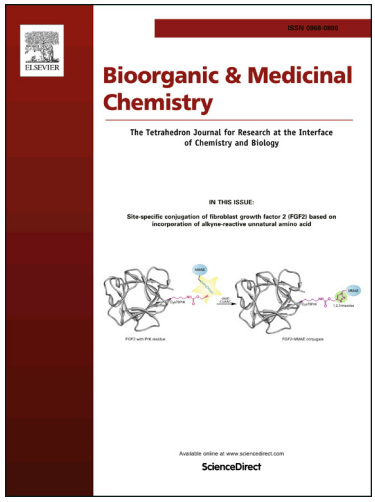
Accepted Manuscript



Design, synthesis, and pharmacological evaluation of a novel series of hormone sensitive lipase inhibitor

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Design, synthesis, and pharmacological evaluation of a novel series of hormone sensitive lipase inhibitor

Tomoko Ogiyama[a]*, Mitsuhiro Yamaguchi[b], Nobuya Kurikawa[c], Shoko Honzumi[d], Koji Terayama[e], Nobumi Nagaoka[a], Yuka Yamamoto[f], Takako Kimura[g], Daisuke Sugiyama[h], Shin-ichi Inoue[h]

[a] Modality Research Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

[b] R&D Planning & Management Department, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

[c] Venture Science Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

[d] Rare Disease & LCM Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

[e] End-Organ Disease Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

[f] Biological Research Department, Daiichi Sankyo RD Novare Co., Ltd., 1-16-13, Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan

[g] Organic Synthesis Department, Daiichi Sankyo RD Novare Co., Ltd., 1-16-13, Kita-Kasai, Edogawa-ku, Tokyo 134-8630, Japan

[h] Drug Metabolism & Pharmacokinetics Research Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

*To whom correspondence should be addressed:

Modality Research Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

Tel.: +81-3-3492-3131

E-mail address: yamamoto.tomoko.me@daiichisankyo.co.jp

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Hormone sensitive lipase (HSL)

Dyslipidemia

Lipolysis

Boronic acid

Abstract

HSL inhibition is a promising approach to the treatment of dyslipidemia. As a result of re-optimization of lead compound **2**, we identified novel compound **25a** exhibiting potent inhibitory activity against HSL enzyme and cell with high selectivity for cholinesterases (AChE and BuChE). Reflecting its potent in vitro activity, compound **25a** exhibited antilipolytic effect in rats at 1 mg/kg p.o., which indicated that this novel compound is the most potent orally active HSL inhibitor. Moreover, compound **25a** did not show bioactivation liability.

1. Introduction

Hormone sensitive lipase (HSL) is an intracellular neutral lipase that mediates the hydrolysis of broad substrates such as tri-, di-, and monoacylglycerol (TG, DG and MG), cholesterylester, retinyl ester and water soluble ester substrates.[1] HSL is highly expressed in adipose tissues (ATs) where it catalyzes lipolysis of triglyceride into glycerol and free fatty acid (FFA).[2] The activity of adipose HSL is controlled by several hormones depending on energy demand.[3] In the fasted state, HSL is stimulated by catecholamines, and FFA is released into circulation as an energy source for most tissues.[4]

Although FFA plays an important role in energy homeostasis, the rise in plasma FFA level is associated with obesity and insulin resistance. These conditions cause dysregulation of lipolysis as a result of enlarged AT mass and attenuated insulin-mediated AT lipolysis metabolism. In addition, the increased FFA flux to the liver contributes to increased secretion of very-low-density lipoprotein (VLDL), one of the hallmarks of dyslipidemia in the metabolic syndrome.[5] For this reason, HSL inhibitors could have significant beneficial effects on lipid profile and thereby may contribute to reducing the CVD risk, but to date only limited efforts have been made to identify HSL inhibitors.[6-15]

We previously reported that phenylboronic acid derivatives as potent and orally active HSL inhibitors (Figure 1).[16, 17] By exchanging a metabolic labile benzyl group in compound **1**, benzanilide **2** was discovered as a novel lead compound with decreased bioactivation liability. To enhance HSL inhibitory activity in enzyme assay, hydrophobic moieties such as a trifluoromethyl benzene ring and a chloro group were introduced into the left- and right-hand moieties, which led to the identification of a

potent HSL inhibitor **3**. Although compound **3** showed potent in vitro enzymatic activity, these optimization resulted in an increase in the lipophilicity ($LogD_{7.4}$ = 4.3) that resulted in poor solubility (JP1 = 1.8 μg/mL, JP2 = 1.5 μg/mL, and FaSSIF = 12 μg/mL). Thus, it appeared that further improvement of HSL inhibitory activity starting from compound **3** was difficult and we reconsidered optimization from lead compound **2** to explore new scaffolds. In this paper, synthesized compounds were evaluated in relation to cellular inhibitory activity as well as enzymatic inhibitory activity, since the significant difference between cellular and enzymatic inhibitory activity was observed in the case of compound **2** and **3** (5.2 and 55-fold decrease, respectively).

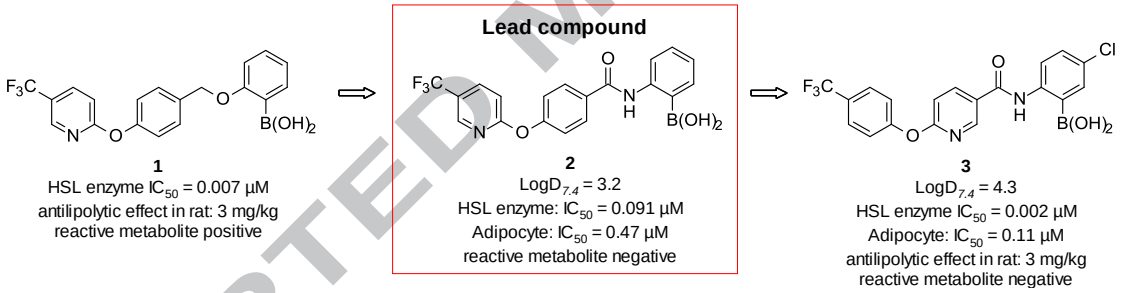
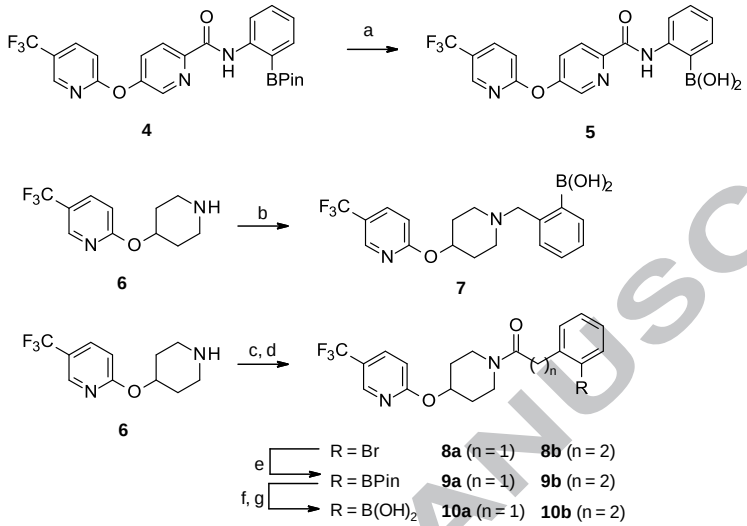


Figure 1. HSL inhibitors

2. Chemistry

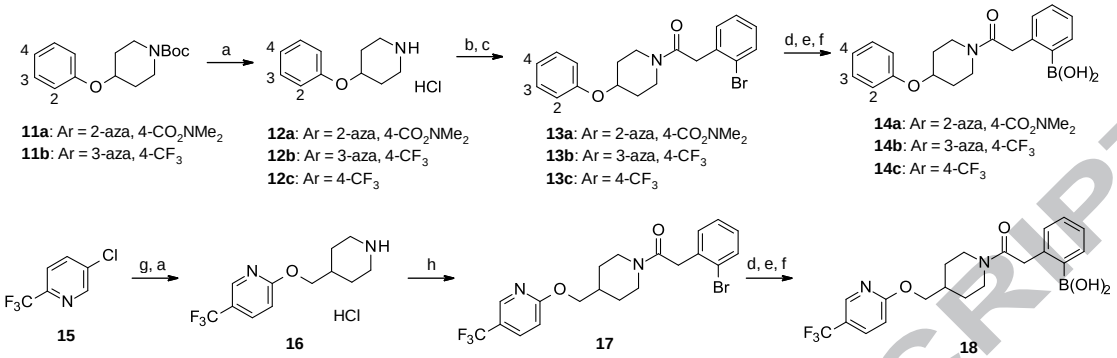
Scheme 1 describes the synthesis of derivatives **5**, **7**, and **10a**-**10b**. Oxidative deprotection of pinacol (Pin) group in compound **4**[17] using sodium periodate led to **5**. Reaction of 2-formylphenylboronic acid under reductive amination conditions with amine **6** gave **7**. Intermediates **8a** and **8b** were synthesized by acylation of **6** with corresponding acids. Compound **10a** was obtained by Miyaura-Ishiyama borylation[18] of **8a** and following two-step deprotection of the pinacol group via fluorinated

intermediates.[19] Compound **10b** was synthesized in a manner similar to that for compound **10a**.



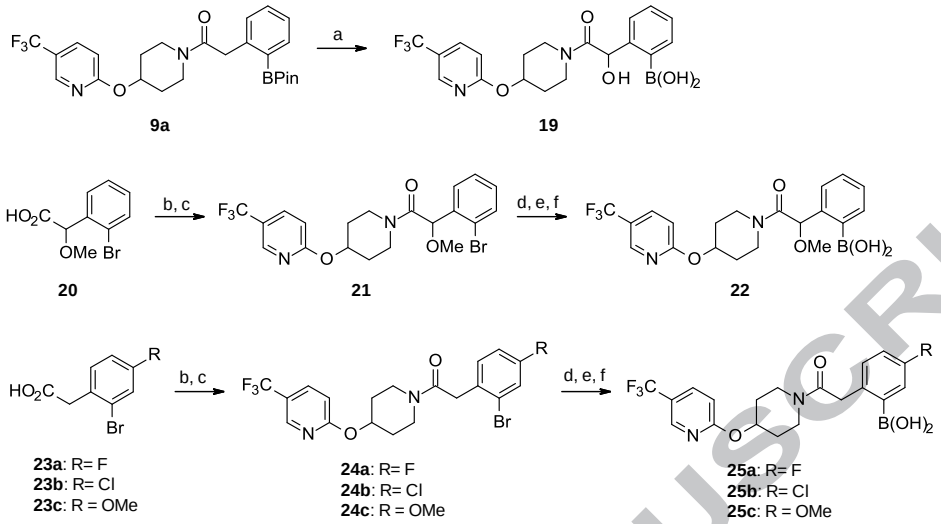
Scheme 1. Reagents and conditions: (a) $NaIO_4$, THF, H_2O, then 1 M HCl; (b) 2-formylphenylboronic acid, $NaBH_4$, MeOH; (c) carboxylic acids, oxalyl chloride, DMF (cat.), CH_2Cl_2; (d) DIPEA, CH_2Cl_2; (e) $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, bis(pinacolato)diboron, potassium acetate, 1,4-dioxane; (f) KHF_2, MeOH, H_2O; (g) TMSCl, CH_3CN, H_2O.

Scheme 2 illustrates the synthesis of compounds **14a**-**14c** and **18**. Deprotection of the Boc group in **11a**[20] and **11b**[21] yielded **12a** and **12b**, respectively. Amines (**12a**, **12b** and **12c**[22]) were condensed with 2-bromophenylacetic acid to give amides (**13a**-**13c**) which led to compounds **14a**-**14c** in a manner similar to that for compound **10a**. Condensation of **15** with 1-(*tert*-butoxycarbonyl)-4-piperidinemethanol furnished intermediate **16**, which was converted to compound **18** in a manner similar to that of compounds **14a**-**14c**.



