition of activity of this enzyme is expected to suppress re-synthesis of TG and lead to reduce fat absorption.

Three isoforms of MGAT enzyme have been identified,³⁻⁵⁾ and MGAT2 is highly expressed in small intestine in both humans and mice.¹⁾ The phenotype of MGAT2 deficient mice has been already reported.⁶⁾ These mice were viable and fertile without apparent abnormalities. After these mice were orally given dietary oil (oral lipid tolerance test), plasma TG levels of these mice were significantly lower than those of the wild type. In addition, MGAT2 knock-out mice showed resistance to obesity, glucose intolerance, and hypercholesterolemia induced by a high fat diet. Furthermore, these mice demonstrated increased oxygen consumption without high-fat feeding, and MGAT2 was expected to modulate energy expenditure.^{7,8)} These results implied that inhibition of MGAT2 could be an attractive mechanism for reduction of fat absorption and treatment of obesity and associated metabolic disorders.

Some small molecule MGAT2 inhibitors have been disclosed.⁹⁻¹⁵⁾ As shown in Fig. 1, AstraZeneca's compound **1** reported in the literature showed potent MGAT2 inhibitory activity and a significant reduction of plasma TG concentration after an oral administration at a dose of 150 mg/kg in mice oral lipid tolerance test.^{16–18)} We have also reported an orally available MGAT2 inhibitor,¹⁹⁾ 2-[2-(4-*tert*-butylphenyl)ethyl]-*N*-(4-fluorophenyl)-1,2,3,4-tetrahydroisoquinoline-6sulfonamide **2**, which demonstrated the significant suppression of fat absorption in mice oral lipid tolerance test at an oral dose of 100 mg/kg.

The identification of compound 2 promoted us to explore a more potent and orally available MGAT2 inhibitor, and our efforts have been focused on optimization of compound 2. As





*To whom correspondence should be addressed. e-mail: t-busujima@so.taisho.co.jp



Reagents and conditions: (a) TFAA, pyridine, DMAP, CHCl₃, rt; (b) ClSO₃H, CHCl₃, 60°C, 62% in two steps; (c) R^1R^2NH , pyridine, CHCl₃, rt; (d) H₂, 10% Pd–C, MeOH–EtOAc, rt; (e) KOH aq., EtOH, rt, 61–94% in three steps; (f) 2-(4-(*tert*-butyl)phenyl)acetic acid, EDC·HCl, HOBt·H₂O, CHCl₃, rt, 59% for **17a**; (g) 10% TFA, CHCl₃, rt, 54% in two steps for **17b**; (h) BH₃–THF, THF, relux; (i) 4N HCl/EtOAc, EtOAc, rt, 91 and 45% in two steps for **2** and **9**, 65 and 63% in three steps for **5** and **8**, 56–84% in two steps for **10–13**; (j) 2-(4-(*tert*-butyl)phenyl)acetaldehyde, NaBH(OAc)₃, ClCH₂CH₂Cl, rt.

Chart 1



Reagents and conditions: (a) RX, K_2CO_3 , DMF, rt; (b) H_2 , 10% Pd–C, EtOH, rt, 55–81% in two steps; (c) 2,4-dimethoxybenzaldehyde, NaBH(OAc)₃, AcOH, THF, rt, 34%-quant.; (d) olefin or borane reagent, Pd catalyst, solvent, reflux, 69–92%; (e) PPh₃, I_2 , imidazole, CHCl₃, rt; (f) PPh₃, CH₃CN, reflux, then 3-fluoro-4-nitrobenzaldehyde, KHMDS, THF, rt, 37% in two steps; (g) H_2 ,10% Pd–C, EtOH, rt, 57%.

Chart 2

a result, introduction of the substituents at the *para* position of the sulfonamide phenyl ring of compound 2 led to identify compound 11 (Fig. 1).

Herein, we describe the synthesis and biological properties of 2-[2-(4-*tert*-butylphenyl)ethyl]-*N*-[4-(3-cyclopentylpropyl)-2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (11) as a more potent MGAT2 inhibitor and its derivatives.

Chemistry

Synthesis of 1,2,3,4-tetrahydroisoquinoline-6-sulfonamide derivatives (compounds 2, 5, 8–13) were shown in Chart 1. The secondary amine of 14 was protected with trifluoroacetic anhydride (TFAA), followed by chlorosulfonylation to afford

15. Intermediates 16a-h were synthesized by condensation with the corresponding anilines, debromination by hydrogenolysis, and subsequent removal of trifluoroacetyl group. The secondary amines 16a and b were condensed with (4-*tert*-butylphenyl)acetic acid, followed by deprotection of the 2,4-dimethoxybenzyl group by treating with trifluoroacetic acid (TFA) to afford the amide compounds 17a and b, respectively. *N*-Alkyl derivatives 2 and 9 were prepared by reduction of these amide compounds using borane-tetrahydrofuran (THF) complex as a reducing reagent. Other intermediates 16c-h were converted into the corresponding *N*-alkylated compounds by reductive amination with 2-(4-(*tert*-butyl)-phenyl)acetaldehyde.



Reagents and conditions: (a) 2,4-dimethoxybenzaldehyde, NaBH(OAc)₃, AcOH, THF, rt, 92%; (b) **27**, pyridine, CHCl₃, rt; (C) H₂, 10% Pd–C, Et₃N, MeOH–EtOAc, rt, 70% in two steps; (d) RX, K_2CO_3 , DMF, rt; (e) KOH aq., EtOH, rt; (f) 2-(4-(*tert*-butyl)phenyl)acetaldehyde, NaBH(OAc)₃, CHCl₃, rt, 36–68% in three steps; (g) 20% TFA, CHCl₃, rt; (h) 4N HCl/EtOAc, EtOAc, rt, 2–33% in two steps.

Chart 3



Fig. 2. MGAT2 Inhibitory Activity of Tetrahydroisoquinoline and Dihydro-1H-isoindole Sulfonamide Derivatives

Preparation of the anilines (20a-22c) was accomplished by the synthetic routes as depicted in Chart 2. Anilines 19a-cwere synthesized by alkylation with the corresponding alkyl halides using 3-fluoro-4-nitrophenol 18 as a starting material and subsequent reduction of the nitro group. These anilines were protected with 2,4-dimethoxybenzyl group to give 20a-c.

Aniline 21 was coupled with the corresponding olefins or borane reagents using Pd-catalyst to afford 22e-g. Aniline 25 was prepared from alcohol 23 by iodination, Wittig reaction with 3-fluoro-4-nitrobenzaldehyde, and reduction of the nitro group.

As shown in Chart 3, *O*-alkyl derivatives 4, 6, and 7 were synthesized using aniline 27 protected with 2,4-dimethoxybenzyl group. The *N*-protected sulfonamide intermediate 28 was obtained by condensation of the sulfonyl chloride 15 with aniline 27, and subsequent hydrogenolysis to remove the bromo group. Compound 28 was alkylated with the corresponding alkyl halides, followed by the same procedure as described for the preparation of 5 to afford 4, 6, and 7.

Results and Discussion

As we previously reported,¹⁹⁾ the structure–activity relationship (SAR) study of 2,3-dihydro-1*H*-isoindole-5-sulfonamide derivative **3** indicated that the longer the alkoxy chain at the *para* positions of sulfonamide-substituted phenyl ring was, the more it improved the enzyme inhibitory activity (**3a** *vs.* **b** in Fig. 2). The plasma exposure level of these isoindoline derivatives after an oral administration to mice, however, was too low due to its insufficient solubility (low absorption), while compound **2** was orally available because its solubility was improved by replacing the isoindolyl-urea scaffold with tetrahydroisoquinoline. Therefore, to identify a more potent and orally available MGAT2 inhibitor, we focused on the optimization of the substituents at the *para* position of the phenyl ring and applied these findings to the tetrahydroisoquinoline scaffold (Table 1).

As expected, compound 4 possessing the same substituents as compound 3b was three times more potent than 2. It was found that further extension of the length of alkoxy group (to *n*-hexyloxy and *n*-heptyloxy) resulted in increase of the potency (5 and 6), while *n*-octyloxy analog (7) was as potent as 6. Incorporation of benzene ring at the terminal position of the side chain was tolerable like 8. Replacement of the benzene ring with cyclohexyl group enhanced the potency (8 vs. 9). In addition, replacement of the ether linker with the methylene linker was also found to be tolerable (9 vs. 10). Introduction of the cyclopentyl group at the terminal position (11) led to the most potent MGAT2 inhibitor among them with an IC_{50} value of 28 nm. Replacement of the cyclopentyl group with cyclopropyl or contraction of the carbon chain to ethylene linker resulted in decrease of the potency drastically (11 vs. 12 and 13).

Having obtained more potent MGAT2 inhibitors than compound **2**, selected compounds (**2**, **7**, **8**, and **11**), which have distinctive substituents such as *n*-alkyl group, phenyl group, and cycloalkyl group, were evaluated for their inhibition activity for mice MGAT2 enzyme, metabolic stability (MS) in human and mice microsomes, and solubility in Fed State Simulated Intestinal Fluid (FeSSIF),²⁰⁾ plasma exposure level in mice,

Table 1. Structure–Activity Relationships of Tetrahydroisoquinoline Derivatives with a Variety of Substituents at the *para* Position



a) Values are the means of two or more separate experiments.

