SHORT COMMUNICATION

Synthesis and biological evaluation of novel thienopyrimidine derivatives as diacylglycerol acyltransferase 1 (DGAT-1) inhibitors

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ABSTRACT
A novel series of thieno[3,2-d]pyrimidine derivatives were synthesised and their inhibitory effects against diacylglycerol acyltransferase 1 (DGAT-1) were assessed. cis-Isomer 17a showed potent and selective inhibitory activity against DGAT-1 in SF9 cells. In addition, 17a had an acceptable pharmacokinetic profile and accumulated mainly in the small intestine and liver. Oral administration of 17a led to a significant reduction in plasma triacylglycerol level during an oral lipid tolerance test (OLTT) in murine and canine models. Taken together, 17a is a high-quality candidate that deserves further investigation.

Introduction
Obesity is characterised by abnormal fat accumulation due to a systemic dysfunction of energy homeostasis. It is a significant risk factor for diabetes, hypertension, cardiovascular disease, and non-alcoholic fatty liver disease. Excessive accumulation of triglycerides (or triacylglycerol, TAG) in adipocytes and non-adipocytes, including in the liver, skeletal muscle, myocardium, and pancreas, is a crucial feature of obesity. In particular, abnormal TAG levels increase the risk of metabolic syndrome, characterised by insulin resistance, dyslipidaemia and cardiomyopathy.

Therefore, preventing excessive accumulation of TAG could be beneficial in the treatment of metabolic diseases such as obesity and type 2 diabetes. Two routes of TAG biosynthesis have been identified: the glycerol phosphate pathway, and the monoacylglycerol pathway. Both routes have a common intermediate, diacylglycerol (DAG), which is then converted to TAG by acyl CoA: diacylglycerol acyltransferase (DGAT). DGAT exists as two isoforms, DGAT-1 and DGAT-2, which share minimal sequence homology. These enzymes catalyse the final dedicated step in TAG synthesis: the esterification of a fatty acid moiety to DAG to generate TAG. DGAT-1 is highly expressed in the small intestine, adipose tissue, and liver. DGAT-1 deficient mice showed a significant reduction in the postprandial increase of plasma TAG and were resistant to diet-induced obesity due to increased energy expenditure. Moreover, the DGAT1 knockout mice had enhanced sensitivity to insulin and leptin. Indeed, DGAT-1 inhibitors had significant pharmacologic effects, including decreased TAG, which were consistent with the DGAT1 knockout mouse phenotypes.

However, DGAT2 knockout mice had extremely low levels of TAG, and their skin could not protect against moisture, so these mice died after birth. Therefore, selective DGAT-1 inhibitors have been developed to manage metabolic diseases such as obesity and type 2 diabetes. Pharmaceutical companies, including AstraZeneca, Novartis, Pfizer, and Abbott, are developing selective DGAT-1 inhibitors, whose structures are described in Figure 1. Other novel DGAT1 inhibitors have also been reported.

Herein, we described the discovery of novel thieno[3,2-d]pyrimidine derivatives as DGAT-1 inhibitors. Target molecules were designed based on a bioisosteric replacement strategy (Figure 2). Introduction of a heterobicycle instead of a phenyl linker was attempted. Thienopyrimidines showed various pharmacologic activities as a privileged scaffold, and the introduction of thieno[3,2-d]pyrimidine was the first approach to designing novel inhibitors based on the structural features of reported DGAT-1 inhibitors.