Scheme 2. Reagents and conditions: (a) 4 M HCl/1,4-dioxane, 1,4-dioxane; (b) 2-bromophenylacetic acid, oxalyl chloride, DMF (cat.), CH_2Cl_2; (c) amines, DIPEA, CH_2Cl_2; (d) Pd(dppf)Cl_2·CH_2Cl_2, bis(pinacolato)diboron, potassium acetate, 1,4-dioxane; (e) KHF_2, MeOH, H_2O; (f) TMSCl, CH_3CN, H_2O; (g) 1-(*tert*-butoxycarbonyl)-4-piperidinemethanol, NaH, DMSO; (h) 2-bromophenylacetic acid, HBTU, DIPEA, DMF.

Scheme 3 depicts the synthesis of derivatives **19**, **22** and **25a**-**25c**. Compound **9a** was converted to compound **19** using sodium periodate. Condensation of **20** with **6** afforded compound **21**, which was converted to compound **22** in a manner similar to that for compound **10a**. Compounds **25a**-**25c** were generated in a manner similar to that used for the synthesis of compound **22**.



Scheme 3. Reagents and conditions: (a) $NaIO_4$, 1 M HCl, THF, H_2O; (b) 2-bromophenylacetic acid, oxalyl chloride, DMF (cat.), CH_2Cl_2; (c) **6**, DIPEA, CH_2Cl_2; (d) $Pd(dppf)Cl_2 \cdot CH_2Cl_2$, bis(pinacolato)diboron, potassium acetate, 1,4-dioxane; (e) KHF_2, MeOH, H_2O; (f) TMSCl, CH_3CN, H_2O.

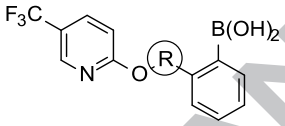
3. Results and discussion

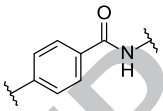
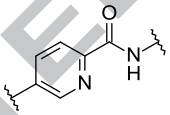
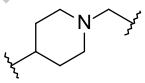
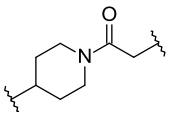
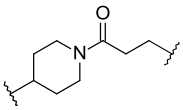
The inhibitory activity against HSL enzyme was measured by colorimetric assay using human HSL fractions and *p*-nitrophenyl butyrate (PNPB) as a substrate.[6] The cellular inhibitory activity was measured by glycerol concentration in rat subcutaneous fat tissue cells.

As mentioned above, the left- and right-hand moiety was thought to play a crucial role in enzyme activity; we first explored a central moiety (Table 1). Exchanging the benzene ring with pyridine ring resulted in a 10-fold decrease in inhibitory activity against HSL enzyme (**5**). Incorporation of methylpiperidine moiety led to a 6-fold decrease in HSL inhibitory activity (**7**). Introduction of piperidine carbonyl maintained HSL inhibitory activity (**10a**). Insertion of the methylene linker into the distal phenyl

group in compound **10a** was not tolerated (**10b**). In cellular assay, inhibitory activity of compounds **5** and **10a** was equivalent to that of enzymatic activity while compound **2**, **7** and **10b** led to 5, 2.5 and 1.7-fold decreases compared to its enzymatic activity, respectively. Above all, compound **10a** exhibited potent inhibitory activity against both HSL enzyme (IC_{50} = 0.10 μM) and adipocytes (IC_{50} = 0.080 μM).

Table 1. Replacement of the central ring moiety of compound **2**.



Compd	R	HSL IC_{50} (μM) Enzyme[a]	Adipocyte[b]
2		0.091	0.47
5		0.86	1.1
7		0.57	1.4
10a		0.10	0.080
10b		0.81	1.4

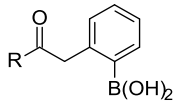
[a] Assay results are the average of triplicates.

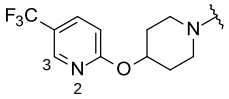
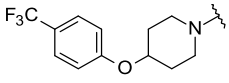
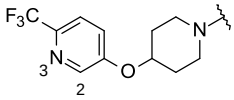
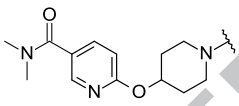
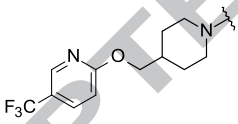
[b] Inhibition of rat adipocyte lipolysis. Assay results are the average of quadruplicates.

Encouraged by this result, further optimization of the left-hand moiety in compound **10a** was investigated (Table 2). In our previous work, replacement of the terminal

pyridine ring with a hydrophobic benzene ring resulted in a significant enhancement of inhibitory activity against HSL enzyme,[17] however, this replacement did not have a positive effect on this series (**14c**). Converting 2-pyridine ring to 3-pyridine ring exhibited an 8-fold decrease in HSL inhibitory activity (**14b**). Exchanging the CF_3 group in compound **10a** with hydrophilic *N*, *N*-dimethyl amide group led to a complete loss of inhibitory activity (**14a**). Insertion of the methylene between piperidine group and hydroxypyridine group retained HSL inhibitory activity (**18**). In cellular assay, the inhibitory activity of compound **14c** and **18** was comparable to those in enzymatic assay. Compound **14b** showed a 4.5-fold increased inhibitory activity compared to enzymatic activity (IC_{50} = 0.18 μM), however, its IC_{50} value was inferior to that of compound **10a** (IC_{50} = 0.080 μM). To better understand quality of the novel scaffold, we evaluated its enzyme selectivity for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE).[23] As a result of this investigation, compounds **10a**, **14c** and **14b** showed high selectivity for AChE, but resulted in moderate selectivity for BuChE. The inhibitory activity against BuChE varied depending on modifications made to the left-hand moiety, and 2-pyridine ring (**10a**) demonstrated adequate selectivity among these derivatives, thereby, we selected compound **10a** for further optimization.

Table 2. SAR of the left-hand moiety



Compd	R	HSL IC_{50} (μM)		Inhibitory activity at 10 μM[c]	
		Enzyme[a]	Adipocyte[b]	AChE	BuChE
10a		0.10	0.080	7%	46%
14c		0.11	0.13	9%	72%
14b		0.81	0.18	6%	62%
14a		> 5	NT[d]	NT[d]	NT[d]
18		0.28	0.26	NT[d]	NT[d]

[a] Assay results are the average of triplicates.

[b] Inhibition of rat adipocyte lipolysis. Assay results are the average of quadruplicates.

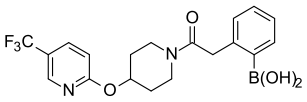
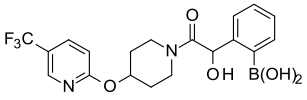
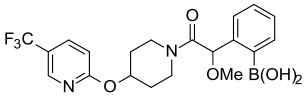
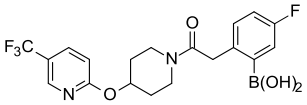
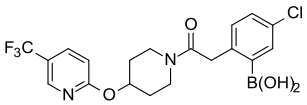
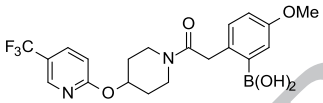
[c] Assay results are the average of duplicates.

[d] Not tested.

The structure–activity relationship of compound **10a** is summarized in Table 3. We first incorporated substituents on the benzyl position of compound **10a**. The benzyl oxidized derivative **19** resulted in a 2-fold decrease in inhibitory activity against HSL enzyme, and its methyl derivative **22** resulted in a 20-fold decline in inhibitory activity.

In keeping with the implications from last investigation,[17] we next examined the introduction of substituents to C-4 position of the right-hand benzene ring. 4-F derivative **25a** showed slightly improved inhibitory activity against HSL enzyme, 4-OMe derivative **25c** led to a 5-fold increase, and 4-Cl derivative **25b** resulted in a 13-fold increase in inhibitory activity against HSL enzyme with an IC_{50} value of 0.008 μM. In these derivatives, cellular inhibitory activity of **19** and **25a** was equal to HSL enzyme inhibitory activity. Compound **25b** and **25c** resulted in 3 and 2.4-fold decreased cellular inhibitory activity, however, an improvement in HSL enzyme inhibitory activity was directly reflected in these IC_{50} values. In particular, compound **25b** showed the most potent cellular inhibitory activity with an IC_{50} value of 0.024 μM. Moreover, compounds **25a-25c** resulted in a significant improvement in selectivity for BuChE compared with compound **10a**, while maintaining high selectivity for AChE.

Table 3. SAR of compound **10a**.

Compd		HSL IC_{50} (μM)		Inhibitory activity at 10 μM[c]	
		Enzyme[a]	Adipocyte[b]	AChE	BuChE
10a		0.10	0.080	7%	46%
19		0.23	0.28	-4%	4%
22		1.9	NT[d]	NT[d]	NT[d]
25a		0.068	0.067	7%	2%
25b		0.008	0.024	8%	5%
25c		0.019	0.045	9%	-4%

[a] Assay results are the average of triplicates.

[b] Inhibition of rat adipocyte lipolysis. Assay results are the average of quadruplicates.

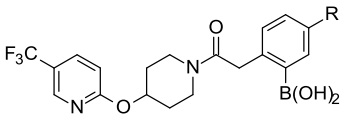
[c] Assay results are the average of duplicates.

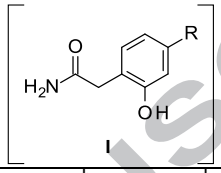
[d] Not tested.

Due to the need for a good safety profile when treating dyslipidemia, we assessed the bioactivation potential of **10a** and **25a**-**25c**. When each compound was incubated with isotope labeled glutathione ([$^{13}C_2$, ^{15}N-Gly]GSH) in the presence of human liver microsomes followed by LC/MS analysis, GSH adduct formation was detected except in the case of 4-F derivative **25a**. It is well known that elimination of bioactivation liability is highly desirable since reactive metabolite formation could cause organ toxicity and carcinogenesis.[25, 26] In the GSH trapping assay, GSH adduct signals

corresponding to the molecular weight of ‘‘parent compound – $B(OH)_2$ + GSH + O - H” (**10a** and **25c**) and “parent compound – $B(OH)_2$ + GSH + 2O - H” (**10a**, **25b** and **25c**) were detected (Table 4).[27, 28]

Table 4. Reactive metabolite trapping assay.





I

Compd	R =	GSH adduct formation[a]		E_{HOMO}[b]	E_{LUMO}[b]	E_{LUMO} -E_{HOMO}[b]
		m/z	Proposed GSH adduct	(eV)	(eV)	(eV)
10a	H	686	P–$B(OH)_2$+GSH+O-H	-5.61	0.09	5.70
		702	P–$B(OH)_2$+GSH+2O-H			
25a	F	ND[c]		-5.83	-0.03	5.80
25b	Cl	736	P–$B(OH)_2$+GSH+2O-H	-5.92	-0.29	5.63
25c	OMe	716	P–$B(OH)_2$+GSH+O-H	-5.48	-0.08	5.40
		732	P–$B(OH)_2$+GSH+2O-H			

[a] Reactive metabolite assessment was conducted using isotope labeled glutathione as a trapping agent in NADPH-supplemented human liver microsomes.

[b] E_{HOMO} and E_{LUMO} of each simplified phenylacetamide **I** were calculated by the software Jaguar using DFT/B3LYP method of Schrödinger Macromodel (Maestro version 9.1) software (**10a-I**, **25a-I**, **25b-I** and **25c-I**).