Table 2. Profiles of Representative Compounds

and *in vivo* efficacy in mice oral lipid tolerance test (OLTT) (Table 2). These compounds showed relatively higher potency in mouse recombinant enzyme assay than that in human. Their plasma exposure levels at 0.5 h were measured after oral administration in mice at a dose of 30 mg/kg. The exposure levels were well correlated with their solubility in FeSSIF.

Their in vivo efficacy was tested using mice oral lipid tolerance test. Triglyceride (TG) was administrated at 0.5h after oral dosing (3, 10 or 30 mg/kg) of the compounds. Then the values of plasma levels of TG were measured at 0.5, 1, 2 and 4h post triglyceride dose and calculated its area under the curve (AUC)_{0-4h} value. Compound 2 demonstrated the significant suppression of fat absorption in mice oral lipid tolerance test at an oral dose of 100 mg/kg, while 2 did not show any effects on plasma AUC_{0-4h} levels of TG at 30 mg/kg per os (p.o.) dosing despite the highest exposure level among these compounds. This result indicated that its intrinsic potency in MGAT2 inhibitory activity was insufficient to show in vivo efficacy at less than 30 mg/kg dosing. On the other hand, other compounds exhibited significant suppression of fat absorption at an oral dose of 30 mg/kg. At lower dose of 30 mg/kg, 7 and 8 resulted in decrease of the efficacy. However, 11 demonstrated significant reduction of fat absorption even at a dose of 3 mg/kg.

Having showed a significant suppression in plasma TG levels of **11**, we studied its pharmacokinetics (PK) in mice after intravenous (i.v.) administration at a dose of 1 mg/kg and oral administration at a dose of 3 mg/kg (Table 3). The C_{max} and AUC of **11** were 127 ng/mL and 402 ng·h/mL, respectively, and the bioavailability was 21.4%. In this study, the plasma concentration of **11** after 4h of the TG dosing was 43.5 ng/mL (75 nM) and 18-fold higher than its IC₅₀ value (4 nM).

Conclusion

In summary, through an optimization of substitutions at the *para* position of the phenyl ring of compound **2**, 2-[2-(4-*tert*-butylphenyl)ethyl]-*N*-[4-(3-cyclopentylpropyl)-2fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (**11**) as an orally active MGAT2 inhibitor was identified. Compound **11** exhibited the significant reduction of TG levels in plasma even at an oral dose of 3 mg/kg in mice oral lipid tolerance test. Further *in vivo* evaluation of compound **11**, such

Compound	MGAT2 IC ₅₀ ^{<i>a</i>)} (nм) (h/m)	MS ^{b)} (%) (h/m)	Solubility	Plasma exposure at 0.5 h ^d - (ng/mL)	% Reduction of in OLTT ^{e)}				
			$(\mu g/mL)$ FeSSIF ^{c)}		1 (mg/kg)	3 (mg/kg)	10 (mg/kg)	30 (mg/kg)	100 (mg/kg)
2	1522/1170	14.6/47.8	385	135	NT ^{f)}	NT ^{f)}	NT ^{/)}	2%	57%##
7	45/20	-5.2/-3.5	207	21.5	NT ^{f)}	-10%	22%##	28%\$\$	NT ^{/)}
8	173/25	23.6/14.3	68.3	13.9	1%	5%	14%	29%*	NT ^{f)}
11	28/4	-9.6/-4.3	288	54.3	19%	25%**	35%***	38%***	41%***

a) Values are the means of two or more separate experiments. b) % metabolized after 15 min of incubation with human/mouse liver microsomes. c) Fed State Simulated Intestinal Fluid, pH 5.0. d) Orally dosed at 30 mg/kg as a 0.5% methyl cellulose (MC) solution to mice. e) Data are the mean±standard error of the mean averaged from 7–8 studies, expressed as percentage reduction of plasma AUC_{0-4h} levels of TG for 4h after an oral administration in mice fat load test (*p<0.05, **p<0.01, ***p<0.001 vs. vehicle (Dunnett's test), ##p<0.01 vs. vehicle (Studies's t-test)). f) NT: Not tested.

Table 3. PK Profiles of Compound 11 in Mice

	CL (mL/h/kg)	Vd _{ss} (mL/kg)	$T_{1/2}$ (h)	$AUC_{\text{last}} (\text{ng} \cdot \text{h/mL})$	$C_{\rm max}~({\rm ng/mL})$	$T_{\rm max}$ (h)	$AUC_{0-24\mathrm{h}}(\mathrm{ng}\cdot\mathrm{h/mL})$	BA (%)
i.v1 mg/kg	1560	6930	7.1	625	_	_	_	_
<i>p.o.</i> -3 mg/kg	—	—	—	—	127	1.67	402	21.4

as C57BL/6 mice on a high-fat diet and other pharmacological studies will be reported in due course.

Experimental

Chemistry All commercially available starting materials and reagents were used without further purification unless otherwise note. Thin layer chromatography was performed to monitor reactions using Merck silica gel 60F254 plates or Fuji Silysia chromatorex NH plates. Silica gel column chromatography was performed using Wakogel[®] C-200, or NH-silica gel Fuji Silysia chromatorex[®] DM1020, or an appropriately sized pre-packed silica cartridge on a Biotage system. ¹H-NMR spectra were recorded on a Varian Instruments INOVA-300 spectrometer at 300 MHz or a JEOL ECA-600 spectrometer at 600MHz, and are referenced to an internal standard of trimethylsilane (TMS, δ 0.00 ppm). Chemical shifts are reported in parts per million (ppm) in the indicated solvent. Multiplicity was defined as s (singlet), d (doublet), t (triplet), g (quartet), dd (double doublet), m (multiplet), brs (broad singlet), brd (broad doublet) or brt (broad triplet). Mass spectra (MS) were recorded on a Shimadzu LCMS-2010EV mass spectrometer, LCMS-iontrap-time-of-flight (LCMS-IT-TOF) mass spectrometer, a Waters Micromass Platform LC mass spectrometer, or a ThermoFisher Scientific LCQ Deca XP with an electrospray ionization (ESI) or an ESI/atmospheric pressure chemical ionization (APCI) dual source. High resolution mass spectra (HR-MS) were recorded on a Shimadzu LCMS-IT-TOF mass spectrometer with an ESI/APCI dual source. Elemental analyses were performed using a Perkin-Elmer 2400II or a Yanaco MT-6, and the results were within $\pm 0.4\%$ of the calculated values.

7-Bromo-2-(trifluoroacetyl)-1,2,3,4-tetrahydroisoquino**line-6-sulfonyl Chloride (15)** Pyridine (18.2 mL, 226 mmol) and 4-N,N-dimethylaminopyridine (184 mg, 1.51 mmol) were added to a solution of 7-bromo-1,2,3,4-tetrahydroisoquinoline (32.0g, 151 mmol) in chloroform (280 mL). After cooling to 0°C, trifluoroacetic acid anhydride (21.9mL, 158mmol) was added dropwise to the mixture. The mixture was warmed to room temperature and stirred for 20h. The mixture was concentrated under reduced pressure, and the resulting residue was diluted with ethyl acetate. One mole/liter hydrochloric acid was added to the mixture, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution and then brine, dried over anhydrous MgSO4, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 10 to 30% ethyl acetate-n-hexane to afford the intermediate (42.4 g, 91%) as a pale yellow oil.

Chlorosulfonic acid (35.6 mL, 535 mmol) was added dropwise to a solution of the above intermediate (25.4 g, 82.3 mmol) in chloroform (35 mL) at 0°C, and the mixture was stirred at room temperature for 1 h and at 60°C for 4 h. After cooling to room temperature, the reaction mixture was added dropwise to ice water, and the mixture was extracted with chloroform. The organic layer was dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. To the residue was added 18% ethyl acetate–*n*-hexane, and the resulting precipitates were collected by filtration to afford crude **15** (22.7 g, 68%) as a pale yellow powder: ¹H-NMR (300 MHz, CDCl₃) δ : 2.96–3.10 (2H, m), 3.83–4.02 (2H, m), 4.76–4.93 (2H, m), 7.62–7.70 (1H, m), 7.97–8.06 (1H, m).

N-(4-Fluorophenyl)-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (16a) Pyridine (3.09 mL, 38.4 mmol) was added to a solution of 4-fluoroaniline (3.73 g, 33.6 mmol) in chloroform (107 mL). After cooling to 0° C, 15 (13.0 g, 32.0 mmol) was added to the mixture. The mixture was warmed to room temperature and stirred for 13 h. To the mixture was added 1 mol/L hydrochloric acid, and the mixture was extracted with chloroform. The organic layer was concentrated under reduced pressure, and to the residue was added 20% ethyl acetate–*n*-hexane. The resulting precipitates were collected by filtration to afford crude product (14.5 g, 94%) as an orange powder.