Materials and methods

Chemistry
All commercial chemicals and solvents were reagent grade and were used without further purification. Completion of the reactions was monitored by analytical thin layer chromatography (TLC) using precoated glass-backed plates (E-Merck, silica gel 60 F 254, 0.25 mm). For normal pressure and flash column chromatography purifications, Merck silica gel 60 (size 70–230 and 230–400 mesh, respectively) was used. 1H and 13C NMR spectra were recorded with an Avance DPX-300 NMR spectrometer (Bruker, Germany) and Jeol 600 MHz spectrometer (Jeol Resonance ECZ 600R, USA). All the 1H and 13C NMR spectra were recorded in deuterated chloroform (CDCl3) with tetramethylsilane (TMS) as an internal standard or deuterated dimethyl sulfoxide (DMSO)-d6 as solvents; chemical shifts are reported in δ values (ppm) relative to the residual solvent peak. Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); m (multiplet); dd (doublet of doublet);
brs (broad singlet). Mass spectra were obtained on Waters Aquity UPLC/QTOF (Waters Corporation, USA). HPLC was used Agilent, 1200 series using capcellpak MGII (4.6 × 150 mm, 5 μm) eluted with a 30 min gradient from 20–70% acetonitrile in water.

**Synthesis of 17a and 17b**

**Tert-butyl (4–(7-bromo-thieno[3,2-d]pyrimidin-4-yl)phenyl)carbamate (8)**

A mixture of 7-bromo-4-chlorothieno[3,2-d]pyrimidine 6 (13 g, 52.10 mmol, CY C37043, KindChem Corporation, China), tert-butyl 4–(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) phenylcarbamate 7 (20 g, 62.70 mmol), tetrakis(triphenylphosphine) palladium (3.16 g, 2.73 mmol) and sodium carbonate (8.28 g, 78.12 mmol) were added to 100 mL of 1,4-dioxane/water (4:1), and then stirred at 80 °C for 18 h under argon atmosphere. The organic layer was extracted with 300 mL of ethyl acetate and 300 mL of water, dried over anhydrous magnesium sulphate, and then concentrated in vacuo. Subsequently, 30 mL of methanol was added, and the resulting mixture was stirred to precipitate out solids, and then filtered to obtain 12.5 g of the yellow title compound.

**Methyl 2–(4–(4–(((tert-butylcarbonyl)amino)phenyl)thieno[3,2-d]pyrimidin-7-yl)cyclohex-3-en-1-yl)acetate (10)**

A mixture of 8 (0.56 g, 1.38 mmol), 2–(4–(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)cyclohex-3-en-1-yl)acetate 9 (0.5 g, 1.78 mmol), which was prepared according to the reported method, and tetrakis(triphenylphosphine) palladium (94.9 mg, 0.08 mmol) was added to a solution of 2.06 mL of 2 N aqueous sodium carbonate and 8.2 mL of 1,4-dioxane, and then stirred at 100 °C for 12 h under argon. The reaction mixture was extracted with 300 mL of ethyl acetate and 300 mL of water. The organic layer was dried over anhydrous magnesium sulphate, and then concentrated in vacuo.
with anhydrous magnesium sulphate, filtered and then concentrated. Subsequently, methanol was added to the resulting solution, stirred to precipitate out solids, and then filtered to obtain 315 mg of the yellow title compound.

$^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 9.30(s, 1H), 8.17(d, $J = 8.7$ Hz, 2H), 7.43(s, 1H), 7.59(d, $J = 8.7$ Hz, 2H), 7.16(bs, 1H), 6.69(s, 1H), 3.71(s, 3H), 2.64 ~ 2.38(m, 3H), 2.26 ~ 2.02(m, 6H), 1.56(s, 9H). LCMS (ESI) m/z 480.2 [M + H]$^+$; HRMS calc'd for C$_{28}$H$_{26}$N$_{2}$O$_{5}$S [M + H]$^+$ 480.1957, found 480.1986.

Methyl 2-[(4-[(t-butoxycarbonyl)amino]phenyl)thieno[3,2-d]pyrimidin-7-yl]cyclohexyl acetate (11)

To a solution of 10 (315 mg, 0.66 mmol) in ethanol (50 mL) and 1,4-dioxane (20 mL) was added 20% charcoal-shaped palladium hydroxide (158 mg, 50%w/w). The mixture was stirred at room temperature under H$_2$ overnight. The reaction mixture was filtered through Celite, and then the filtrate was concentrated to obtain the title compound (11) (190 mg), which was used in the following step without further purification.