[c] Not detected

CYP450 enzymes are considered to be mainly responsible for bioactivation in the liver,[29] therefore, these findings led to two hypotheses: (i) the right-hand benzene group is a major activation site because GSH adduct formation differed with respect to the functional groups on the benzene ring and (ii) reactive metabolites are formed through a two-step process in which the phenylboronic acid resulted in a corresponding phenol via CYP450-catalyzed oxidative deboronation,[30] which is subsequently transformed into

reactive metabolite species due to the electron-rich nature of an aryl ring (Figure 2, path A). The rate-limiting step for aromatic oxidation is formation of the bond between a hem oxygen in CYP450 and an aromatic carbon atom,[31] and the first step is considered to be a reaction between electrophilic iron-oxo species and the highest occupied molecular orbital (HOMO) of compounds.[32] Consequently, a close correlation between the calculated HOMO energy ($E_{HOMO)}$ value of parent compounds and the formation of reactive metabolite or mutagenicity was observed.[33, 34] As depicted in Table 4, E_{HOMO} values of phenols **10-I** and **25c-I**, which are simplified versions of each metabolite, were higher than that of **25a-I** (values are -5.61 eV, -5.48 eV and -5.83 eV, respectively), and those were in good agreement with the results of GSH adduct formation.

On the other hand, compound **25b** showed GSH adduct formation despite the lowest E_{HOMO} of **25b-I** (-5.92 eV) among these derivatives. Thus, we speculated that bioactivation of **25b** might be oxidative deboronation and the subsequent oxidation of a benzyl carbon (Figure 2, path B). The rate-limiting step for aliphatic oxidation by CYP450 is thought to be carbon-hydrogen abstraction.[35] The C-H bond dissociation energies of alkanes reportedly have a good correlation with the gap of the lowest unoccupied molecular orbital energy (E_{LUMO}) and the E_{HOMO}.[36, 37] As seen in Table 4, the calculated E_{HOMO}-E_{LUMO} gap of **25b-I** (5.63 eV) was lower than that of **25a-I** (5.80 eV), and the values were in accord with the results of reactive metabolite trapping assay.

Taken together, the reason why **25a** did not show bioactivation liability might be attributed to its low intrinsic reactivity of the phenol intermediate with CYP450. Moreover, flavin mono oxygenases, monoamine oxidases and the heme-containing

peroxidases have also been noted to participate in the catalysis of bioactivation in the liver, [38] and further investigation would be needed.

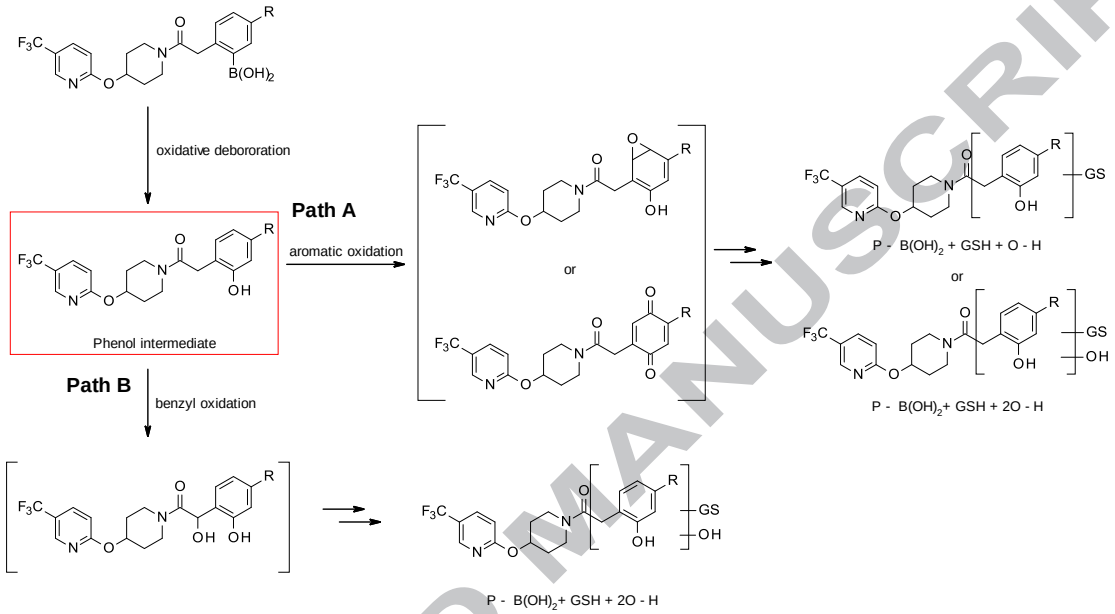


Figure 2. Proposed bioactivation of **10a** and **25b-c**.

In vitro pharmaceutical properties of compound **25a** are summarized in Table 5. Compound **25a** demonstrated high permeability coefficients (pH 5.0: > 50 × 10^{-6} cm/s, pH 7.4: > 50 × 10^{-6} cm/s) in a parallel artificial membrane permeability assay (PAMPA). In addition, compound **25a** showed good aqueous solubility (190.0 μg/mL in JP1 solution, 84.0 μg/mL in JP2 solution, 99.0 μg/mL in fasted state simulated fluid) presumably because of moderate lipophilicity ($LogD_{7.4}$ = 3.7). These favorable pharmaceutical profiles effected rat pharmacokinetic profiles, the C_{max} and AUC values of compound **25a** at 3 mg/kg p.o. were 0.65 μg/mL and 3.18 μg·h/mL in 0.5% methylcellulose suspension, respectively.

Table 5. Pharmaceutical profiles of compound **25a**.

PAMPA, Peff[a] (10^{-6} cm/s)	pH 5.0	> 50
	pH 7.4	> 50
$LogD_{7.4}$[b]		3.7
Solubility[c] (μg/mL)	JP1	190.0
	JP2	84.0
	FaSSIF	99.0
C_{max}[d] (μg/mL)		0.65
AUC[d] (μg·h/mL)		3.18

[a] Permeability coefficient.

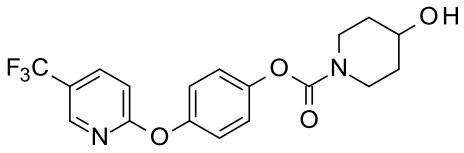
[b] The distribution coefficients (LogD) were measured between 1-octanol and phosphate buffer saline (pH 7.4).

[c] JP1/JP2: Japanese pharmacopoeia first/second test fluid (pH = 1.2/6.8). FaSSIF: Fasted state simulated fluid.

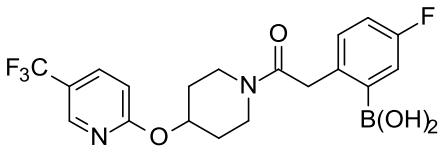
[d] 3 mg/2 mL/kg of compound suspension in 0.5% (w/v) methylcellulose solution.

Compound **25a** showed excellent profiles and we examined its antilipolytic effect in rats. To evaluate potency, a head-to-head comparison was performed by measuring the plasma glycerol level as a lipolytic end point.[39] For comparison, compound **26** (Figure 3), which was identified by Ebdrup et al. as a potent orally active HSL inhibitor and exerted an antilipolytic effect at a dose of 10 mg/kg p.o. due to its good PK profiles,[12] was selected.

As shown in Figure 4, oral administration of compound **25a** relatively lowered plasma glycerol level and the value of compound **25a** at 1 mg/kg p.o. was comparable to that of **26** at 10 mg/kg p.o. with a value of 22%, owing to its potent cellular inhibitory activity (IC_{50} = 67 nM) compared with that of compound **26** (IC_{50} = 950 nM). This indicates that compound **25a** is an orally active HSL inhibitor that has the potential to act as an antilipolytic agent.



26
HSL enzyme: IC_{50} = 270 nM
Adipocyte: IC_{50} = 950 nM



25a
HSL enzyme: IC_{50} = 68 nM
Adipocyte: IC_{50} = 67 nM

Figure 3. Structures of **26** and **25a**.

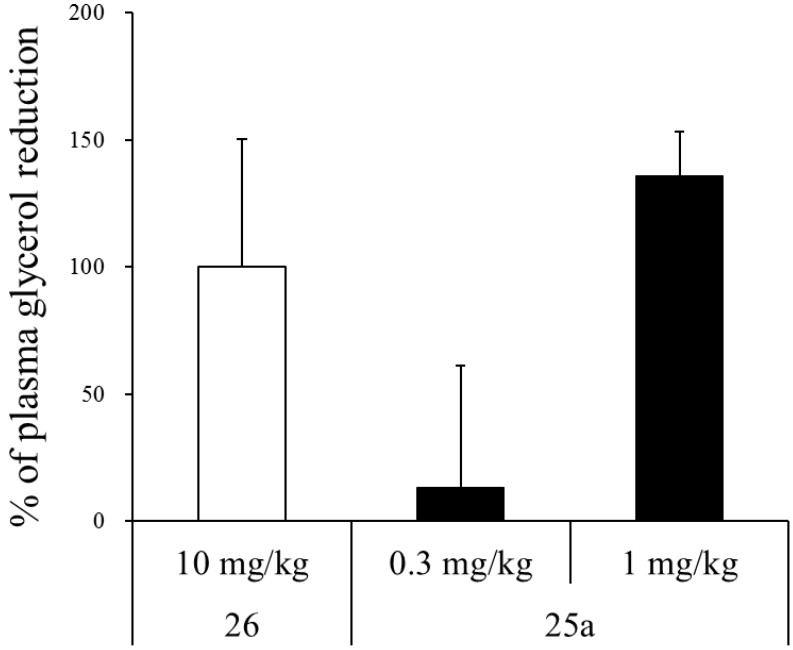


Figure 4. The acute effect of HSL inhibition in rats treated with **26** at 10 mg/kg (white square) or **25a** at 0.3 and 1 mg/kg (black square). Data are reported as a percentage of plasma glycerol reduction of the area under the curve (AUC) (0–7 h), setting at 100% the reduction measured in **26**. Data are mean ± SEM *n* = 3–4.

4. Conclusion

Starting from lead compound **2**, we have identified compound **25a**. Compound **25a** showed potent inhibitory activity against HSL enzyme with an IC_{50} value of 0.068 μM, improved cellular inhibitory activity with an IC_{50} value of 0.067 μM. Reflecting its potent cellular inhibitory activity, compound **25a** led to a decrease in the plasma glycerol level by oral administration when dosed at 1 mg/kg in rats. Compound **25a** shows potential as an HSL inhibitor for use in the treatment of dyslipidemia associated with increased lipolysis.

5. Experimental

5.1. Chemistry

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. NMR spectra were recorded on a Varian Mercury 400 or 500 spectrometer with tetramethylsilane as an internal reference. Mass spectra were recorded on an Agilent Technologies Agilent 1100 series LC/MS. TLC analysis was performed on 60F354 plates (Merck). Flash column chromatography was performed on Shoko scientific SI series on a Shoko Scientific Purif-α2. Purities of assayed compounds were in all cases greater than 90%, as determined by NMR analysis and LC/MS. The following abbreviations are used: DMF, *N*,*N*-dimethylformamide; AcOEt, ethyl acetate; THF, tetrahydrofuran; MeOH, methanol; Et_2O, diethyl ether; EtOH, ethanol; DMSO, dimethylsulfoxide; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; PNPB, *p*-nitrophenyl butyrate.

5.1.1.