To a solution of the above product (14.5 g, 30.2 mmol) in methanol (200 mL) and ethyl acetate (100 mL) was added 10% palladium activated carbon (4.36 g), and the mixture was stirred under a hydrogen atmosphere at room temperature for 15 h. Then to the mixture was added 10% palladium activated carbon (2.00 g), and the mixture was stirred under a hydrogen atmosphere at room temperature for 5 h. The mixture was filtered through a pad of Celite[®] and the filtrate was concentrated under reduced pressure. Ethanol was added to the residue, and the resulting precipitates were collected by filtration to afford the intermediate (10.6 g, 88%) as a colorless powder.

An aqueous solution (10 mL) of potassium hydroxide (2.24 g, 40.0 mmol) was added to a suspension of the above intermediate (8.05 g, 20.0 mmol) in ethanol (40 mL), and the mixture was stirred at room temperature for 15 h. The mixture was concentrated under reduced pressure, and the resulting residue was diluted with water. After cooling to 0°C, 3 mol/L hydrochloric acid was added dropwise to adjust the pH to 7 to 8. The resulting precipitates were collected by filtration to afford **16a** (6.04 g, 99%) as a colorless powder: ¹H-NMR (300 MHz, DMSO- d_6) δ : 2.65–2.72 (2H, m), 2.87–2.94 (2H, m), 3.84 (2H, s), 7.05–7.10 (4H, m), 7.16 (1H, d, *J*=8.1 Hz), 7.38–7.45 (2H, m); MS (ESI/APCI dual) *m/z*: 307 [M+H]⁺.

Compounds **16b** to **h** were prepared from the corresponding aniline in the same procedure described for **16a**.

N-[4-(2-Cyclohexylethoxy)-2-fluorophenyl]-*N*-(2,4-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (16b) Colorless amorphous (yield 68%): ¹H-NMR (600 MHz, CDCl₃) δ: 0.89–1.00 (2H, m), 1.11–1.30 (4H, m), 1.40–1.50 (1H, m), 1.54–1.77 (6H, m), 2.79–2.86 (2H, m), 3.15–3.17 (2H, m), 3.57 (3H, s), 3.75 (3H, s), 3.85–3.95 (2H, m), 4.08 (2H, s), 4.67 (2H, s), 6.25–6.29 (1H, m), 6.35–6.40 (1H, m), 6.45–6.52 (2H, m), 6.87–6.93 (1H, m), 7.07–7.12 (1H, m), 7.19–7.25 (1H, m), 7.46–7.50 (2H, m); MS (ESI): *m/z* 583 [M+H]⁺.

N-(2,4-Dimethoxybenzyl)-*N*-[2-fluoro-4-(hexyloxy)phenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (16c) Pale yellow oil (yield 81%): ¹H-NMR (300 MHz, CDCl₃) δ : 0.85–0.94 (3H, m), 1.24–1.50 (4H, m), 1.63–1.81 (4H, m), 2.82 (2H, t, *J*=5.8 Hz), 3.16 (2H, t, *J*=5.8 Hz), 3.57 (3H, s), 3.75 (3H, s), 3.86 (2H, t, *J*=6.5 Hz), 4.08 (2H, s), 4.67 (2H, s), 6.27 (1H, d, *J*=2.3 Hz), 6.37 (1H, dd, *J*=8.2, 2.3 Hz), 6.43–6.52 (2H, m), 6.84–6.94 (1H, m), 7.08–7.13 (1H, m), 7.20–7.27 (1H, m), 7.44–7.52 (2H, m); MS (ESI/APCI dual) *m/z*: 557 [M+H]⁺.

N-(2,4-Dimethoxybenzyl)-*N*-[2-fluoro-4-(3-phenylpropoxy)phenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (16d) Colorless amorphous (yield 70%): ¹H-NMR (300 MHz, CDCl₃) δ : 1.97–2.16 (2H, m), 2.71–2.86 (4H, m), 3.11–3.20 (2H,

m), 3.57 (3H, s), 3.75 (3H, s), 3.83–3.89 (2H, m), 4.08 (2H, s), 4.67 (2H, s), 6.26–6.28 (1H, m), 6.35–6.40 (1H, m), 6.43–6.51 (2H, m), 6.86–6.94 (1H, m), 7.06–7.34 (7H, m), 7.44–7.52 (2H, m); MS (ESI/APCI dual) *m/z*: 591 [M+H]⁺.

N-[4-(3-Cyclohexylpropyl)-2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (16e) Colorless powder (yield 66%): ¹H-NMR (300 MHz, DMSO- d_6) δ : 0.73–0.88 (2H, m), 1.01–1.26 (6H, m), 1.42–1.73 (7H, m), 2.42–2.51 (2H, m), 2.65–2.75 (2H, m), 2.90–3.00 (2H, m), 3.89 (2H, s), 6.86–7.02 (2H, m), 7.05–7.20 (2H, m), 7.34–7.46 (2H, m); MS (ESI/APCI dual) *m/z*: 431 [M+H]⁺.

N-[4-(3-Cyclopentylpropyl)-2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (16f) Colorless powder (yield 61%): ¹H-NMR (300 MHz, DMSO- d_6) δ : 0.93–1.09 (2H, m), 1.17–1.29 (2H, m), 1.40–1.60 (6H, m), 1.63–1.78 (3H, m), 2.40–2.53 (2H, m), 2.65–2.75 (2H, m), 2.90–2.97 (2H, m), 3.88 (2H, s), 6.87–6.99 (2H, m), 7.05–7.19 (2H, m), 7.37–7.44 (2H, m); MS (ESI/APCI dual) *m/z*: 417 [M+H]⁺.

N-[4-(3-Cyclopropylpropyl)-2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (16g) Colorless powder (yield 91%): ¹H-NMR (300 MHz, DMSO- d_6) δ : 0.07–0.01 (2H, m), 0.33–0.40 (2H, m), 0.59–0.72 (1H, m), 1.09–1.19 (2H, m), 1.54–1.66 (2H, m), 2.48–2.57 (2H, m), 2.81–2.89 (2H, m), 3.12–3.20 (2H, m), 4.10 (2H, s), 6.91–7.03 (2H, m), 7.08–7.16 (1H, m), 7.26–7.32 (1H, m), 7.46–7.52 (2H, m); MS (ESI/APCI dual) *m/z*: 389 [M+H]⁺.

N-[4-(2-Cyclopentylethyl)-2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (16h) Colorless powder (yield 94%): ¹H-NMR (300 MHz, DMSO-*d*₆) δ: 0.99–1.16 (2H, m), 1.37–1.79 (9H, m), 2.45–2.56 (2H, m), 2.80–2.89 (2H, m), 3.09–3.20 (2H, m), 4.10 (2H, s), 6.90–7.03 (2H, m), 7.06–7.16 (1H, m), 7.28 (1H, d, *J*=8.7Hz), 7.45–7.53 (2H, m); MS (ESI/ APCI dual) *m/z*: 403 [M+H]⁺.

2-[(4-tert-Butylphenyl)acetyl]-N-(4-fluorophenyl)-1,2,3,4tetrahydroisoquinoline-6-sulfonamide (17a) To a suspension of 16a (153 mg, 0.500 mmol) in chloroform (10 mL) were added (4-tert-butylphenyl)acetic acid (106 mg, 0.650 mmol), 1-hydroxybenzotriazole monohydrate (100 mg, 0.650 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (125 mg, 0.650 mmol). The reaction mixture was stirred at room temperature for 15h. Water was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 25% and then 60% ethyl acetate-n-hexane. The fractions incruding product were collected and evaporated by rotary evaporation. To the residue was added ethyl acetate-diisopropylether-nhexane, and the resulting precipitates were collected by filtration to afford 17a (142 mg, 59%) as a colorless powder: ¹H-NMR (300 MHz, DMSO- d_6) δ : 1.26 (9H, s), 2.73–2.83 (2H, m), 3.62-3.76 (4H, m), 4.62-4.78 (2H, m), 7.04-7.19 (6H, m), 7.24-7.39 (3H, m), 7.47-7.56 (2H, m), 10.20 (1H, brs); MS (ESI/APCI dual) m/z: 481 [M+H]⁺.

2-[(4-tert-Butylphenyl)acetyl]-*N*-[**4-(2-cyclohexylethoxy)**-**2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (17b)** To a suspension of **16b** (468 mg, 0.803 mmol) in *N*,*N*-dimethylformamide (10 mL) were added (4-*tert*butylphenyl)acetic acid (132 mg, 0.879 mmol), 1-hydroxybenzotriazole monohydrate (207 mg, 1.04 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (199 mg, 1.04 mmol). The reaction mixture was stirred at room temperature for 19 h. Water was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated aqueous NaHCO₃ and brine, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 30 to 50% ethyl acetate–*n*-hexane to afford the intermediate (530 mg, 80%) as a colorless powder.