$^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 9.26(s, 2H), 8.17(d, $J = 8.5$ Hz, 4H), 7.69(s, 1H), 7.63(s, 1H), 7.62(d, $J = 8.2$ Hz, 4H), 6.71(s, 2H), 3.69(s, 6H), 3.32 ~ 3.17(m, 2H), 2.50(d, $J = 7.5$ Hz, 2H), 2.4 ~ 2.35(m, 1H), 2.33(d, $J = 6.6$ Hz, 2H), 2.28 ~ 2.18(m, 2H), 2.07 ~ 1.19(m, 9H), 1.73 ~ 1.60(m, 4H), 1.57(s, 18H), 1.40 ~ 1.25(m, 2H). LCMS (ESI) m/z 482.2 [M + H]$^+$; HRMS calc'd for C$_{27}$H$_{25}$N$_{3}$O$_{5}$S [M + H]$^+$ 482.2114, found 482.2258.

Methyl 2-[(4-[(4-aminophenyl)ureido]phenyl)thieno[3,2-d]pyrimidin-7-yl]cyclohexylacetate (12)

To a solution of 11 (280 mg, 0.58 mmol) in dichloromethane (5 mL) was added 0.6 mL of trifluoroacetic acid. The mixture was stirred at room temperature for 12 h. Then the reaction mixture was concentrated to a residue that was partitioned between dichloromethane and saturated aqueous sodium bicarbonate. The organic layer was separated from the aqueous layer, washed with brine, dried over anhydrous magnesium sulphate, and then concentrated to afford 190 mg of the title compound.

$^1$H-NMR (300 MHz, DMSO-d$_6$): $\delta$ 9.08(s, 2H), 8.13(s, 1H), 8.04(s, 1H), 8.01(d, $J = 8.6$ Hz, 4H), 6.76(d, $J = 8.6$ Hz, 4H), 5.90(s, 4H), 3.60(s, 6H), 3.30 ~ 3.10(m, 2H), 2.48(d, 2H), 2.28(d, $J = 6.5$ Hz, 2H), 2.25 ~ 2.13(m, 1H), 2.28 ~ 2.16(m, 3H), 2.10 ~ 1.98(m, 2H), 1.90 ~ 1.46(m, 14H), 1.30 ~ 1.10(m, 2H).

Methyl cis-2-[(4-[(4-[(3-chlorophenyl)ureido]phenyl)thieno[3,2-d]pyrimidin-7-yl]cyclohexyl]acetate (13)

To a solution of 12 (3.1 g, 8.13 mmol) in anhydrous tetrahydrofuran (60 mL) was added 3-chlorophenyl isocyanate (1.37 g, 8.92 mmol). The mixture was stirred at room temperature for 18 h. The mixture was concentrated in part and diluted with diethyl ether to give a precipitate, which was filtered to afford 4.06 g of a light-yellow title compound.

$^1$H-NMR (300 MHz, DMSO-d$_6$): $\delta$ 9.21(s, 2H), 9.16(s, 2H), 9.03(s, 2H), 8.24(s, 1H), 8.19(d, $J = 8.8$ Hz, 2H), 8.15(s, 1H), 7.75 ~ 7.72(m, 3H), 7.30 ~ 7.31(m, 2H), 7.0 ~ 7.00(m, 1H), 3.32 ~ 3.20(m, 1H), 2.39(d, $J = 7.5$ Hz, 2H), 2.28 ~ 2.14(m, 1H), 1.90 ~ 1.50(m, 8H). $^{13}$C-NMR (150 MHz, DMSO-d$_6$): $\delta$ 174.08, 160.01, 158.32, 154.04, 152.20, 142.37, 142.10, 141.00, 133.20, 131.36, 130.43, 129.97, 129.20, 127.20, 121.70, 118.21, 117.66, 117.75, 103.37, 35.51, 30.03, 29.31, 27.18. LCMS (ESI) m/z 521.1 [M + H]$^+$; HRMS calc'd for C$_{28}$H$_{27}$ClN$_{3}$O$_{5}$S [M + H]$^+$ 521.1414, found 521.1441.
**Sodium cis-2-(4-(4-(3-(3-chlorophenyl)ureido)phenyl)thieno[3,2-d]pyrimidin-7-yl)cyclohexyl)acetate (17a)**