(2-{[(5-{[5-(trifluoromethyl)pyridin-2-yl]oxy}pyridin-2-yl)carbonyl]amino}phenyl) boronic acid (5)

A mixture of **4** (0.25 g, 0.51 mmol), $NaIO_4$ (0.65 g, 3.0 mmol), THF (20 mL) and H_2O (5 mL) was stirred at room temperature for 30 min. To the mixture was added 1M HCl aq. (1.8 mL). After stirring overnight, the reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane-AcOEt). The obtained solid was triturated in hexane/AcOEt and filtered to give **5** (0.14 g, 69%) as a colorless solid. ^{1}H-NMR (CD_3OD) δ: 8.98 (1H, dd, *J* = 2.3, 0.8 Hz), 8.53 (1H, dd, *J* = 8.6, 0.8 Hz), 8.34 (1H, br s), 8.28 (1H, dd, *J* = 8.4, 2.5 Hz), 7.82 (1H, dd, *J* = 9.6, 2.5 Hz), 7.55 (1H, dd, *J* = 7.2, 1.8 Hz), 7.50 (1H, dd, *J* = 7.8, 1.2 Hz), 7.39–7.35 (2H, m), 6.79 (1H, d, *J* = 9.8 Hz). ^{13}C-NMR (DMSO-*d6*) δ: 161.53, 160.77, 149.87, 146.92, 143.32, 139.35 (q, ^3J(C, F) = 5.4 Hz), 138.48, 136.71, 136.36, 135.71, 131.09, 124.74, 123.10, 122.64, 121.37, 119.32, 108.32 (q, ^2J(C, F) = 34.7 Hz). MS (ESI$^-$) *m/z*: 402 $(M-H)^-$. HRMS (ESI$^-$) *m/z*: 402.0873 $(M-H)^-$ (calcd for $C_{18}H_{12}BF_3N_3O_4$: 402.0878).

5.1.2.

{2-[(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)methyl]phenyl}boronic acid (7)

A solution of **6** (0.77 g, 3.1 mmol) and 2-formylphenylboronic acid (0.49 g, 3.3 mmol) in MeOH (125 mL) was stirred at room temperature for 1 h. To the solution was added sodium borohydride (0.18 g, 4.7 mmol). After stirring at room temperature for 1

h, the reaction mixture was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and filtered. The filtrate was concentrated in vacuo and the residue was triturated with hexane and AcOEt to give **7** (0.068 g, 5.7%) as a colorless solid. ^{1}H-NMR (CD_3OD) δ: 8.46 (1H, dd, *J* = 1.6, 0.8 Hz), 7.95 (1H, dd, *J* = 8.8, 2.5 Hz), 7.62 (1H, d, *J* = 7.0 Hz), 7.27–7.23 (1H, m), 7.18–7.15 (2H, m), 6.98 (1H, d, *J* = 9.0 Hz), 5.44–5.38 (1H, m), 4.17 (2H, s), 3.24–3.20 (2H, m), 3.08–3.04 (2H, m), 2.25–2.19 (2H, m), 2.10–2.06 (2H, m). ^{13}C-NMR (CD_3OD) δ: 166.72, 146.29 (q, ^3J(C, F) = 4.5 Hz), 137.80 (q, ^3J(C, F) = 3.2 Hz), 135.33, 131.04, 130.12, 129.6, 128.95, 128.05, 127.18, 124.46, 121.74 (q, ^2J(C, F) = 33.1 Hz), 113.23, 70.12, 62.91, 31.89, 29.24. MS (ESI^+) *m/z*: 381 $(M+H)^+$. HRMS (ESI^+) *m/z*: 381.1624 $(M+H)^+$ (calcd for $C_{18}H_{21}BF_3N_2O_3$: 381.1597).

5.1.3

2-(2-bromophenyl)-1-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethanone (8a)

Step A: To a solution of 2-bromophenylacetic acid (0.22 g, 1.0 mmol) in dichloromethane (4.5 mL) was added oxalyl chloride (0.25 mL, 3.0 mmol) followed by DMF (1 drop) at room temperature. The reaction mixture was stirred at room temperature for 1 h and concentrated in vacuo to give crude (2-bromophenyl)acetyl chloride, which was used directly in the next step. Step B: Crude (2-bromophenyl)acetyl chloride was dissolved in dichloromethane (4.5 mL) at 0°C. To the solution was added DIPEA (0.19 mL, 1.1 mmol) and 2-(piperidin-4-yloxy)-5-(trifluoromethyl)pyridine (0.27 g, 1.1 mmol). After stirring at room temperature for 1 h, the mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel

chromatography (hexane-AcOEt) to give **8a** (0.43 g, 97%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 8.40 (1H, s), 7.77 (1H, dd, *J* = 8.6, 2.3 Hz), 7.57 (1H, dd, *J* = 7.8, 1.2 Hz), 7.32–7.29 (2H, m), 7.14 (1H, ddd, *J* = 8.5, 6.4, 1.7 Hz), 6.80 (1H, d, *J* = 8.6 Hz), 5.34–5.32 (1H, m), 4.01–3.95 (1H, m), 3.87 (2H, s), 3.73–3.70 (1H, m), 3.61–3.55 (1H, m), 3.43–3.40 (1H, m), 2.04–1.91 (2H, m), 1.84–1.68 (2H, m). ^{13}C-NMR (CD_3OD) δ: 170.94, 166.76, 146.09 (q, ^{3}J (C, F) = 4.5 Hz), 137.51 (q, ^{3}J(C, F) = 3.2 Hz), 136.99, 133.94, 132.54, 130.00, 128.94, 126.06, 124.35, 121.29 (q, ^{2}J(C, F) = 64.8 Hz), 113.13, 72.27, 44.45, 41.68, 40.57, 32.24, 31.63. MS (ESI^+) *m/z*: 443 $(M+H)^+$. HRMS (ESI^+) *m/z*: 443.0605 $(M+H)^+$ (calcd for $C_{19}H_{19}BrF_3N_2O_2$: 443.0576).

5.1.4.

3-(2-bromophenyl)-1-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)propan-1-one (8b)

Compound **8b** was prepared in a manner similar to that used for **8a**. Yield: 100%. ^{1}H-NMR ($CDCl_3$) δ: 8.40 (1H, s), 7.77 (1H, dd, *J* = 8.8, 2.4 Hz), 7.54 (1H, d, *J* = 7.8 Hz), 7.31 (1H, dd, *J* = 7.8, 1.5 Hz), 7.24 (1H, d, *J* = 7.3 Hz), 7.09 (1H, td, *J* = 7.6, 1.5 Hz), 6.79 (1H, d, *J* = 8.3 Hz), 5.34–5.30 (1H, m), 3.98–3.93 (1H, m), 3.71–3.66 (1H, m), 3.54–3.49 (1H, m), 3.39–3.34 (1H, m), 3.11 (2H, t, *J* = 7.8 Hz), 2.68 (2H, t, *J* = 8.1 Hz), 2.00–1.89 (2H, m), 1.79–1.65 (2H, m). ^{13}C-NMR (CD_3OD) δ: 172.80, 166.71, 146.06 (q, ^{3}J(C, F) = 4.5 Hz), 141.49, 137.46 (q, ^{3}J(C, F) = 3.2 Hz), 134.08, 132.16, 129.57, 129.10, 127.06, 125.29, 121.23 (q, ^{2}J(C, F) = 66.3 Hz), 113.10, 72.16, 44.24, 40.22, 34.02, 33.39, 32.25, 31.59. MS (ESI^+) *m/z*: 457 $(M+H)^+$. HRMS (ESI^+) *m/z*: 457.0742 $(M+H)^+$ (calcd for $C_{20}H_{21}BrF_3N_2O_2$: 457.0733).

5.1.5.

2-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-1-(4-{[5-(trifluoromethyl) pyridin-2-yl]oxy}piperidin-1-yl)ethanone (9a)

A mixture of **8a** (14 g, 33 mmol), Pd(dppf)Cl_2·CH_2Cl_2 (5.3 g, 6.5 mmol), bis(pinacolato)diboron (9.9 g, 39 mmol), potassium acetate (9.6 g, 98 mmol), and dioxane (330 mL) was stirred at 90°C for 6 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and evaporated. The resulting residue was purified by silica gel chromatography (hexane-AcOEt) to give **9a** (6.0 g, 38%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 8.39 (1H, s), 7.84 (1H, d, *J* = 7.3 Hz), 7.76 (1H, dd, *J* = 8.8, 2.4 Hz), 7.41–7.38 (1H, m), 7.30–7.23 (2H, m), 6.78 (1H, d, *J* = 8.8 Hz), 5.33–5.29 (1H, m), 4.11 (2H, s), 4.02–3.97 (1H, m), 3.78–3.76 (1H, m), 3.52–3.47 (1H, m), 3.44–3.38 (1H, m), 2.00–1.98 (1H, m), 1.90–1.86 (1H, m), 1.77–1.74 (1H, m), 1.67–1.63 (1H, m), 1.33 (12H, s). MS (ESI^+) *m/z*: 491 $(M+H)^+$.

5.1.6.

3-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-1-(4-{[5-(trifluoromethyl) pyridin-2-yl]oxy}piperidin-1-yl)propan-1-one (9b)

Compound **9b** was prepared in a manner similar to that used for **9a**. Yield: 88%. ^{1}H-NMR ($CDCl_3$) δ: 8.40 (1H, s), 7.80–7.77 (2H, m), 7.39–7.35 (1H, m), 7.24–7.22 (2H, m), 6.79 (1H, d, *J* = 8.8 Hz), 5.33–5.29 (1H, m), 4.00–3.96 (1H, m), 3.71–3.69 (1H, m), 3.51–3.46 (1H, m), 3.37–3.32 (1H, m), 3.23–3.19 (2H, m), 2.68–2.65 (2H, m), 1.98–1.95 (1H, m), 1.89–1.85 (1H, m), 1.75–1.73 (1H, m), 1.65–1.62 (1H, m), 1.34 (12H, s). MS (ESI^+) *m/z*: 505 $(M+H)^+$.

5.1.7.

{2-[2-oxo-2-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethyl]phenyl}boronic acid (10a)

Step A: To a solution of **9a** (6.0 g, 12 mmol) in MeOH (80 mL) was added 4.5 M KHF_2 aq. (15 mL). After stirring at room temperature overnight, the reaction mixture was concentrated in vacuo. The residue was dissolved in hot acetone and filtered. The filtrate was concentrated in vacuo to give crude potassium trifluoro{2-[2-oxo-2-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethyl]phenyl}borate(1-), which was used directly in the next step. Step B: Crude potassium trifluoro{2-[2-oxo-2-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethyl]phenyl}borate(1-), which was used directly was dissolved in CH_3CN (80 mL) and H_2O (1.3 mL) to which chlorotrimethylsilane (9.3 ml, 74 mmol) was added. After stirring overnight, the resulting mixture was evaporated. The resulting residue was diluted with saturated $NaHCO_3$ solution and extracted with AcOEt. The organic layer was washed with H_2O (twice) and brine, dried over Na_2SO_4, filtered and evaporated. The obtained residue was triturated with AcOEt and hexane to give **10a** (2.2 g, 45%) as a colorless solid. ^{1}H-NMR (CD_3OD) δ: 8.48 (1H, s), 7.95 (1H, dd, *J* = 8.8, 2.4 Hz), 7.45 (1H, dd, *J* = 5.4, 3.4 Hz), 7.22–7.15 (3H, m), 6.97 (1H, d, *J* = 8.8 Hz), 5.52–5.49 (1H, m), 4.09–3.86 (6H, m), 2.21–2.17 (2H, m), 2.03–1.98 (2H, m). ^{13}C-NMR (CD_3OD) δ: 175.25, 166.52, 146.02 (q, ^3J(C, F) = 4.5 Hz), 137.51 (q, ^3J(C, F) = 3.2 Hz), 135.66, 132.04, 128.28, 128.01, 127.33, 126.93, 124.21, 121.40 (q, ^2J(C, F) = 32.4 Hz), 113.07, 70.89, 44.80, 42.11, 33.89, 31.66, 31.14. HRMS (ESI$^-$) *m/z*: 407.1420 $(M-H)^-$ (calcd for $C_{19}H_{19}BF_3N_2O_4$: 407.1395).