To a solution of the above intermediate (500 mg, 0.661 mmol) in chloroform (1.8 mL) was added trifluoroacetic acid (1.77 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure, and ethyl acetate–*n*-hexane was added to the residue. The resulting precipitate was collected by filtration to afford **17b** (270 mg, 67%) as a colorless powder: ¹H-NMR (300 MHz, DMSO-*d*₆) δ : 0.80–1.02 (2H, m), 1.09–1.27 (2H, m), 1.26 (9H, s), 1.34–1.46 (1H, m), 1.49–1.78 (8H, m), 2.74–2.84 (2H, m), 3.64–3.81 (4H, m), 3.88–3.99 (2H, m), 4.64–4.81 (2H, m), 6.65–6.81 (2H, m), 6.97–7.08 (1H, m), 7.11–7.20 (2H, m), 7.24–7.41 (3H, m), 7.43–7.51 (2H, m), 9.77 (1H, s); MS (ESI) *m/z*: 607 [M+H]⁺.

2-[2-(4-tert-Butylphenyl)ethyl]-N-(4-fluorophenyl)-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (2) A borane-THF complex (0.9 mol/L THF solution, 0.550 mL, 0.500 mmol) was added to a solution of 17a (120 mg, 0.250 mmol) in THF (10 mL) at 0°C, and the mixture was heated at reflux temperature for 5h. After cooling to room temperature, 6 mol/L hydrochloric acid (6 mL) was added, and the mixture was stirred at reflux temperature for 3 h. The mixture was cooled to 0°C, 6mol/L aqueous sodium hydroxide was added to adjust the pH to 8 to 9, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with chloroform to 5% methanol-chloroform to afford 2 (115 mg, 99%) as a colorless amorphous. To a solution of 2 in ethyl acetate (4mL) was added 4mol/L hydrogen chloride in ethyl acetate (1 mL), and the mixture was stirred at room temperature for 15h. After the volatiles were removed by rotary evaporation, diethyl ether-ethyl acetate was added to the residue, and the resulting precipitates were collected by filtration to afford the monohydrochloride salt of 2 (115 mg, 92%) as a colorless powder: ¹H-NMR (600 MHz, DMSO- d_6) δ : 1.27 (9H, s), 3.04–3.16 (3H, m), 3.20-3.31 (2H, m), 3.34-3.48 (2H, m), 3.72-3.82 (1H, m), 4.35-4.45 (1H, m), 4.63-4.73 (1H, m), 7.06-7.15 (4H, m), 7.18–7.25 (2H, m), 7.33–7.42 (3H, m), 7.57–7.68 (2H, m), 10.35 (1H, s), 11.01 (1H, brs); MS (ESI/APCI dual) m/z: 467 $[M+H]^+$, 465 $[M-H]^-$; Anal. Calcd for $C_{27}H_{31}FN_2O_2S$ HCl: C, 64.46; H, 6.41; N, 5.57. Found: C, 64.31; H, 6.41; N, 5.49.

2-[2-(4-*tert*-Butylphenyl)ethyl]-*N*-[4-(2-cyclohexylethoxy)-2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (9) Compound 9 was prepared by the same procedure described for 2 using 17b instead of 17a.

Colorless powder (yield 45%): ¹H-NMR (600 MHz, DMSO- d_6) δ : 0.88–0.98 (2H, m), 1.08–1.34 (4H, m), 1.27 (9H, s), 1.36–1.46 (1H, m), 1.52–1.74 (7H, m), 3.04–3.17 (3H, m), 3.18–3.50 (3H, m), 3.75–3.84 (1H, m), 3.87–4.01 (2H, m), 4.38–4.49 (1H, m), 4.67–4.78 (1H, m), 6.66–6.74 (1H, m), 6.75–6.82 (1H, m), 7.02–7.09 (1H, m), 7.18–7.28 (2H, m), 7.32–7.44 (3H, m), 7.52–7.64 (2H, m), 9.90 (1H, brs),

10.79 (1H, brs); HR-MS (ESI/APCI Dual) m/z: Calcd for $C_{35}H_{45}FN_2O_3S$ [M+H]⁺; 593.3208. Found 593.3208.

2-[2-(4-*tert*-Butylphenyl)ethyl]-*N*-[2-fluoro-4-(hexyloxy)phenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (5) i) To a solution of 16c (520 mg, 0.935 mmol) in 1,2-dichloroethane (3 mL) were added a solution of 2-(4-(*tert*-butyl)phenyl)acetaldehyde (181 mg, 1.03 mmol) in 1,2-dichloroethane (2 mL) and sodium triacetoxyborohydride (297 mg, 1.40 mmol), and the mixture was stirred overnight at room temperature. Saturated aqueous NaHCO₃ was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 20 to 34% ethyl acetate–*n*-hexane to afford the intermediate (622 mg, 93%) as a pale yellow oil.

ii) To a solution of the above intermediate (593 mg, 0.827 mmol) in chloroform (5 mL) was added anisole (5 mL), the mixture was cooled to 0°C. To the mixture was added trifluoroacetic acid (1 mL), and the mixture was stirred at room temperature for 21 h. Saturated aqueous NaHCO₃ was added to the reaction mixture, and the mixture was extracted with chloroform. The organic layer was washed with brine, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified using a NH silica gel column eluted with 50 to 100% ethyl acetate–*n*-hexane to afford **5** (378 mg, 76%) as a pale yellow amorphous.

iii) To a solution of **5** in ethyl acetate (5 mL) was added 4 mol/L hydrogen chloride in ethyl acetate (1 mL), and the mixture was stirred overnight at room temperature. To the reaction mixture was added *n*-hexane, and the mixture was stirred for 1 h. The resulting precipitates were collected by filtration to afford the monohydrochloride salt of **5** (376 mg, 92%) as a colorless powder: ¹H-NMR (600 MHz, DMSO- d_6) δ : 0.80–0.92 (3H, m), 1.25–1.30 (4H, m), 1.27 (9H, s), 1.33–1.42 (2H, m), 1.62–1.70 (2H, m), 3.02–3.28 (4H, m), 3.34–3.50 (3H, m), 3.76–3.85 (1H, m), 3.91 (2H, t, *J*=6.4Hz), 4.40–4.52 (1H, m), 4.68–4.79 (1H, m) 6.67–6.72 (1H, m), 6.75–6.79 (1H, m), 7.03–7.10 (1H, m), 7.23 (2H, d, *J*=8.3Hz), 7.35–7.43 (3H, m), 7.55–7.62 (2H, m), 9.90 (1H, s), 10.46 (1H, brs); MS (ESI) *m/z*: 607 [M+H]⁺; *Anal.* Calcd for C₃₃H₄₃FN₂O₃S HCl: C, 65.71; H, 7.35; N, 4.64. Found: C, 65.53; H, 7.29; N, 4.53.

Compound 8 was prepared from the corresponding 16d in the same procedure described for 5. Compounds 10 to 13 were prepared from the corresponding 16e to h in the same procedure described for 5-(i) and -(iii).

2-[2-(4-*tert***-Butylphenyl)ethyl]**-*N*-[**2-fluoro-4-(3-phenylpropoxy)phenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (8)** Colorless powder (yield 63%): ¹H-NMR (600 MHz, DMSO- d_6) δ : 1.27 (9H, s), 1.94–2.02 (2H, m), 2.68–2.73 (2H, m), 3.02–3.24 (4H, m), 3.35–3.51 (3H, m), 3.76–3.84 (1H, m), 3.89–3.96 (2H, m), 4.41–4.52 (1H, m), 4.69–4.79 (1H, m), 6.68–6.82 (2H, m), 7.03–7.12 (1H, m), 7.15–7.31 (7H, m), 7.35–7.43 (3H, m), 7.55–7.64 (2H, m), 9.91 (1H, s), 10.28 (1H, brs); MS (ESI) *m/z*: 601 [M+H]⁺; *Anal.* Calcd for C₃₆H₄₁FN₂O₃S HCl: C, 67.85; H, 6.64; N, 4.40. Found: C, 67.83; H, 6.63; N, 4.32.

2-[2-(4-*tert*-Butylphenyl)ethyl]-*N*-[4-(3-cyclohexylpropyl)-2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (10) Colorless powder (yield 56%): ¹H-NMR (300 MHz, DMSO- d_{δ}) δ : 0.77–0.86 (2H, m), 1.05–1.23 (6H, m), 1.27 (9H, s), 1.48–1.54 (2H, m), 1.56–1.68 (5H, m), 2.44–2.53 (2H, m), 3.04–3.17 (3H, m), 3.19–3.48 (4H, m), 3.75–3.83 (1H, m), 4.40–4.48 (1H, m), 4.68–4.75 (1H, m), 6.92–6.96 (1H, m), 6.97–7.02 (1H, m), 7.10–7.14 (1H, m), 7.20–7.25 (2H, m), 7.34–7.43 (3H, m), 7.57–7.65 (2H, m), 10.10 (1H, s), 10.60 (1H, br s); MS (ESI/APCI dual) m/z: 591 [M+H]⁺, 589 [M–H]⁻; *Anal.* Calcd for C₃₆H₄₇FN₂O₂S HCI: C, 68.93; H, 7.71; N, 4.47. Found: C, 68.83; H, 7.66; N, 4.42.