To a solution of 16a (7.6 g, 14.59 mmol) in 100 mL of methanol was added 14 mL of a 1 N sodium hydroxide. The resulting mixture was stirred at room temperature for 2 h. The solvent was removed to give 8.3 g of the yellow title compound 17a.

1H-NMR (300 MHz, DMSO-d6): δ 12.50(s, 1H), 12.37(s, 1H), 9.21(s, 1H), 8.23(s, 1H), 8.17(d, J = 8.9 Hz, 2H), 7.7 5 ~ 7.72(m, 3H), 7.56(d, J = 9.0 Hz, 1H), 7.27(t, J = 8.1 Hz, 1H), 6.96(d, J = 7.9 Hz, 1H), 3.3 2 ~ 3.18(m, 1H), 2.4 0 ~ 2.20(m, 3H), 2.0 0 ~ 1.6(m, 8H).

**DGAT-1 inhibition assay (IC50)**

The activity of DGAT-1 inhibitors in vitro was evaluated by using a human recombinant DGAT1 enzyme expressed in insect cells (SF9 cells). SF9 cells were homogenised by washing them with DPBS (Dulbecco’s phosphate-buffered saline) and then suspending cell pellets with a tris buffer (250 mM sucrose; 10 mM Tris-HCl [pH 7.4]; protease inhibitor). The resulting mixture was centrifugally separated at 10,000 x g for 30 min to remove the cell debris remaining in the lower layer thereof, and was centrifugally separated at 100,000 x g for 60 min to obtain a microsomal membrane. Further, membrane fractions were resuspended by the tris buffer, and then stored at -80°C.

The activity of DGAT1 was measured according to the reported method59. Specifically, 0.0001 – 10 μM (final concentration, FC) of the test compounds were cultured at room temperature (25°C) for 15 min with a 10 μM of SF9 microsomal protein solution and 100 mM of MgCl2 solution, and were then further cultured at room temperature (25°C) for 30 min after the addition of 100 μM (FC in 12.5% EtOH) of 1,2-dioleyl-sn-glycerol and 30 μM (final conc.) of 14C-oleyl coenzyme A. The reaction was completed by the addition of 300 μL of a mixed solution of 2-propanol/heptane (7:1), and radioactive triglyceride was separated from an organic solvent layer by using 200 μL of heptane and 200 μL of a 0.1 M carbonate buffer (pH 9.5). The amount of triglyceride was measured by liquid scintillography (Perkin Elmer, USA) after mixing with an organic solvent and an equivalent amount of scintillation solvent (MicroScint-O, Perkin Elmer, USA). The effects of inhibiting DGAT1 were calculated as percent in comparison with a control material.

**Oral lipoid tolerance test (OLTT)**

*Mouse*

Male, Institute of Cancer Research (ICR) mice were obtained from OrientBio Inc. (Republic of Korea) (n = 5/group) fasted 16 h, received 5 mL/kg corn oil via oral administration (p.o.) 30 min after compound administration. Plasma was collected at different time points (0, 1, 2, and 4 h).

*Dog*

Beagle dogs (9 months old, n = 2/group) were obtained from Wo jung Bio Inc. (Republic of Korea) were fasted for 16 h and received heavy cream (2.7 mL/kg), glucose (1 g/kg), water (5.3 mL/kg), and acetaminophen (20 mg/kg p.o.) 1 h after compound administration. Blood samples were collected at −0.5, 0, 1, 2, and 4 h.