5.1.8.

{2-[3-oxo-3-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)propyl]phenyl} boronic acid (10b)

Compound **10b** was prepared in a manner similar to that used for **10a**. Yield: 53%. ^{1}H-NMR (CD_3OD) δ: 8.45 (1H, s), 7.93 (1H, dd, *J* = 9.0, 2.3 Hz), 7.30–7.22 (4H, m), 6.91 (1H, d, *J* = 9.0 Hz), 5.34–5.30 (1H, m), 3.92–3.89 (1H, m), 3.70–3.68 (1H, m), 3.51–3.45 (1H, m), 3.40–3.36 (1H, m), 2.91 (2H, t, *J* = 7.6 Hz), 2.70 (2H, t, *J* = 7.6 Hz), 2.02–1.94 (1H, m), 1.86–1.80 (1H, m), 1.73–1.67 (1H, m), 1.59–1.51 (1H, m). ^{13}C-NMR (CD_3OD) δ: 173.57, 166.89, 146.23 (q, ^{3}J(C, F) = 4.5 Hz), 144.86, 137.64 (q, ^{3}J(C, F) = 3.2 Hz), 133.03, 130.37, 130.17, 127.03, 121.41 (q, ^{2}J(C, F) = 33.7 Hz), 113.24, 72.41, 44.40, 40.28, 36.49, 33.89, 32.33, 31.67. HRMS (ESI$^-$) *m/z*: 421.1573 $(M-H)^-$ (calcd for $C_{20}H_{21}BF_3N_2O_4$: 421.1552).

5.1.9. *N*,*N*-dimethyl-6-(piperidin-4-yloxy)pyridine-3-carboxamide hydrochloride (1:1) (12a)

A mixture of **11a** (0.10 g, 0.29 mmol), 4 M HCl in 1,4-dioxane (1.7 mL) and 1,4-dioxane (1.7 mL) was stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo and the resulting residue was triturated with Et_2O to give **12a** (0.27 g, 100%) as a colorless solid. Yield: 100%. ^{1}H-NMR (CD_3OD) δ: 8.32 (1H, d, *J* = 2.3 Hz), 7.90 (1H, dd, *J* = 8.4, 1.8 Hz), 7.01 (1H, dd, *J* = 6.1, 2.5 Hz), 5.41–5.39 (1H, m), 3.45–3.41 (2H, m), 3.27–3.25 (2H, m), 3.09 (6H, d, *J* = 13.3 Hz), 2.29–2.24 (2H, m), 2.14–2.06 (2H, m). ^{13}C-NMR (CD_3OD) δ: 169.69, 163.27, 144.68, 143.55, 127.70,

112.77, 71.41, 68.27, 42.06, 40.24, 36.09, 28.43. MS (ESI^+) *m/z*: 250 $(M+H)^+$. HRMS (ESI^+) *m/z*: 250.1547 $(M+H)^+$ (calcd for $C_{13}H_{20}N_3O_2$: 250.1550).

5.1.10. 5-(piperidin-4-yloxy)-2-(trifluoromethyl)pyridine hydrochloride (1:1) (12b)

Compound **12b** was prepared in a manner similar to that used for **12a**. Yield: 91%. ^{1}H-NMR (CD_3OD) δ: 8.43 (1H, d, *J* = 2.9 Hz), 7.78 (1H, d, *J* = 8.8 Hz), 7.64 (1H, dd, *J* = 8.8, 2.9 Hz), 4.93–4.90 (1H, m), 3.46–3.41 (2H, m), 3.28–3.25 (2H, m), 2.26–2.22 (2H, m), 2.09–2.07 (2H, m). ^{13}C-NMR (CD_3OD) δ: 156.99, 141.01, 126.88, 123.94, 123.21 (q, ^3J(C, F) = 2.9 Hz), 121.40, 71.02, 68.28, 41.94, 28.26. MS (ESI^+) *m/z*: 247 $(M+H)^+$. HRMS (ESI^+) *m/z*: 247.1063 $(M+H)^+$ (calcd for $C_{11}H_{14}F_3N_2O$: 247.1053).

5.1.11. 6-({1-[(2-bromophenyl)acetyl]piperidin-4-yl}oxy)-*N*,*N*-dimethylpyridine-3-carboxamide (13a)

Compound **13a** was prepared in a manner similar to that used for **8a**. Yield: 72%. ^{1}H-NMR ($CDCl_3$) δ: 8.24 (1H, d, *J* = 2.4 Hz), 7.69 (1H, dd, *J* = 8.8, 2.4 Hz), 7.57 (1H, dd, *J* = 8.1, 1.2 Hz), 7.33–7.27 (2H, m), 7.13 (1H, td, *J* = 7.6, 1.8 Hz), 6.73 (1H, d, *J* = 7.8 Hz), 5.33–5.29 (1H, m), 4.00–3.95 (1H, m), 3.87 (2H, d, *J* = 2.0 Hz), 3.73–3.69 (1H, m), 3.60–3.55 (1H, m), 3.48–3.37 (1H, m), 3.08 (6H, s), 2.06–1.91 (2H, m), 1.80–1.71 (2H, m). MS (ESI^+) *m/z*: 448 $(M+H)^+$.

5.1.12. 2-(2-bromophenyl)-1-(4-{[6-(trifluoromethyl)pyridin-3-yl]oxy}piperidin-1-yl)ethanone (13b)

Compound **13b** was prepared in a manner similar to that used for **8a**. Yield: 67%. ^{1}H-NMR ($CDCl_3$) δ: 8.40 (1H, d, *J* = 2.9 Hz), 7.64 (1H, d, *J* = 8.8 Hz), 7.60 (1H, dd, *J* = 6.6, 2.7 Hz), 7.36–7.30 (3H, m), 7.17 (1H, td, *J* = 7.6, 1.6 Hz), 4.70–4.66 (1H, m), 3.90 (2H, s), 3.86–3.78 (2H, m), 3.75–3.70 (1H, m), 3.51–3.49 (1H, m), 2.03–1.99 (1H, m), 1.93–1.87 (2H, m), 1.83–1.79 (1H, m). ^{13}C-NMR (CD_3OD) δ: 170.97, 157.46, 140.91, 136.96, 133.94, 132.56, 130.01, 128.95, 126.05, 124.79, 123.81, 123.13 (q, ^{3}J(C, F) = 2.9 Hz), 122.06, 74.23, 44.04, 41.63, 40.13, 31.90, 31.24. MS (ESI^+) *m/z*: 443 $(M+H)^+$. HRMS (ESI^+) *m/z*: 443.0563 $(M+H)^+$ (calcd for $C_{19}H_{19}BrF_3N_2O_2$: 443.0576).

5.1.13.

2-(2-bromophenyl)-1-{4-[4-(trifluoromethyl)phenoxy]piperidin-1-yl}ethanone (13c)

Compound **13c** was prepared in a manner similar to that used for **8a**. Yield: 78%. ^{1}H-NMR ($CDCl_3$) δ: 7.57–7.55 (3H, m), 7.34–7.28 (2H, m), 7.15–7.13 (1H, m), 6.96 (2H, d, *J* = 8.6 Hz), 4.62–4.59 (1H, m), 3.87 (2H, s), 3.79–3.65 (3H, m), 3.47–3.44 (1H, m), 1.94–1.93 (1H, m), 1.84–1.78 (3H, m). ^{13}C-NMR (CD_3OD) δ: 171.33, 170.94, 161.57, 136.98, 133.95, 132.53, 130.00, 128.95, 128.19 (q, ^{3}J(C, F) = 3.8 Hz), 127.47, 127.25, 126.29, 126.07, 125.51, 124.76, 124.74, 124.22, 123.90, 117.13, 73.20, 44.12, 41.66, 40.22, 32.06, 31.42. MS (ESI^+) *m/z*: 444 $(M+H)^+$. HRMS (ESI^+) *m/z*: 442.0633 $(M+H)^+$ (calcd for $C_{20}H_{20}BrF_3NO_2$: 442.0624).

5.1.14.

{2-[2-(4-{[5-(dimethylcarbamoyl)pyridin-2-yl]oxy}piperidin-1-yl)-2-oxoethyl]phenyl}boronic acid (14a)

N,*N*-dimethyl-6-[(1-{[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]acetyl}piperidin-4-yl)oxy]pyridine-3-carboxamide was prepared in a manner similar to that used for **9a**. Yield: 54%. ^{1}H-NMR ($CDCl_3$) δ: 8.24 (1H, d, *J* = 2.4 Hz), 7.84 (1H, dd, *J* = 7.6, 1.2 Hz), 7.68 (1H, dd, *J* = 8.3, 2.4 Hz), 7.41–7.38 (1H, m), 7.30–7.23 (2H, m), 6.72 (1H, d, *J* = 8.8 Hz), 5.30–5.26 (1H, m), 4.11 (2H, s), 4.01–3.96 (1H, m), 3.79–3.74 (1H, m), 3.52–3.47 (1H, m), 3.43–3.38 (1H, m), 3.08 (6H, br s), 2.01–1.97 (1H, m), 1.90–1.86 (1H, m), 1.78–1.74 (1H, m), 1.67–1.61 (1H, m), 1.33 (12H, s). MS (ESI^+) *m/z*: 494 $(M+H)^+$. Compound **14a** was prepared in a manner similar to that used for **10a**. Yield: 39%. ^{1}H-NMR (CD_3OD) δ: 8.27 (1H, dd, *J* = 2.3, 0.8 Hz), 7.78 (1H, dd, *J* = 8.6, 2.3 Hz), 7.45–7.43 (1H, m), 7.22–7.14 (3H, m), 6.88 (1H, dd, *J* = 8.6, 0.8 Hz), 5.47–5.44 (1H, m), 4.06–3.86 (7H, m), 2.24–2.12 (2H, m), 2.03–1.97 (2H, m). ^{13}C-NMR (DMSO-*d6*) δ: 170.63, 167.97, 162.82, 146.05, 139.76, 138.76, 134.35, 129.21, 125.58, 110.75, 70.43, 43.25, 31.14, 30.32. MS (ESI^-) *m/z*: 410 $(M-H)^-$. HRMS (ESI^-) *m/z*: 410.1886 $(M-H)^-$ (calcd for $C_{21}H_{25}BN_3O_5$: 410.1893).

5.1.15. {2-[2-oxo-2-(4-{[6-(trifluoromethyl)pyridin-3-yl]oxy}piperidin-1-yl)ethyl]phenyl}boronic acid (14b)

2-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-1-(4-{[6-(trifluoromethyl)pyridin-3-yl]oxy}piperidin-1-yl)ethanone was prepared in a manner similar to that used for **9a**. Yield: 57%. ^{1}H-NMR ($CDCl_3$) δ: 8.36 (1H, d, *J* = 2.7 Hz), 7.84 (1H, dd, *J* = 7.4, 1.6 Hz), 7.61 (1H, d, *J* = 8.6 Hz), 7.40 (1H, td, *J* = 7.4, 1.6 Hz), 7.28–7.25 (3H, m), 4.64–4.59 (1H, m), 4.11 (2H, s), 3.82–3.73 (3H, m), 3.51–3.46 (1H, m), 1.98–1.94 (1H, m), 1.85–1.66 (3H, m), 1.34 (12H, s). MS (FAB^+) *m/z*: 491 $(M+H)^+$. Compound **14b**

was prepared in a manner similar to that used for **10a**. Yield: 34%. ^{1}H-NMR (CD_3OD) δ: 8.42 (1H, d, *J* = 3.1 Hz), 7.77 (1H, d, *J* = 8.6 Hz), 7.63 (1H, dd, *J* = 9.0, 2.7 Hz), 7.45–7.43 (1H, m), 7.22–7.15 (3H, m), 4.97–4.92 (1H, m), 4.06 (2H, s), 4.04–3.99 (3H, m), 3.92–3.89 (1H, m), 2.22–2.11 (2H, m), 2.01–1.98 (2H, m). ^{13}C-NMR (DMSO-*d6*) δ: 170.63, 155.62, 139.68, 138.46 (q, ^{2}J(C, F) = 68.2 Hz), 134.33, 129.14, 125.62, 123.05, 122.64, 121.99, 120.89, 72.81, 42.75, 38.61, 30.63, 29.82. MS (ESI^-) *m/z*: 407 $(M-H)^-$. HRMS (ESI^-) *m/z*: 407.1410 $(M-H)^-$ (calcd for $C_{19}H_{19}BF_3N_2O_4$: 407.1395).