2-[2-(4-*tert*-**Butylphenyl)ethyl]**-*N*-[**4-(3-cyclopentylpropyl)**-**2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfon-amide (11)** Colorless powder (yield 60%): ¹H-NMR (600 MHz, DMSO- d_6) δ : 0.94–1.06 (2H, m), 1.20–1.29 (2H, m), 1.27 (9H, s), 1.41–1.59 (6H, m), 1.66–1.76 (3H, m), 2.49–2.54 (2H, m), 3.06–3.19 (3H, m), 3.28–3.45 (4H, m), 3.73–3.81 (1H, m), 4.39–4.48 (1H, m), 4.66–4.75 (1H, m), 6.92–6.97 (1H, m), 6.98–7.03 (1H, m), 7.08–7.13 (1H, m), 7.22 (2H, d, *J*=8.3 Hz), 7.34–7.42 (3H, m), 7.57–7.65 (2H, m), 10.12 (1H, s), 11.64 (1H, br s); MS (ESI/APCI dual) *m/z*: 577 [M+H]⁺, 575 [M–H]⁻; *Anal.* Calcd for C₃₅H₄₅FN₂O₂S HCI: C, 68.55; H, 7.56; N, 4.57. Found: C, 68.46; H, 7.57; N, 4.55.

2-[2-(4-*tert*-**Butylphenyl)ethyl]**-*N*-[**4-(3-cyclopropylpropyl)**-**2-fluorophenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfon-amide (12)** Colorless powder (yield 84%): ¹H-NMR (600 MHz, DMSO- d_6) δ : -0.05-0.02 (2H, m), 0.33-0.40 (2H, m), 0.62-0.70 (1H, m), 1.11-1.18 (2H, m), 1.27 (9H, s), 1.56-1.64 (2H, m), 2.44-2.57 (2H, m), 3.02-3.49 (7H, m), 3.75-3.84 (1H, m), 4.40-4.49 (1H, m), 4.68-4.76 (1H, m), 6.92-7.03 (2H, m), 7.10-7.16 (1H, m), 7.19-7.26 (2H, m), 7.35-7.43 (3H, m), 7.56-7.66 (2H, m), 10.11 (1H, s), 10.62 (1H, brs); HR-MS (ESI/APCI dual) *m/z*: Calcd for C₃₃H₄₁FN₂O₂S [M+H]⁺; 549.2946. Found 549.2935.

2-[2-(4-*tert*-**Butylphenyl)ethyl]**-*N*-[**4-(2-cyclopentylethyl)**-**2-fluorophenyl]**-**1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (13)** Colorless powder (yield 72%): ¹H-NMR (600 MHz, DMSO- d_6) δ : 1.04–1.12 (2H, m), 1.27 (9H, s), 1.41–1.60 (6H, m), 1.63–1.74 (3H, m), 2.45–2.55 (2H, m), 3.05–3.16 (3H, m), 3.20–3.46 (4H, m), 3.74–3.83 (1H, m), 4.40–4.48 (1H, m), 4.66–4.76 (1H, m), 6.93–6.97 (1H, m), 6.98–7.03 (1H, m), 7.09–7.15 (1H, m), 7.20–7.24 (2H, m), 7.35–7.42 (3H, m), 7.57–7.64 (2H, m), 10.10 (1H, s), 10.82 (1H, brs); HR-MS (ESI/APCI dual) *m/z*: Calcd for C₃₄H₄₃FN₂O₂S [M+H]⁺; 563.3102. Found 563.3089.

4-(2-Cyclohexylethoxy)-2-fluoroaniline (19a) Potassium carbonate (4.15 g, 30.0 mmol) and (2-bromoethyl)cyclohexane (3.76 mL, 24.0 mmol) were added to a solution of 3-fluoro-4-nitrophenol (3.14 g, 20.0 mmol) in N,N-dimethylformamide (50 mL) at room temperature, and the mixture was stirred overnight. Water was added to the reaction mixture and the mixture was extracted three times with ethyl acetate. The organic layer was washed with 1 mol/L hydrochloric acid and brine, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 10 to 20% ethyl acetate–n-hexane to afford the target intermediate (2.31 g, 43%) as a pale yellow oil.

To a solution of the above intermediate (2.28g, 8.52 mmol) in ethanol (25 mL) was added 10% palladium activated carbon (2.28g) at room temperature, and the mixture was stirred overnight under a hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite[®], and the filtrate was concentrated under reduced pressure. The resulting residue

was purified using a silica gel column eluted with 10 to 20% ethyl acetate–*n*-hexane to afford **19a** (1.63 g, 81%) as a red oil: ¹H-NMR (300 MHz, CDCl₃) δ : 0.84–1.05 (2H, m), 1.09–1.34 (3H, m), 1.40–1.53 (1H, m), 1.57–1.81 (7H, m), 3.41 (2H, brs), 3.90 (2H, t, *J*=6.7Hz), 6.48–6.76 (3H, m); MS (ESI/APCI dual) *m/z*: 238 [M+H]⁺.

Compounds **19b** and **c** were prepared from the corresponding alkyl halide in the same procedure described for **19a**.

2-Fluoro-4-(hexyloxy)aniline (19b) Pale yellow oil (yield 55%): ¹H-NMR (300 MHz, CDCl₃) δ : 0.82–1.01 (3H, m), 1.20–1.52 (6H, m), 1.63–1.83 (2H, m), 3.42 (2H, br s), 3.86 (2H, t, *J*=6.6 Hz), 6.45–6.84 (3H, m); MS (ESI/APCI dual) *m/z*: 212 [M+H]⁺.

2-Fluoro-4-(3-phenylpropoxy)aniline (19c) Brown oil (yield 64%): ¹H-NMR (300MHz, CDCl₃) δ: 1.95–2.14 (2H, m), 2.74–2.83 (2H, m), 3.42 (2H, brs), 3.87 (2H, t, *J*=6.3 Hz), 6.48–6.77 (3H, m), 7.12–7.34 (5H, m); MS (ESI/APCI dual) *m/z*: 246 [M+H]⁺.

4-(2-Cyclohexylethoxy)-N-(2,4-dimethoxybenzyl)-2-fluoroaniline (20a) To a solution of 19a (1.39g, 5.84 mmol) in THF (20mL) were added acetic acid (2.01mL, 35.1mmol), 2,4-dimethoxybenzaldehyde (1.17g, 7.01 mmol) and sodium triacetoxyborohydride (3.72g, 17.6 mmol). The reaction mixture was stirred overnight at room temperature. To the reaction mixture was added 1 mol/L aqueous sodium hydroxide to adjust the pH to 8 to 9, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 10% ethyl acetate-n-hexane to afford **20a** (2.28 g, quant.) as a purple oil: ¹H-NMR (300 MHz, CDCl₃) *b*: 0.84–1.06 (2H, m), 1.07–1.34 (3H, m), 1.36–1.87 (8H, m), 3.79 (3H, s), 3.83 (3H, s), 3.88 (2H, t, J=6.7 Hz), 4.01 (1H, brs), 4.23 (2H, s), 6.34-6.76 (5H, m), 7.17 (1H, d, $J = 8.2 \, \text{Hz}$).

Compounds **20b** and **c** were prepared from the corresponding aniline in the same procedure described for **20a**.

N-(2,4-Dimethoxybenzyl)-2-fluoro-4-(hexyloxy)aniline (20b) Light brown oil (yield 34%): ¹H-NMR (300 MHz, CDCl₃) δ: 0.84–0.96 (3H, m), 1.26–1.48 (6H, m), 1.64–1.81 (2H, m), 3.80 (3H, s), 3.84 (3H, s), 3.82–3.88 (2H, m), 4.01 (1H, brs), 4.23 (2H, s), 6.32–6.78 (5H, m), 7.17 (1H, d, *J*=8.2 Hz).

N-(2,4-Dimethoxybenzyl)-2-fluoro-4-(3-phenylpropoxy)aniline (20c) Pale yellow oil (yield 90%): ¹H-NMR (300MHz, CDCl₃) δ: 1.99–2.11 (2H, m), 2.73–2.82 (2H, m), 3.79 (3H, s), 3.83 (3H, s), 3.82–3.89 (2H, m), 4.03 (1H, br s), 4.23 (2H, s), 6.38–6.73 (5H, m), 7.14–7.22 (4H, m), 7.25–7.32 (2H, m).

4-[(1*E*)-3-Cyclohexylprop-1-en-1-yl]-2-fluoroaniline (22e) Toluene (5 mL) was added to 4-bromo-2-fluoroaniline (950 mg, 5.00 mmol), allylcyclohexane (1.15 mL, 7.50 mmol), palladium acetate (112 mg, 0.50 mmol), tris(2-methylphenyl)phosphine (457 mg, 1.50 mmol) and triethylamine (2.09 mL, 15.0 mmol), and the mixture was stirred at reflux temperature for 10 h. After cooling to room temperature, the mixture was diluted with ethyl acetate and filtered through a pad of Celite[®]. The filtrate was concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 10% ethyl acetate–*n*-hexane to afford **22e** (805 mg, 69%) as a pale yellow oil: ¹H-NMR (300 MHz, CDCl₃) δ : 0.82–1.02 (2H, m), 1.03–1.44 (5H, m), 1.58–1.80 (4H, m), 2.01–2.10 (2H, m), 3.67 (2H, brs), 5.96–6.07 (1H, m), 6.16–6.24 (1H, m), 6.65–6.72 (1H, m), 6.87–6.93 (1H, m), 6.96–7.04 (1H, m); MS (ESI/APCI dual) *m/z*: 234 [M+H]⁺.