**Statistical analysis**

Statistical analysis was performed using one-way ANOVA, followed by Dunnett’s multiple comparison test using Prism 6.0 (GraphPad, USA). The Kruskal-Wallis test was applied when data did not pass the normality test.

**Results and discussion**

We found that thieno[3,2-d]pyrimidine could be a core scaffold connecting an aryl moiety at one end to a cyclo-aliphatic carboxylic acid group at the other. Synthetic schemes for a series of thieno[3,2-d]pyrimidine derivatives are outlined in Schemes 1 and 2.

Desired compounds 14a–14i were synthesised from commercially available 7-bromo-4-chlorothieno[3,2-d]pyrimidine (6), as shown in Scheme 1. Consecutive Suzuki coupling reactions gave 8 and 10. Compound 9 was prepared using the reported method19. Hydrogenation of 10, followed by de-protection of the Boc group with trifluoroacetic acid afforded the key intermediate 12. The aniline 12 was converted to amides 14a–14h or ureas 14i–14l by coupling with acid chlorides or aryl isocyanates, respectively, followed by hydrolysis with sodium hydroxide or lithium hydroxide.

The activities of synthesised compounds were evaluated using SF9 insect cells expressing human DGAT-122. The IC50 values are summarised in Table 1. A potent DGAT-1 inhibitor, LQC-908 (2 or pradigastat) was used as a positive control. To optimise the alkylic chain length of the acid moiety, compound 14b (n = 1) was more potent than short chain 14a (n = 0) and long chain 14c (n = 2). The calculated lowest energy conformation model of 14c provided that the distance between the terminal carboxylic acid and NH of urea was over 16 Å, which may result in reduced activity. 14a exhibited a loss of activity, which suggested that the terminal carboxylic acid should be placed one-carbon away from cyclohexane. Next, structure-activity relationships for the aryl moiety on the left side were investigated, with derivatives bearing chain lengths the same as that of 14b, 5-chloro-2-nitro phenyl (14d), 2-trifluomethylpyridinyl (14f), 4-chloropyridinyl (14k), and 5-bromopyridinyl (14l) derivatives had no activity (IC50 > 1 μM), while 14b, 14c, 14e, 14h, and 14j had good activity (IC50 values of 0.3 μM–0.4 μM). Compounds 14g and 14i were equipotent to the known compound 2. The most potent compound, 14i, was subjected to further investigation. cis and trans isomers of 14i were separated to determine the activity and properties of each isomer. As shown in Scheme 2, the racemic mixture 13 was conveniently separated.
into cis-isomer 15a and trans-isomers 15b by the treatment with ethyl acetate. 15a and 15b exhibited greatly different solubility for ethyl acetate, and were soluble and insoluble for ethyl acetate, respectively. Hydrolysis of 15a and 15b afforded cis isomer 17a and trans isomer 17b, respectively.

Interestingly, the isomers 17a and 17b were more potent than mixture 14i, and even compound 2, in SF9 cells (Table 2). However, pharmacokinetic studies demonstrated that cis-isomer 17a had a much better profile than trans-isomer 17b (Table 3). 17a had a shorter half-life (1.2 h), but had much better bioavailability, compared to 17b. In enzymatic assays, 17a had an IC50 of 61 nM for DGAT-1 and displayed high off-target selectivity against DGAT-2, acyl-coenzyme A (CoA):cholesterol acyltransferases (ACAT1 and ACAT2) (Table 2). ACATs have sequence homology to...
### Table 1. DGAT-1 inhibitors and their in vitro data.

| Compound | n | R₁ | DGAT-1 IC₅₀ (µM)ᵃ |
|----------|---|----|------------------|
| 2        | 0 |    | 0.157            |
| 14a      | 1 |    | >1               |
| 14b      | 1 |    | 0.276            |
| 14c      | 2 |    | 0.409            |
| 14d      | 1 |    | >1               |
| 14e      | 1 |    | 0.326            |
| 14f      | 1 |    | >1,000           |
| 14g      | 1 |    | 0.152            |
| 14h      | 1 |    | 0.398            |
| 14i      | 1 |    | 0.121            |
| 14j      | 1 |    | 0.351            |
| 14k      | 1 |    | >1,000           |
| 14l      | 1 |    | >1,000           |

ᵃData present mean values.