5.1.16.

[2-(2-oxo-2-{4-[4-(trifluoromethyl)phenoxy]piperidin-1-yl}ethyl)phenyl]boronic acid (14c)

2-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-1-{4-[4-(trifluoromethyl)phenoxy]piperidin-1-yl}ethanone was prepared in a manner similar to that used for **9a**. Yield: 53%. ^{1}H-NMR ($CDCl_3$) δ: 7.84 (1H, dd, *J* = 7.4, 1.6 Hz), 7.54 (2H, d, *J* = 9.0 Hz), 7.40 (1H, td, *J* = 7.5, 1.4 Hz), 7.30–7.27 (2H, m), 6.94 (2H, d, *J* = 8.6 Hz), 4.59–4.56 (1H, m), 4.11 (2H, s), 3.79–3.65 (3H, m), 3.48–3.44 (1H, m), 1.92–1.70 (4H, m), 1.27 (6H, d, *J* = 3.1 Hz), 1.24 (6H, s). MS (ESI^+) *m/z*: 490 $(M+H)^+$. Compound **14c** was prepared in a manner similar to that used for **10a**. Yield: 24%. ^{1}H-NMR (CD_3OD) δ: 7.60 (2H, d, *J* = 8.8 Hz), 7.45–7.44 (1H, m), 7.23–7.14 (5H, m), 4.89–4.87 (1H, m), 4.06 (2H, s), 4.02–3.99 (3H, m), 3.92–3.89 (1H, m), 2.16–2.12 (2H, m), 2.02–1.94 (2H, m). ^{13}C-NMR (DMSO-*d6*) δ: 170.63, 159.92, 139.78, 139.74, 134.34, 129.18, 129.11, 127.05 (q, ^{3}J(C, F) = 3.5 Hz), 125.63, 123.52, 121.18 (q, ^{2}J(C, F) = 32.1 Hz), 116.06, 72.03, 42.86, 38.72, 30.83, 30.02. MS (ESI^-) *m/z*: 406 $(M-H)^-$. HRMS (ESI^-) *m/z*: 406.1455 $(M-H)^-$ (calcd for $C_{20}H_{20}BF_3NO_4$: 406.1443).

5.1.17. 2-(piperidin-4-ylmethoxy)-5-(trifluoromethyl)pyridine hydrochloride (1:1) (16)

To a solution of NaH (0.26 g, 6.1 mmol) in DMSO (16 mL) was added 1-(*tert*-Butoxycarbonyl)-4-piperidinemethanol (1.3 g, 6.1 mmol) and the reaction mixture was stirred at room temperature for 15 min. To the mixture was added a solution of 2-chloro-5-(trifluoromethyl)pyridine **15** (1.0 g, 5.5 mmol) in DMSO (6 mL) at room temperature. After stirring overnight, the reaction mixture was diluted with water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography (hexane-AcOEt) to give *tert*-butyl 4-({[5-(trifluoromethyl)pyridin-2-yl]oxy}methyl)piperidine-1-carboxylate (1.7 g, 88%) as a colorless solid. ^{1}H-NMR ($CDCl_3$) δ: 8.42 (1H, s), 7.77 (1H, dd, *J* = 9.0, 2.3 Hz), 6.81 (1H, d, *J* = 8.6 Hz), 4.21 (2H, d, *J* = 6.7 Hz), 4.18–4.08 (2H, m), 2.76–2.73 (2H, m), 1.98–1.96 (1H, m), 1.80 (2H, d, *J* = 13.3 Hz), 1.47 (9H, s), 1.30–1.26 (2H, m). HRMS (ESI^+) *m/z*: 361.1743 $(M+H)^+$ (calcd for $C_{17}H_{24}F_3N_2O_3$: 361.1734). Compound **16** was prepared in a manner similar to that used for **12b**. Yield: 100%. ^{1}H-NMR (CD_3OD) δ: 8.47 (1H, s), 7.97 (1H, dd, *J* = 8.8, 2.5 Hz), 6.97 (1H, d, *J* = 8.6 Hz), 4.32 (2H, d, *J* = 6.3 Hz), 3.45 (2H, *J* = 12.9 Hz), 3.08–3.01 (2H, m), 2.24–2.17 (1H, m), 2.07 (2H, d, *J* = 8.0 Hz), 1.66–1.55 (2H, m). ^{13}C-NMR (CD_3OD) δ: 167.05, 145.19 (q, ^3J(C, F) = 4.5 Hz), 138.77 (q, ^3J(C, F) = 2.9 Hz), 126.75, 124.03, 121.73 (q, ^2J(C, F) = 35.6 Hz), 112.82, 71.90, 45.00, 34.83, 26.76. MS (ESI^+) *m/z*: 261 $(M+H)^+$. HRMS (ESI^+) *m/z*: 261.1227 $(M+H)^+$ (calcd for $C_{12}H_{16}F_3N_2O$: 261.1209).

5.1.18.

2-(2-bromophenyl)-1-[4-({[5-(trifluoromethyl)pyridin-2-yl]oxy}methyl)piperidin-1-yl]ethanone (17)

Compound **17** was prepared in a manner similar to that used for **11b**. Yield: 96%. ^{1}H-NMR ($CDCl_3$) δ: 8.41 (1H, s), 7.77 (1H, dd, *J* = 8.8, 2.5 Hz), 7.56 (1H, d, *J* = 8.2 Hz), 7.30–7.27 (2H, m), 7.14–7.10 (1H, m), 6.80 (1H, d, *J* = 9.0 Hz), 4.72 (1H, d, *J* = 13.3 Hz), 4.20 (2H, dd, *J* = 6.7, 2.7 Hz), 3.89–3.81 (3H, m), 3.08–3.05 (1H, m), 2.68–2.64 (1H, m), 2.08–2.04 (1H, m), 1.85 (2H, t, *J* = 14.9 Hz), 1.32–1.29 (1H, m), 1.18–1.15 (1H, m). ^{13}C-NMR (CD_3OD) δ: 170.81, 167.67, 146.08 (q, ^{3}J(C, F) = 4.5 Hz), 137.38 (q, ^{3}J(C, F) = 3.2 Hz), 137.02, 133.93, 132.46, 129.96, 128.92, 126.06, 121.27 (q, ^{2}J(C, F) = 32.1 Hz), 112.56, 71.80, 47.25, 43.36, 41.74, 37.12, 30.47, 29.80. HRMS (ESI^+) *m/z*: 457.0750 $(M+H)^+$ (calcd for $C_{20}H_{21}BrF_3N_2O_2$: 457.0733).

5.1.19.

(2-{2-oxo-2-[4-({[5-(trifluoromethyl)pyridin-2-yl]oxy}methyl)piperidin-1-yl]ethyl}phenyl)boronic acid (18)

Step A: 2-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-1-[4-({[5-(trifluoromethyl)pyridin-2-yl]oxy}methyl)piperidin-1-yl]ethanone was prepared in a manner similar to that used for **9a**. Yield: 45%. ^{1}H-NMR ($CDCl_3$) δ: 8.41 (1H, s), 7.83 (1H, d, *J* = 7.3 Hz), 7.77 (1H, dd, *J* = 8.5, 2.2 Hz), 7.38 (1H, t, *J* = 7.6 Hz), 7.28–7.22 (2H, m), 6.79 (1H, d, *J* = 8.8 Hz), 5.00–4.95 (2H, m), 4.71 (1H, d, *J* = 12.7 Hz), 4.19 (2H, d, *J* = 6.3 Hz), 4.08 (2H, s), 4.03 (1H, *J* = 13.7 Hz), 2.97 (1H, t, *J* = 12.2 Hz), 2.62–2.59 (1H, m), 2.05–2.01 (1H, m), 1.84 (1H, d, *J* = 12.7 Hz), 1.75 (1H, d, *J* = 13.2 Hz), 1.32 (12H, s). HRMS

(ESI$^+$) *m/z*: 505.2490 $(M+H)^+$ (calcd for $C_{26}H_{33}BF_3N_2O_4$: 505.2480). Step B: Compound **18** was prepared in a manner similar to that used for **10a**. Yield: 27%. ^{1}H-NMR (CD_3OD) δ: 8.45 (1H, s), 7.93 (1H, dd, *J* = 8.5, 2.7 Hz), 7.45–7.43 (1H, m), 7.22–7.14 (3H, m), 6.94 (1H, d, *J* = 8.8 Hz), 4.79 (1H, d, *J* = 13.2 Hz), 4.38 (1H, d, *J* = 14.2 Hz), 4.31 (2H, d, *J* = 6.3 Hz), 4.04 (2H, s), 3.41–3.38 (1H, m), 3.08–3.05 (1H, m), 2.29–2.26 (1H, m), 2.08–2.05 (2H, m), 1.57–1.42 (2H, m). ^{13}C-NMR (DMSO-*d6*) δ: 170.56, 165.79, 144.97 (q, ^3J(C, F) = 4.8 Hz), 139.86 (q, ^3J(C, F) = 5.4 Hz), 136.55, 134.39, 129.23, 125.66, 123.15, 118.83 (q, ^2J(C, F) = 32.4 Hz), 111.47, 70.30, 59.88, 45.62, 41.36, 35.15, 29.00, 28.11, 20.81, 14.15. MS (ESI$^-$) *m/z*: 421 $(M-H)^-$. HRMS (ESI$^-$) *m/z*: 421.1584 $(M-H)^-$ (calcd for $C_{20}H_{21}BF_3N_2O_4$: 421.1552).

5.1.20.

{2-[1-hydroxy-2-oxo-2-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethyl]phenyl}boronic acid (19)

Compound **19** was prepared in a manner similar to that used for **5**. Yield: 11%. ^{1}H-NMR (CD_3OD) δ: 8.48 (1H, s), 7.97–7.95 (1H, m), 7.51–7.44 (2H, m), 7.39–7.32 (2H, m), 6.98 (1H, t, *J* = 8.4 Hz), 6.31 (1H, d, *J* = 2.3 Hz), 5.52–5.49 (1H, m), 4.08–3.92 (4H, m), 2.23–2.17 (2H, m), 2.04–1.99 (2H, m). ^{13}C-NMR (DMSO-d_6) δ: 171.24, 167.39, 152.78, 144.92, 131.14, 130.77, 127.57, 125.28, 118.83 (q, ^2J(C, F) = 32.1 Hz), 68.26, 42.77, 42.31, 31.45, 31.11, 30.44, 30.06, 26.97. MS (ESI$^+$) *m/z*: 425 $(M+H)^+$. HRMS (ESI$^-$) *m/z*: 423.1302 $(M-H)^-$ (calcd for $C_{19}H_{19}BF_3N_2O_5$: 423.1345).

5.1.21.