4-[(1E)-3-Cyclopentylprop-1-en-1-yl]-2-fluoroaniline (22f) 1,4-Dioxane (5 mL) and water (1.2 mL) were added to a mixture of 4-bromo-2-fluoroaniline (285 mg, 1.50 mmol), 2-[(1E)-3-cyclopentylprop-1-en-1-yl]-4,4,5,5-tetramethyl-1,3,2dioxaborolane (425 mg, 1.80 mmol), tris(dibenzylideneacetone)dipalladium (137 mg, 0.15 mmol), tri-2-furylphosphine (210 mg, 0.90 mmol) and cesium carbonate (976 mg, 3.00 mmol). The mixture was stirred at reflux temperature for 6h under an argon atmosphere. After cooling to room temperature, the mixture was diluted with ethyl acetate, washed with brine. The organic layer was dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 10% ethyl acetate-n-hexane to afford 22f (302 mg, 92%) as a pale yellow oil: ¹H-NMR (300 MHz, CDCl₂) δ : 1.10–1.24 (2H, m), 1.44-1.67 (4H, m), 1.69-1.82 (2H, m), 1.83-2.00 (1H, m), 2.12-2.22 (2H, m), 3.68 (2H, brs), 5.96-6.09 (1H, m), 6.17-6.28 (1H, m), 6.64-6.74 (1H, m), 6.86-6.94 (1H, m), 6.96–7.05 (1H, m); MS (ESI/APCI dual) m/z: 220 [M+H]⁺.

4-(2-Cyclopentylethyl)-2-fluoroaniline (22g) 9-Borabicyclo-[3,3,1]nonane (9-BBN) (THF solution, 0.5 mol/L, 33 mL, 16.5 mmol) was added dropwise to a solution of vinylcyclopentane (1.59g, 16.5 mmol) in THF (7.5 mL) at 0°C under a nitrogen atomosphere. The mixture was warmed gradually to room temperature and stirred for 15 h. To the reaction mixture were added dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) (367 mg, 0.45 mmol), 4-bromo-2-fluoroaniline (2.85g, 15.0 mmol) and 3 mol/L aqueous sodium hydroxide (15 mL, 45.0 mmol), and the mixture was heated at reflux temperature for 10h. After cooling to room temperature, the mixture was diluted with ethyl acetate and filtered through a pad of Celite[®]. The filtrate was concentrated under reduced pressure. Water was added to the residue, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 10% ethyl acetate-n-hexane to afford **22g** (3.01 g, 88%) as a pale vellow oil. To a solution of 22g (3.01g) in ethyl acetate (75 mL) was added 4 mol/L hydrogen chloride in ethyl acetate (8 mL), and the mixture was stirred at room temperature for 3h. After the volatiles were removed by rotary evaporation, diethyl ether was added to the residue, and the resulting precipitates were collected by filtration to afford the monohydrochloride salt of 22g (2.34g, 64%) as a colorless powder: ¹H-NMR (300MHz, DMSO-d₆) δ : 1.02–1.17 (2H, m), 1.40–1.79 (9H, m), 2.53–2.60 (2H, m), 6.99-7.04 (1H, m), 7.11-7.27 (2H, m); MS (ESI/APCI dual) m/z: 208 [M+H]⁺.

4-[(1*E***)-3-Cyclopropylprop-1-en-1-yl]-2-fluoro-1-nitrobenzene (24)** Iodine (3.05 g, 24.0 mmol) and imidazole (1.63 g, 24.0 mmol) were added to a solution of triphenylphosphine (6.29 g, 24.0 mmol) in chloroform (50 mL) at 0°C under a nitrogen atmosphere, and the mixture was stirred at the same temperature for 15 min. A solution of 2-cyclopropylethanol (1.72 g, 20.0 mmol) in chloroform (50 mL) was added dropwise to the reaction mixture, and the mixture was stirred at room temperature for 3 h. To the reaction mixture were added saturated aqueous sodium thiosulfate solution (60 mL) and water (60 mL), and the mixture was extracted with chloroform. The organic layer was concentrated under reduced pressure, and the resulting residue was purified using a silica gel column eluted with 100% *n*-hexane to afford the desired product (2.14 g, 55%) as a pale yellow oil.

Triphenylphosphine (2.86 g, 10.9 mmol) was added to a solution of the above product (2.14 g, 10.9 mmol) in acetonitrile (5 mL), and the mixture was heated at reflux temperature for 15 h. After cooling to room temperature, diethyl ether was added to the mixture, and the resulting precipitates were collected by filtration to afford the target intermediate (3.87 g, 77%) as a colorless powder.

To a suspension of the above intermediate (3.83 g, 8.36 mmol) in THF (60 mL) was added dropwise potassium hexamethyldisilazane (toluene solution, 0.5 mol/L) (16.7 mL, 8.36 mmol) at 0°C under a nitrogen atmosphere, and the mixture was stirred at room temperature for 1h. After cooling to 0°C, a solution of 3-fluoro-4-nitrobenzaldehyde (1.23 g, 7.27 mmol) in THF (10 mL) was added dropwise to the reaction mixture, and the mixture was stirred at room temperature for 1h. Saturated aqueous ammonium chloride solution was added to the reaction mixture, and the mixture was extracted twice with ethyl acetate. The organic layer was washed with brine, dried over anhydrous MgSO4, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 25% ethyl acetate-nhexane to afford 24 (1.42g, 88%) as a brown oil: ¹H-NMR (300 MHz, CDCl₃) δ: 0.07–0.21 (2H, m), 0.45–0.60 (2H, m), 0.74-0.92 (1H, m), 2.14-2.30 (2H, m), 5.96-6.11 (1H, m), 6.36-6.45 (1H, m), 7.12-7.31 (2H, m), 7.98-8.08 (1H, m).

4-(3-Cyclopropylpropyl)-2-fluoroaniline (25) To a solution of **24** (1.42 g, 6.42 mmol) in ethanol (40 mL) was added 10% palladium activated carbon (150 mg) at room temperature, and the mixture was stirred under a hydrogen atmosphere for 5 h. The reaction mixture was filtered through a pad of Celite[®], and the filtrate was concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 25% ethyl acetate–*n*-hexane to afford **25** (709 mg, 57%) as a brown oil: ¹H-NMR (300 MHz, CDCl₃) δ : 0.04–0.02 (2H, m), 0.36–0.43 (2H, m), 0.60–0.73 (1H, m), 1.16–1.25 (2H, m), 1.60–1.72 (2H, m), 2.48–2.55 (2H, m), 6.65–6.77 (2H, m), 6.78–6.85 (1H, m); MS (ESI) *m/z*: 194 [M+H]⁺.

4-[(2,4-Dimethoxybenzyl)amino]-3-fluorophenol (27) To a solution of 4-amino-3-fluorophenol (5.00g, 39.3 mmol) in THF (120 mL) were added acetic acid (16.2 mL, 283 mmol), 2,4-dimethoxybenzaldehyde (7.84g, 47.2 mmol) and sodium triacetoxyborohydride (25.0g, 118 mmol). The reaction mixture was stirred at room temperature for 5h. After the volatiles were removed by rotary evaporation, saturated aqueous NaHCO₃ (500 mL) was added to the residue, and the mixture was extracted three times with ethyl acetate. The organic layer was dried over anhydrous MgSO4, filtered, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 30 to 45% ethyl acetate-n-hexane to afford 27 (12.0 g, 92%) as an orange oil: ¹H-NMR (300 MHz, CDCl₃) δ: 3.79 (3H, s), 3.83 (3H, s), 4.22 (2H, s), 6.33-6.73 (5H, m), 7.16 (1H, d, J=8.1 Hz); MS (ESI/ APCI dual) m/z: 276 [M-H]⁻.

N-(2,4-Dimethoxybenzyl)-*N*-(2-fluoro-4-hydroxyphenyl)-2-(trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-6-sulfon**amide (28)** Pyridine (2.22 mL, 27.4 mmol) was added to a solution of **27** (3.80 g, 13.7 mmol) in chloroform (50 mL), and **15** (5.07 g, 12.5 mmol) was added to the mixture. The mixture was stirred at room temperature for 5 h. The mixture was concentrated under reduced pressure, and the resulting residue was purified using a silica gel column eluted with 30 to 50% ethyl acetate–n-hexane to afford the target intermediate (6.52 g, 81%) as a colorless amorphous.

To a solution of the above intermediate (6.50g, 10.0 mmol) in methanol (50 mL) and ethyl acetate (50 mL) was added triethylamine (1.68 mL, 12.1 mmol) and 10% palladium activated carbon (650 mg), and the mixture was stirred under a hydrogen atmosphere at room temperature for 4 h. The mixture was filtered through a pad of Celite[®], and the filtrate was concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 2 to 50% ethyl acetate–*n*-hexane to afford **28** (4.94 g, 87%) as a colorless amorphous: ¹H-NMR (300 MHz, DMSO-*d*₆) δ : 2.90–3.10 (2H, m), 3.54 (3H, s), 3.69 (3H, s), 3.80–3.91 (2H, m), 4.54 (2H, s), 4.83–4.93 (2H, m), 6.37–6.53 (4H, m), 6.75–6.80 (1H, m), 7.04–7.13 (1H, m), 7.43–7.63 (3H, m), 10.05 (1H, brs); MS (ESI/APCI dual) *m/z*: 591 [M+Na]⁺.