### Table 2. Enzyme and cellular inhibitory potencies for selected compounds.

| Compound | DGAT(SF9) IC₅₀ (nM) | hDGAT1 IC₅₀ (nM) | hDGAT2 IC₅₀ (nM) | hACAT1 IC₅₀ (nM) | hACAT2 IC₅₀ (nM) |
|----------|---------------------|------------------|------------------|------------------|------------------|
| 2        | 157                 | 57               | >10,000          | >10,000          | >10,000          |
| 17a      | 74                  | 61               | >10,000          | >10,000          | >10,000          |
| 17b      | 89                  | –                | –                | –                | –                |

### Table 3. Pharmacokinetic profiles of compounds 17a, 17b in ICR mice.ᵃ

| Compounds | AUC ₀⁻₂₄ (ng h/mL) | Cₘₐₓ (ng/mL) | T₁/₂ (h) | Bioavailability (%) |
|-----------|------------------|--------------|----------|---------------------|
| 17a       | 861.2            | 479.3        | 1.2      | 19                  |
| 17b       | 233.7            | 74.8         | 5.2      | 0.7                 |

ᵃAll parameters were determined after oral administration (10 mg/kg, n = 3) and intravenous (iv) injection (3 mg/kg, n = 2) in ICR mice; both compounds were dissolved in a vehicle solution of DMSO/Tween80/saline (1:6:23) for iv., and 0.5% methylcellulose/0.5% Tween80 in distilled water for oral administration. Data represent mean values.

### Table 4. Oral pharmacokinetic profile of 17a in rat and dog.

| Parameters | AUC ₀⁻₂₄ (ng h/mL) | T₁/₂ (h) | Cl (L/hr/kg) | Bioavailability (%) |
|------------|--------------------|----------|--------------|---------------------|
| Ratᵃ       | 8,594.5 ± 6764.2   | 3.0 ± 0.4| 0.5 ± 0.2    | 30                  |
| Dogᵇ       | 8,243.0 ± 2296.3   | 3.6 ± 0.7| 0.4 ± 0.1    | 53                  |

ᵃDose; 3 mg/kg for iv, 10 mg/kg for po.; Vehicle: DMSO/Tween80/saline (1:6:23) for iv.; 0.5% methylcellulose/0.5% Tween80 in distilled water for po.

ᵇDose: 1 mg/kg for iv, 3 mg/kg for po.; Vehicle: DMSO/Tween80/saline (1:3:26) for iv; 0.5% methylcellulose/0.5% Tween80 in distilled water for po.

### Table 5. In vitro microsomal stability and cytochrome P450 (CYP) inhibition assay of compound 17a.

| Microsomal stability, (%)ᵃ | CYPs enzyme inhibition, IC₅₀ (µM)ᵇ |
|----------------------------|-----------------------------------|
| No Human Dog Mouse CYP3A4 CYP1A2 CYP2C9 CYP2C19 CYP2D6 |                       |
| 17a | 94 | 82 | 82 | 16.2 | 10.6 | 2.9 | >20 | 8.7 |

ᵃRemaining percent of metabolism by incubation of the parent molecule (5 µM) with liver microsomes of mouse, rat, dog, and human for 60 min (duplicate).