2-(2-bromophenyl)-2-methoxy-1-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethanone (21)

Compound **21** was prepared in a manner similar to that used for **8a**. Yield: 100%. ^{1}H-NMR ($CDCl_3$) δ: 8.38 (1H, s), 7.76 (1H, t, *J* = 8.0 Hz), 7.59 (1H, d, *J* = 7.8 Hz), 7.54 (1H, dd, *J* = 7.8, 1.6 Hz), 7.37 (1H, td, *J* = 7.0, 1.7 Hz), 7.22 (1H, td, *J* = 7.6, 1.6 Hz), 6.82–6.70 (1H, m), 5.40 (1H, s), 5.31-5.24 (1H, m), 4.11–4.07 (1H, m), 3.82–3.76 (1H, m), 3.67–3.59 (1H, m), 3.46 (3H, s), 3.39–3.30 (1H, m), 1.97–1.84 (2H, m), 1.76–1.65 (1H, m), 1.52–1.50 (1H, m). ^{13}C-NMR (CD_3OD) δ: 169.90, 166.64, 146.06, 137.39, 134.36, 131.88, 130.62, 129.47, 127.05, 125.62, 124.32, 121.29 (q, ^{2}J(C, F) = 33.1 Hz), 113.12, 81.15, 72.37, 58.46, 43.91, 41.16, 31.66. MS (ESI^+) *m/z*: 473 $(M+H)^+$. HRMS (ESI^+) *m/z*: 473.0698 $(M+H)^+$ (calcd for $C_{20}H_{21}BrF_3N_2O_3$: 473.0682).

5.1.22.

{2-[1-methoxy-2-oxo-2-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethyl]phenyl}boronic acid (22)

Step A: 2-methoxy-2-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-1-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethanone was prepared in a manner similar to that used for **9a**. Yield: 57%. ^{1}H-NMR ($CDCl_3$) δ: 8.37 (1H, s), 7.74 (1H, d, *J* = 8.3 Hz), 7.47–7.43 (2H, m), 7.39–7.30 (2H, m), 6.76–6.74 (1H, m), 5.27–5.25 (1H, m), 5.08 (1H, s), 4.05–3.88 (2H, m), 3.68–3.62 (2H, m), 3.41 (3H, s), 2.02–1.61 (4H, m), 1.27 (4H, s), 1.24 (8H, s). MS (ESI^+) *m/z*: 521 $(M+H)^+$. Step B: Compound **22** was prepared in manner similar to that used for **10a**. Yield: 12%. ^{1}H-NMR (CD_3OD) δ: 8.44 (1H, d, *J* =

16.6 Hz), 7.94–7.90 (1H, m), 7.38–7.32 (3H, m), 7.19 (1H, d, *J* = 7.3 Hz), 6.93–6.89 (1H, m), 5.34–5.33 (1H, m), 5.23 (1H, s), 4.04–4.00 (1H, m), 3.79–3.75 (1H, m), 3.66–3.58 (1H, m), 3.51–3.48 (4H, m), 2.14–2.08 (1H, m), 1.89–1.80 (3H, m). ^{13}C-NMR (DMSO-d_6) δ: 164.84, 144.88, 136.66, 127.06, 125.22, 123.07, 118.89, 118.63, 111.94, 80.96, 80.60, 71.24, 70.53, 57.10, 42.19, 30.63, 30.33, 29.60. MS (ESI$^-$) *m/z*: 437 $(M-H)^-$. HRMS (ESI$^-$) *m/z*: 437.1511 $(M-H)^-$ (calcd for $C_{20}H_{21}BF_3N_2O_5$: 437.1501).

5.1.23.

2-(2-bromo-4-fluorophenyl)-1-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethanone (24a)

Compound **24a** was prepared in a manner similar to that used for **8a**. Yield: 94%. ^{1}H-NMR ($CDCl_3$) δ: 8.41 (1H, s), 7.78 (1H, dd, *J* = 8.8, 2.5 Hz), 7.34–7.28 (2H, m), 7.03 (1H, td, *J* = 8.3, 2.6 Hz), 6.80 (1H, d, *J* = 8.6 Hz), 5.38–5.33 (1H, m), 4.01–3.95 (1H, m), 3.82 (2H, d, *J* = 2.0 Hz), 3.76–3.72 (1H, m), 3.61–3.54 (1H, m), 3.47–3.41 (1H, m), 2.05–1.95 (2H, m), 1.82–1.70 (2H, m). ^{13}C-NMR (CD_3OD) δ: 170.68, 166.77, 163.03 (d, ^1J(C, F) = 247.0 Hz), 146.10 (q, ^3J(C, F) = 4.8 Hz), 137.51 (q, ^3J(C, F) = 3.2 Hz), 133.79 (d, ^3J(C, F) = 8.6 Hz), 133.35, 126.15 (d, ^3J(C, F) = 10.5 Hz), 121.29 (q, ^2J(C, F) = 33.4 Hz), 120.80 (d, ^2J(C, F) = 25.7 Hz), 115.80 (d, ^2J(C, F) = 20.0 Hz), 113.14, 72.28, 44.33, 40.71, 40.59, 32.29, 31.64. MS (ESI$^+$) *m/z*: 461 $(M+H)^+$. HRMS (ESI$^+$) *m/z*: 461.0485 $(M+H)^+$ (calcd for $C_{19}H_{18}BrF_4N_2O_2$: 461.0482).

5.1.24.

2-(2-bromo-4-chlorophenyl)-1-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethanone (24b)

Compound **24b** was prepared in a manner similar to that used for **8a**. Yield: 80%. ^{1}H-NMR ($CDCl_3$) δ: 8.41 (1H, s), 7.78 (1H, dd, *J* = 9.0, 2.3 Hz), 7.59 (1H, d, *J* = 2.0 Hz), 7.29–7.26 (2H, m), 6.80 (1H, d, *J* = 8.6 Hz), 5.37–5.33 (1H, m), 3.98–3.94 (1H, m), 3.82 (2H, d, *J* = 2.0 Hz), 3.73–3.70 (1H, m), 3.61–3.55 (1H, m), 3.45–3.42 (1H, m), 2.01–1.97 (2H, m), 1.82–1.72 (2H, m). ^{13}C-NMR (CD_3OD) δ: 173.07, 166.73, 146.08 (q, ^{3}J(C, F) = 4.5 Hz), 137.49 (q, ^{3}J(C, F) = 3.2 Hz), 136.15, 134.65, 133.74, 133.26, 129.01, 126.57, 125.68 (q, ^{1}J(C, F) = 270.2 Hz), 121.30 (q, ^{2}J(C, F) = 67.7 Hz), 113.12, 72.40, 44.34, 40.93, 40.59, 32.33, 31.62. MS (ESI^+) *m/z*: 477 $(M+H)^+$. HRMS (ESI^+) *m/z*: 477.0231 $(M+H)^+$ (calcd for $C_{19}H_{18}BrClF_3N_2O_2$: 477.0187).

5.1.25.

2-(2-bromo-4-methoxyphenyl)-1-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethanone (24c)

Compound **24c** was prepared in a manner similar to that used for **8a**. Yield: 97%. ^{1}H-NMR ($CDCl_3$) δ: 8.41 (1H, s), 7.77 (1H, dd, *J* = 8.6, 2.7 Hz), 7.23 (1H, d, *J* = 8.6 Hz), 7.13 (1H, d, *J* = 2.3 Hz), 6.85 (1H, dd, *J* = 8.4, 2.5 Hz), 6.80 (1H, d, *J* = 9.0 Hz), 5.37–5.31 (1H, m), 4.00–3.97 (1H, m), 3.80–3.79 (5H, m), 3.73–3.70 (1H, m), 3.59–3.55 (1H, m), 3.43–3.39 (1H, m), 2.03–1.91 (2H, m), 1.81–1.70 (2H, m). ^{13}C-NMR (CD_3OD) δ: 171.40, 166.77, 161.02, 146.11, 137.51, 132.76, 128.65, 126.09, 121.30 (q, ^{3}J(C, F) = 68.7 Hz), 119.22, 114.79, 113.13, 72.30, 56.20, 44.43, 40.75,

40.58, 32.25, 31.63. MS (ESI^+) *m/z*: 473 $(M+H)^+$. HRMS (ESI^+) *m/z*: 473.0693 $(M+H)^+$ (calcd for $C_{20}H_{21}BrF_3N_2O_3$: 473.0682).

5.1.26. {5-fluoro-2-[2-oxo-2-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethyl]phenyl}boronic acid (25a)

Step A: 2-[4-fluoro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-1-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethanone was prepared in a manner similar to that used for **9a**. Yield: 59%. ^{1}H-NMR ($CDCl_3$) δ: 8.40 (1H, d, *J* = 2.3 Hz), 7.77 (1H, dd, *J* = 8.6, 2.3 Hz), 7.51 (1H, dd, *J* = 9.4, 2.7 Hz), 7.24 (1H, dd, *J* = 8.6, 5.1 Hz), 7.07 (1H, td, *J* = 8.4, 3.0 Hz), 6.79 (1H, d, *J* = 8.6 Hz), 5.35–5.29 (1H, m), 4.04 (2H, s), 3.99–3.96 (1H, m), 3.80–3.76 (1H, m), 3.49–3.43 (2H, m), 2.00–1.94 (2H, m), 1.79–1.66 (2H, m), 1.33 (12H, s). MS (ESI^+) *m/z*: 509 $(M+H)^+$. Step B: Compound **25a** was prepared in a manner similar to that used for **10a**. Yield: 49%. ^{1}H-NMR (CD_3OD) δ: 8.48 (1H, s), 7.96 (1H, dd, *J* = 8.6, 2.7 Hz), 7.18 (1H, dd, *J* = 8.2, 5.1 Hz), 7.12 (1H, dd, *J* = 9.0, 3.1 Hz), 6.98 (1H, d, *J* = 8.6 Hz), 6.91 (1H, td, *J* = 8.6, 2.7 Hz), 5.54–5.49 (1H, m), 4.05–4.01 (5H, m), 3.94–3.90 (1H, m), 2.28–2.14 (2H, m), 2.06–2.00 (2H, m). ^{13}C-NMR (CD_3OD) δ: 175.21, 166.48, 164.71, 162.26, 146.03 (q, ^3J(C, F) = 4.5 Hz), 137.53 (q, ^3J(C, F) = 3.2 Hz), 131.02, 129.76 (d, ^3J(C, F) = 7.6 Hz), 126.92, 124.21, 121.40 (q, ^2J(C, F) = 65.8 Hz), 117.69 (d, ^2J(C, F) = 19.1 Hz), 114.84 (d, ^2J(C, F) = 23.8 Hz), 113.09, 70.78, 44.88, 42.24, 31.63, 31.10. HRMS (ESI^-) *m/z*: 425.1302 $(M-H)^-$ (calcd for $C_{19}H_{18}BF_4N_2O_4$: 425.1301).

5.1.27.