2-[2-(4-tert-Butylphenyl)ethyl]-N-(2,4-dimethoxybenzyl)-N-[2-fluoro-4-(pentyloxy)phenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (29a) To a solution of 28 (400 mg, 0.704 mmol) in N,N-dimethylformamide (1 mL) were added 1-iodopentane (119 μ L, 0.915 mmol) and potassium carbonate (146 mg, 1.06 mmol), and the mixture was stirred at room temperature overnight. To the reaction mixture was added saturated aqueous NaHCO₃, and the mixture was extracted with ethyl acetate. The organic layer was filtered through a phase separator and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 15 to 30% ethyl acetate–*n*-hexane to afford the target intermediate (307 mg, 68%) as a colorless amorphous.

An aqueous solution (0.5 mL) of potassium hydroxide (59 mg, 1.06 mmol) was added to a solution of the above intermediate (307 mg, 0.48 mmol) in ethanol (3 mL), and the mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated under reduced pressure, and the resulting residue was diluted with water. The mixture was extracted with ethyl acetate, and the organic layer was filtered through a phase separator, concentrated under reduced pressure to afford the crude product (252 mg) as a colorless amorphous.

To a solution of the above product (125 mg, 0.23 mmol) in chloroform (1mL) were added a solution of 2-(4-(tert-butyl)phenyl)acetaldehyde (50.0 mg, 0.28 mmol) in chloroform (1 mL) and sodium triacetoxyborohydride (146 mg, 0.69 mmol), and the mixture was stirred at room temperature for 19h. Saturated aqueous NaHCO₃ was added to the reaction mixture, and the mixture was extracted with chloroform. The organic layer was filtered through a phase separator and concentrated under reduced pressure to afford 29a (162 mg, quant.) as a colorless amorphous: ¹H-NMR (600 MHz, CDCl₂) δ : 0.88–0.94 (3H, m), 1.31 (9H, s), 1.32–1.42 (4H, m), 1.69–1.77 (2H, m), 2.76-2.86 (4H, m), 2.86-2.92 (2H, m), 2.92-2.97 (2H, m), 3.56 (3H, s), 3.74 (3H, s), 3.76 (2H, s), 3.84 (2H, t, J=6.6 Hz), 4.65 (2H, s), 6.24-6.27 (1H, m), 6.34-6.39 (1H, m), 6.42-6.49 (2H, m), 6.83-6.88 (1H, m), 7.09-7.14 (1H, m), 7.15-7.23 (3H, m), 7.29-7.37 (2H, m), 7.43-7.52 (2H, m); MS (ESI/APCI dual) m/z: 703 [M+H]⁺.

Compounds **29b** and **c** were prepared from **28** using 1-iodoheptane and 1-iodooctane, respectively, in the same procedure described for **29a**.

2-[2-(4-*tert*-**Butylphenyl)ethyl]**-*N*-(**2,4-**dimethoxybenzyl)-*N*-[**2-fluoro-4-(heptyloxy)phenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (29b)** Colorless amorphous (total yield 36%): ¹H-NMR (300 MHz, CDCl₃) δ : 0.83–0.95 (3H, m), 1.18–1.44 (8H, m), 1.32 (9H, s), 1.67–1.80 (2H, m), 2.76–3.02 (8H, m), 3.57 (3H, s), 3.75 (3H, s), 3.78 (2H, s), 3.81–3.89 (2H, m), 4.67 (2H, s), 6.25–6.29 (1H, m), 6.34–6.41 (1H, m), 6.43–6.53 (2H, m), 6.84–6.93 (1H, m), 7.08–7.25 (4H, m), 7.31–7.39 (2H, m), 7.44–7.55 (2H, m); MS (ESI) *m/z*: 731 [M+H]⁺.

2-[2-(4-*tert*-**Butylphenyl)ethyl]**-*N*-(**2,4-**dimethoxybenzyl)-*N*-[**2-fluoro-4-(octyloxy)phenyl]-1,2,3,4-tetrahydroisoquin-oline-6-sulfonamide (29c)** Colorless amorphous (total yield 42%): ¹H-NMR (600 MHz, CDCl₃) δ : 0.85–0.91 (3H, m), 1.23–1.35 (8H, m), 1.31 (9H, s), 1.36–1.43 (2H, m), 1.69–1.75 (2H, m), 2.76–2.99 (8H, m), 3.56 (3H, s), 3.74 (3H, s), 3.77 (2H, s), 3.82–3.86 (2H, m), 4.66 (2H, s) 6.24–6.28 (1H, m) 6.33–6.39 (1H, m), 6.43–6.51 (2H, m), 6.82–6.91 (1H, m), 7.10–7.14 (1H, m) 7.16–7.23 (3H, m), 7.31–7.36 (2H, m), 7.44–7.53 (2H, m); MS (ESI) *m/z*: 745 [M+H]⁺.

2-[2-(4-*tert***-Butylphenyl)ethyl]**-*N*-[**2-fluoro-4-(pentyloxy)phenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide (4)** To a solution of **29a** (162 mg, 0.230 mmol) in chloroform (4 mL) was added trifluoroacetic acid (880 μ L), and the mixture was stirred overnight at room temperature. Saturated aqueous NaHCO₃ was added to the reaction mixture, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, filtered through a phase separator, and concentrated under reduced pressure. The resulting residue was purified using a silica gel column eluted with 30 to 70% ethyl acetate–*n*-hexane to afford **4** (20 mg, 16%) as a colorless amorphous.

To a solution of **4** (20 mg, 0.036 mmol) in ethyl acetate (0.5 mL) was added 4 mol/L hydrogen chloride in ethyl acetate (90 μ L), and the mixture was stirred at room temperature for 5 min. After the volatiles were removed by rotary evaporation, ethyl acetate was added to the residue, and the resulting precipitates were collected by filtration to afford the monohydrochloride salt of **4** (3.0 mg, 14%) as a colorless powder: ¹H-NMR (600 MHz, DMSO-*d*₆) δ : 0.84–0.92 (3H, m), 1.27 (9H, s), 1.29–1.38 (4H, m), 1.64–1.70 (2H, m), 3.02–3.50 (7H, m), 3.76–3.84 (1H, m), 3.91 (2H, t, *J*=6.4 Hz), 4.41–4.48 (1H, m), 4.68–4.77 (1H, m), 6.67–6.73 (1H, m), 6.75–6.81 (1H, m), 7.02–7.09 (1H, m), 7.23 (2H, d, *J*=7.8 Hz), 7.35–7.44 (3H, m), 7.54–7.61 (2H, m), 9.91 (1H, s), 10.79 (1H, brs); HR-MS (ESI/APCI dual) *m/z*: Calcd for C₃₂H₄₁FN₂O₃S [M+H]⁺, 553.2895; Found 553.2883.

Compounds 6 and 7 were prepared from 29b and c, respectively, in the same procedure described for 4.

2-[2-(4-*tert*-**Butylphenyl)ethyl]**-*N*-**[2-fluoro-4-(heptyloxy)-phenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide** (6) Colorless amorphous (total yield 29%): ¹H-NMR (600 MHz, DMSO- d_6) δ : 0.83–0.89 (3H, m), 1.24–1.32 (6H, m), 1.27 (9H, s), 1.33–1.40 (2H, m), 1.63–1.70 (2H, m), 3.04–3.49 (7H, m), 3.77–3.85 (1H, m), 3.88–3.94 (2H, m), 4.40–4.51 (1H, m), 4.66–4.78 (1H, m), 6.66–6.81 (2H, m), 7.02–7.10 (1H, m), 7.19–7.27 (2H, m), 7.33–7.45 (3H, m), 7.52–7.63 (2H, m), 9.90 (1H, s), 10.67 (1H, brs); HR-MS (ESI/APCI dual) m/z: Calcd for $C_{34}H_{45}FN_2O_3S$ [M+H]⁺, 581.3208; Found 581.3204.

2-[2-(4-*tert*-**Butylphenyl)ethyl]**-*N*-[**2-fluoro-4-(octyloxy)-phenyl]-1,2,3,4-tetrahydroisoquinoline-6-sulfonamide** (7) Colorless amorphous (total yield 33%): ¹H-NMR (600MHz, DMSO- d_6) δ : 0.81–0.90 (3H, m), 1.22–1.32 (8H, m), 1.27 (9H, s), 1.34–1.39 (2H, m), 1.62–1.70 (2H, m), 3.00–3.48 (7H, m), 3.76–3.85 (1H, m), 3.88–3.93 (2H, m), 4.40–4.51 (1H, m), 4.67–4.78 (1H, m), 6.66–6.82 (2H, m), 7.01–7.09 (1H, m), 7.20–7.25 (2H, m), 7.34–7.44 (3H, m), 7.53–7.64 (2H, m), 9.90 (1H, s), 10.80 (1H, brs); HR-MS (ESI/APCI dual) *m/z*: Calcd for C₃₅H₄₇FN₂O₃S [M+H]⁺; 595.3364. Found 595.3346.