ᵇData were analysed by a fluorescence detection method.
Figure 3. Effect of 17a in the oral lipid tolerance test (n = 5, ***p < .001). Serum TG (or TAG) concentration was measured before and 1, 2, and 4h after oral administration of corn oil in ICR mice treated with 17a (3 mg/kg) or 2 (1 mg/kg). Vehicle for 2: 0.5% methylcellulose/0.5% Tween80 in distilled water, vehicle for 17a: 0.5% Tween80 in distilled water.

Figure 4. Effect of 17a in a canine oral lipid tolerance test (n = 2, *p < .05; ***p < .001). The dog was treated with 17a (po. 1 mg/kg) or 2 (po. 1 mg/kg) before oral administration of corn oil. Vehicle for 2: 0.5% methylcellulose/0.5% Tween80 in distilled water, vehicle for 17a: 0.5% Tween80 in distilled water.

Figure 5. Tissue distribution of 17a in mice. 17a and 2 were orally administered to ICR mice at 30 mg/kg (n = 3). (a) AUC values of 17a and 2 in indicated tissues. Each bar shows AUC obtained from concentrations measured at 0.5h, 1h, 2h, 4h, and 24h after dosing. Data represent mean value. (b) Tissue-to-plasma (T/P) ratio of 17a and 2. S.I.: small intestine, L.I.: large intestine.

Conclusion

We found that a novel series of thieno[3,2-d]pyrimidine derivatives had potent DGAT-1 inhibitory activity. A representative compound, cis-isomer 17a showed potent DGAT-1 inhibitory activity, metabolic stability in four species, an acceptable pharmacokinetic profile in rodents and dogs, and a significant reduction of TAG in the OLTT in mice and dogs. In a tissue-distribution study, 17a was mainly distributed to the liver and small intestine. Therefore, 17a was investigated for further pharmacokinetic properties in four species, and results are summarised in Tables 4 and 5. 17a exhibited low to moderate clearance and acceptable oral bioavailability in rat and dog.

17a exhibited durable metabolic stabilities, as shown in Table 5. In particular, 17a was metabolically stable in four species: human, dog, rat, and mouse. Further evaluation of 17a was carried out for inhibition of clinically relevant cytochrome 450 (CYP) isoforms (3A4, 1A2, 2C9, 2C19, 2D6). No significant inhibition by 17a was observed (Table 5). Thus, 17a would be very unlikely to affect the pharmacokinetics of other drugs.

To determine in vivo efficacy, we evaluated the activity of 17a against serum TAG level induced by the oral administration of corn oil (oral lipid tolerance test, OLTT; Figures 3 and 4), since intestinal targeted DGAT-1 inhibition results in a reduced serum TAG level. 17a was orally administered to ICR mice at 3 mg/kg or to dogs at 1 mg/kg before administration of corn oil. As shown in Figure 3, 17a showed significant activity, as potent as compound 2, in reducing plasma TAG level.

In addition, tissue distribution of 17a was investigated. 17a was orally administered to mice at 30 mg/kg, and 2 was administrated as a positive control (Figure 5). The concentration levels of 17a in the liver and small intestine were much higher than in other tissues, indicating that liver and small intestine are the main target organs of 17a, while 2 showed much high concentrations in the liver and large intestine. Analysis of tissue-to-plasma ratios indicated that 17a had favourable distributions in the liver and small intestine. Significant activity of 17a in the OLTT appeared to result from high concentration in the small intestine, even though bioavailability of 17a was not great in mice. This result could be proof of a pharmacokinetic-pharmacodynamic correlation.

DGAT-1 and play essential roles in cholesterol homeostasis. 17b was not explored in the enzymatic assays due to its poor pharmacokinetic profile.

Considering potency, selectivity, and pharmacokinetic profiles, 17a was investigated to determine if 17a would be very unlikely to affect the pharmacokinetics of other drugs.

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could be a candidate with considerable potential and deserves for further investigation.

Disclosure statement
No potential conflict of interest was reported by the authors.

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