Solubility An excess amount of each compound was added to FeSSIF (Fed State Simulated Intestinal Fluid,²⁰⁾ pH 5.0) and shaken on a shaker (model SR-2s; Yamato Kagaku) at 25°C for 2 h and keep on 37°C for 22 h on a water bath (model LT-10s; Yamato Kagaku).

The suspensions were centrifuged at 3000 and 11000 rpm for 10 min, and the supernatant was diluted with 50% aqueous acetonitrile or 50% aqueous methanol solution. The concentrations were measured by HPLC. The HPLC analysis was performed using a Shimadzu HPLC system composed of a LC-20AD, SPD-20 A and SIL-20AC. The conditions for HPLC were as follows: mobile phase, 0.1% phosphoric acid aqueous solution–acetonitrile; flow rate, 0.8 mL/min; column, reversed-phase (Shimpack XR-ODS, 2.2 μ m, 2.0×75 mm; Shimadzu) at 40°C; and detection wavelength, 210 nm.

Metabolic Stability in Liver Microsomes Test substances (5 μ M) were incubated at 37°C in 1 mg/mL human or mouse microsomes supplemented with 1.5 mM glucose-6-phosphate, 0.16 mM β -nicotinamide-adenine dinucleotide phosphate, 0.18 units/mL glucose-6-phosphate dehydrogenase, 2.4 mM magnesium chloride and 69 mM potassium chloride in 250 mM phosphate buffer (pH 7.4). Concentrations of the test compounds were determined by LC-MS/MS. The metabolized % was determined by comparing a peak area of the test substance at 15 min incubation with the peak area at 0 min.

Biology

MGAT2 Assay

This assay detects CoA, a product of the MGAT2-catalyzed deacylation of oleoyl-CoA. The free thiol of CoA can react with 7-diethylamino-3-(4'-maleimidylphenyl)-4methylcoumarin (CPM), a pro-fluorescent coumarin maleimide derivative that becomes fluorescent upon reaction with thiols.

In this assay, recombinant human and mouse MGAT2 were produced in the baculovirus expression system. The MGAT2 activity was determined as follows: Assay buffer [final concentration: 100 mM Tris-HCl (pH 7.5), 100 mM sucrose, 5 mM MgCl₂] and recombinant MGAT2 and test compound were added to each well of a 96-well black plate (Corning). Substrate solution [final concentration: $5.3 \,\mu\text{M}$ oleoyl-CoA, $0.78 \,\mu\text{M}$ 2-oleoylglycerol, $7.5 \,\mu\text{M}$ phosphatidylcholine] were added to start the reaction, which was allowed to proceed for 20 min. The reaction was terminated upon the addition of CPM with final concentration $5\,\mu$ M. The plates were sealed, incubated for 20 min. Fluorescence that emits at 460 nm when excited at 380nm was counted on a SpectraMax Plate Reader (Molecular Devices, LLC, Japan). Oleoyl-CoA, 2-oleoylglycerol and phosphatidylcholine were obtained from Sigma-Aldrich and CPM from Life Technologies.

Oral Lipid Tolerance Test in Mice

C57BL/6J male mice (10 weeks old, n=7 to 8) were used. To inhibit the clearance of plasma triacylglycerol, we administered $100 \,\mu\text{L}$ of the surfactant tyloxapol (10% in phosphate buffered saline (PBS)) through a tail vein. Immediately, we administered orally 4mL/kg of test compound or 0.5% methvlcellulose solution as vehicle. After 30 min, we had challenged orally with 4.4 mL/kg of triolein containing 0.1 mCi/ mL 3H triolein. We collected blood from tail vein at 2, 3 and 4h after lipid challenge and counted scintillation. Efficacy was calculated as the percentage reduction in area under the curve of plasma scintillation for 4h (AUC_{0-4h}) compared to vehicletreated control animals. Bartlett's test was applied to evaluate the equality of variance for AUC_{0-4h} between vehicle and treated mice. If the equality of variance isn't statistically significant in Bartlett's test, Dunnett's test is applied to establish statistical significance of AUC_{0-4h} between vehicle and treated mice. If the equality of variance is statistically significant in Bartlett's test, Steel's test is applied to establish statistical significance of AUC_{0-4h} between vehicle and treated mice. Student's t-test was used to establish statistical significance in the comparison between 2 groups.

Acknowledgments We are grateful to Dr. N. Ohtake and Dr. T. Yoshizumi for their helpful and critical reading of the manuscript. We acknowledge the analytical support (the high-resolution mass spectra) by Mr. A. Higuchi.

Conflict of Interest The authors declare no conflict of interest.

References and Notes

- Yen C.-L. E., Farese R. V. Jr., J. Biol. Chem., 278, 18532–18537 (2003).
- Bell R. M., Coleman R. A., Annu. Rev. Biochem., 49, 459–487 (1980).
- Yen C.-L. E., Stone S. J., Cases S., Zhou P., Farese R. V. Jr., Proc. Natl. Acad. Sci. U.S.A., 99, 8512–8517 (2002).
- Cao J., Lockwood J., Burn P., Shi Y., J. Biol. Chem., 278, 13860– 13866 (2003).
- Cheng D., Nelson T. C., Chen J., Walker S. G., Wardwell-Swanson J., Meegalla R., Taub R., Billheimer J. T., Ramaker M., Feder J. N.,

J. Biol. Chem., 278, 13611-13614 (2003).

- Yen C.-L. E., Cheong M.-L., Grueter C., Zhou P., Moriwaki J., Wong J. S., Hubbard B., Marmor S., Farese R. V. Jr., *Nat. Med.*, 15, 442–446 (2009).
- Nelson D. W., Gao Y., Spencer N. M., Banh T., Yen C.-L. E., J. Lipid Res., 52, 1723–1732 (2011).
- Yen C.-L. E., Nelson D. W., Yen M.-I., J. Lipid Res., 56, 489–501 (2015).
- Nakamura K., Nakagawa H., Kan Y., PCT Int. Appl., WO 2008038768 (2008) [Chem. Abstr., 148, 426906 (2008)].
- Suwa A., Takada T., Nishida T., PCT Int. Appl., WO 2012091010 (2012) [*Chem. Abstr.*, **157**, 165601 (2012)].
- Arakawa K., Kawamoto H., Sakaki J., Shimamura T., PCT Int. Appl., WO 2010095767 (2010) [*Chem. Abstr.*, **153**, 359049 (2010)].
- Turdi H., Hangeland J. J., Lawrence R. M., Cheng D., Ahmad S., Meng W., Brigance R. P., Devasthale P., PCT Int. Appl., WO 2013082345 (2013) [*Chem. Abstr.*, **159**, 75803 (2013)].
- Ahmad S., Megash L. A., PCT Int. Appl., WO 2014193884 (2014) [Chem. Abstr., 162, 37205 (2014)].
- 14) Fernandez M. C., Gonzalez-garacia M. R., Liu B., Pfeifer L. A., PCT Int. Appl., WO 2013112323 (2013) [*Chem. Abstr.*, **159**, 305466 (2013)].
- 15) Berger R., Blizzard T. A., Campbell B. T., Chen H. Y., Debenham J. S., Dewnani S. V., Dubois B., Gude C., Guo Z., Harper B., Hu Z., Lin S., Liu P., Wang M., Ujjainwalla F., Xu J., Xu L., Zhang R., PCT Int. Appl., WO 2015112465 (2015) [*Chem. Abstr.*, **163**, 271104 (2015)].
- 16) Barlind J. G., Buckett L. K., Crosby S. G., Davidsson Ö., Emtenäs H., Ertan A., Jurva U., Lemurell M., Gutierrez P. M., Nilsson K., O'Mahony G., Petersson A. U., Redzic A., Wågberg F., Yuan Z.-Q., *Bioorg. Med. Chem. Lett.*, 23, 2721–2726 (2013).
- 17) Recently *N*-phenylindoline-5-sufonamide derivatives as MGAT2 inhibitor has been reported. Sato K., Takahagi H., Yoshikawa T., Morimoto S., Takai T., Hidaka K., Kamaura M., Kubo O., Adachi R., Ishii T., Maki T., Mochida T., Takekawa S., Nakakariya M., Amano N., Kitazaki T., *J. Med. Chem.*, **58**, 3892–3909 (2015).
- Sato K., Takahagi H., Kubo O., Hidaka K., Yoshikawa T., Kamaura M., Nakakariya M., Amano N., Adachi R., Maki T., Take K., Takekawa S., Kitazaki T., Maekawa T., *Bioorg. Med. Chem.*, 23, 4544–4560 (2015).
- Busujima T., Tanaka H., Shirasaki Y., Munetomo E., Saito M., Kitano K., Minagawa T., Yoshida K., Osaki N., Sato N., *Bioorg. Med. Chem.*, 23, 5922–5931 (2015).
- 20) Galia E., Nicolaides E., Horter D., Lobenberg R., Reppas C., Dressman J. B., *Pharm. Res.*, **15**, 698–705 (1998).