{5-chloro-2-[2-oxo-2-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethyl] phenyl}boronic acid (25b)

Step A: 2-[4-chloro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-1-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethanone was prepared in a manner similar to that used for **9a**. Yield: 39%. ^{1}H-NMR ($CDCl_3$) δ: 8.40 (1H, s), 7.79–7.77 (2H, m), 7.35 (1H, dd, *J* = 8.2, 2.3 Hz), 7.21 (1H, d, *J* = 8.2 Hz), 6.79 (1H, d, *J* = 8.6 Hz), 5.35–5.29 (1H, m), 4.04 (2H, s), 4.01–3.95 (1H, m), 3.80–3.75 (1H, m), 3.53–3.39 (2H, m), 2.00–1.94 (2H, m), 1.77–1.70 (2H, m), 1.33 (12H, s). MS (FAB^+) *m/z*: 525 $(M+H)^+$. Step B: Compound **25b** was prepared in a manner similar to that used for **10a**. Yield: 53%. ^{1}H-NMR (CD_3OD) δ: 8.49 (1H, s), 7.96 (1H, dd, *J* = 8.6, 2.7 Hz), 7.40 (1H, d, *J* = 2.0 Hz), 7.19–7.16 (2H, m), 6.98 (1H, d, *J* = 8.6 Hz), 5.53–5.49 (1H, m), 4.08–4.01 (5H, m), 3.93–3.90 (1H, m), 2.27–2.14 (2H, m), 2.09–1.96 (2H, m). ^{13}C-NMR (CD_3OD) δ: 174.92, 166.48, 146.03 (q, ^{3}J(C, F) = 4.8 Hz), 137.53 (q, ^{3}J(C, F) = 3.2 Hz), 134.00, 133.74, 131.59, 129.67, 128.09, 126.93, 124.22, 121.41 (q, ^{2}J(C, F) = 33.1 Hz), 113.05, 70.72, 44.91, 42.27, 31.57, 31.06. HRMS (ESI^-) *m/z*: 441.1018 $(M-H)^-$ (calcd for $C_{19}H_{18}BClF_3N_2O_4$: 441.1006).

5.1.28.

{5-methoxy-2-[2-oxo-2-(4-{[5-(trifluoromethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethyl]phenyl}boronic acid (25c)

Step A: 2-[4-methoxy-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]-1-(4-{[5-(trifluor

omethyl)pyridin-2-yl]oxy}piperidin-1-yl)ethanone was prepared in a manner similar to that used for **9a**. Yield: 61%. ^{1}H-NMR ($CDCl_3$) δ: 8.40 (1H, s), 7.77 (1H, dd, *J* = 8.4, 2.5 Hz), 7.37 (1H, d, *J* = 2.7 Hz), 7.22 (1H, d, *J* = 8.6 Hz), 6.95 (1H, dd, *J* = 8.4, 2.9 Hz), 6.79 (1H, d, *J* = 8.6 Hz), 5.32–5.30 (1H, m), 4.04 (2H, s), 4.01–3.96 (1H, m), 3.83 (3H, s), 3.56–3.44 (3H, m), 1.99–1.90 (3H, m), 1.79–1.74 (1H, m), 1.33 (12H, s). MS (FAB^+) *m/z*: 521 $(M+H)^+$. Step B: Compound **25c** was prepared in a manner similar to that used for **10a**. Yield: 30%. ^{1}H-NMR (CD_3OD) δ: 8.48 (1H, s), 7.95 (1H, dd, *J* = 8.8, 2.4 Hz), 7.08 (1H, d, *J* = 8.3 Hz), 7.01 (1H, d, *J* = 2.9 Hz), 6.97 (1H, d, *J* = 8.3 Hz), 6.77 (1H, dd, *J* = 8.5, 2.7 Hz), 5.52–5.48 (1H, m), 4.07–3.94 (5H, m), 3.90–3.87 (1H, m), 3.78 (3H, s), 2.24–2.13 (2H, m), 2.06–1.95 (2H, m). ^{13}C-NMR (CD_3OD) δ: 175.79, 166.76, 160.00 , 146.29 (q, ^{3}J(C, F) = 4.5 Hz), 137.77 (q, ^{3}J(C, F) = 3.2 Hz), 129.32, 127.70, 127.21, 124.4, 121.66 (q, ^{2}J(C, F) = 66.3 Hz), 116.76, 114.56, 113.33, 71.19, 55.82, 45.06, 42.32, 31.95, 31.40. HRMS (ESI^-) *m/z*: 437.1479 $(M-H)^-$ (calcd for $C_{20}H_{21}BF_3N_2O_5$: 437.1501).

5.2. In vitro assay

5.2.1. HSL enzyme assay

Recombinant human HSL was expressed in a *Spodoptera frugiperda* Sf9 insect cell using the Bac-to-Bac Baculovirus Expression System (Invitrogen. Inc.). The infected Sf9 cells were suspended in 3 pellet volumes with homogenization buffer (25 mM sucrose pH 7.5, 1.0 mM EDTA, 1.0 mM DTT and protease inhibitors) for a total volume of 30 mL. The homogenate was sonicated in an ice bath. Insoluble material was removed by centrifugation at 18000 rpm for 20 min. The obtained 20 mL of clear supernatant was utilized for in vitro assay. Inhibition of HSL is determined by a

colorimetric assay that measures PNPB. A solution of human HSL extract diluted 6-fold (10 µL) and various concentrations of compounds in DMSO (10 µL) were added to a solution of 1.0 mM PNPB in a phosphate buffer (0.1 M NaH_2PO_4 pH 7.25, 0.15 M NaCl, and 1.0 mM dithiothreitol) (180 µL). The enzymatic reactions were carried out at 37°C for 5 min. The solution absorbance was measured spectrophotometrically at λ of 405 nm at 0 and 5 min. Each assay was carried out in triplicate. IC_{50} values were obtained by fitting data to a nonlinear regression equation (GraphPad Software, Inc., La Jolla CA).

5.2.2. Cellular assay

Lipolysis was measured in rat subcutaneous fat tissue cells (White Adipocyte Culture kit, f-8 purchased from Primary Cell Co., Ltd.) differentiated for 7 days. After removal of the culture medium, each well in the plate was washed with 200 µL of pre-warmed rat lipolysis assay buffer (a solution of 137 mM NaCl, 5 mM KCl, 4.2 mM $NaHCO_3$, 1.3 mM $CaCl_2 \cdot 2H_2O$, 0.5 mM KH_2PO_4, 0.5 mM $MgCl_2 \cdot 6H_2O$, 0.5 mM $MgSO_4 \cdot 7H_2O$, 5 mM D-(+)-glucose, 20 mM HEPES, 11.1 µg/mL adenosine and 1% bovine serum albumin (w/v), and the pH was adjusted to 7.0 by the addition of 1 M NaOH aq.). Then, 50 µL of various concentrations of compounds in DMSO was added to each well in the plate. The cells were incubated in the CO_2 incubator at 37°C with 5% CO_2 for 30 min. After incubation, all of the culture supernatants in each well in the plate were collected. Glycerol concentration in the culture supernatants was measured by Free Glycerol Determination Kit (Sigma-Aldrich). The solution absorbance was measured spectrophotometrically at λ of 540 nm using a spectrophotometer (SPECTRAMax PLUS384, Molecular Devices Corporation Japan). Each assay was carried out in

triplicate. IC_{50} values were obtained by fitting data to a nonlinear curve fitting program (GraphPad Software, Inc., La Jolla CA).

5.2.3. In vitro AChE and BuChE assay

A solution of the inhibitor (50 µL) was added to a 100 U/mL solution of AChE or BChE (50 µL) and preincubated for 10 min at room temperature. A phosphate buffer (0.1 M potassium phosphate-HCl, pH 8.0) (100 µL), acetylthiocholin iodide (final 500 µM) or S-butyrylthiocholin iodide (final 1000 µM), and 0.01 M DTNB in aqueous sodium hydrogen carbonate (1 mg/mL, 10 µL) were mixed with the preincubation mixture in microtiter plates and incubated at 37°C for 15 min. The absorption at 405 nm was measured with a spectrophotometer at t = 0 and after incubation for 15 min at 37°C with shaking. Inhibitory activity was measured at 1 µM, 10 µM, and 100 µM respectively.

5.3. In vivo inhibition of lipolysis

Male Wistar rats 8 weeks of age were purchased from Japan SLC, Inc. All animals were held under standard laboratory conditions (12 h light per day, light on at 7:00 AM, 23 ± 2°C, 55 ± 10% humidity) with food and water available ad libitum. Overnight-fasted rats received vehicle (0.5% (w/v) methylcellulose) (n = 4) or HSL inhibitor in the vehicle (0.1 mg/kg, 0.4 mg/kg and 1.3 mg/kg body weight p.o.; n = 3 per group). EDTA (ethylenediaminetetraacetic acid)-treated blood samples were collected just prior to dosing and at 0.5, 1, 3, 5, and 7 h from tail vein. The blood was centrifuged at 12000 rpm for 5 min at 4°C to obtain the plasma. The plasma samples were stored at -20°C before analysis. The plasma glycerol level was determined using Free Glycerol

Determination Kit (Sigma-Aldrich). All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Daiichi Sankyo. The relative value was calculated using the following formula:

Plasma glycerol reduction of the $AUC_{0\text{-}7\ h}$ (%) relative to compound **26** = {[Plasma glycerol $AUC_{0\text{-}7\ h}$ value (vehicle group) - Plasma glycerol $AUC_{0\text{-}7\ h}$ value (test group)] / [Plasma glycerol $AUC_{0\text{-}7\ h}$ value (vehicle group) - Plasma glycerol $AUC_{0\text{-}7\ h}$ value (compound **26** group)]} × 100

5.4. Parallel artificial membrane permeability assay

Parallel artificial membrane permeability assay (PAMPA) was carried out using a Freedom EVO200 system (Tecan, Männedorf, Switzerland). The filter membrane of the acceptor plate (Stirwell PAMPA Sandwich; Pion, Billerica, MA, USA) was coated with GIT−0 lipid solution (Pion). Each compound solution in DMSO (10 mM) was added to Prisma HT buffer (Pion) to obtain 5 μM donor solutions (containing 0.05% DMSO, pH 5.0 and pH 7.4) and placed on a donor plate. The acceptor plate was filled with an acceptor sink buffer (Pion). The donor plate was placed on top of the acceptor plate and incubated for 4 h at 25°C. After incubation, the concentration of each compounds in both plates was measured by an LC−MS/MS system. The permeability coefficient was calculated using PAMPA Evolution DP software (Pion).

5.5. Distribution coefficient

The distribution coefficient between 1-octanol and phosphate buffered saline (PBS) was assayed using a shaking flask method. Equal amounts of PBS and 1-octanol were shaken and left overnight. The upper layer (1-octanol) and lower layer (PBS) were

collected individually. Each test compound was dissolved in 1-octanol or PBS (200 μM). The same amount of either PBS or 1-octanol was added and the mixture was shaken vigorously for 30 min at room temperature followed by centrifugation at 2100 *g* for 5 min at room temperature. Then, both phases were separated and assayed by HPLC and LC-MS. Log $D_{7.4}$ was calculated by the following equation: Log $D_{7.4}$ = log (peak area of compound in 1-octanol/peak area of compound in PBS).

5.6. Solubility

A solution (50 μL) of each sample in DMSO was freeze-dried to remove DMSO. To the residue, 250 μL of Japanese Pharmacopoeia First Fluid, Japanese Pharmacopoeia Second Fluid, or FaSSIF were added, and the each mixture was stirred at 1700 rpm for 4 h. After filtration of the mixture by Whatman Unifilter, the resulting filtrate was diluted with DMSO to obtain the measurement sample solution. Concentration of the filtrate was analyzed by HPLC-UV using Acquity UPLC (Waters) based on the calibration curve of each sample.

5.7. GSH trapping experiment

For the GSH trapping experiments, each test compound (500 μM) was incubated with human liver microsomes (2 mg of protein/mL) supplemented with an NADPH generating system and an equimolar mixture of GSH and stable isotope-labeled GSH ([$^{13}C_2$, ^{15}N-Gly]GSH). After 60-min incubation, the reaction was terminated with acetonitrile containing propranolol, followed by centrifugation and concentration of the supernatant. Incubation without NADP or a substrate was performed to obtain control samples. The analytical samples were subjected to LC-MS analysis in full scan MSE

mode using a Q-Tof Xevo mass spectrometer (Waters). The data were analyzed by MetaboLynx software (Waters).

5.8. Calculation of HOMO-LUMO gap

The conformations of each compound were obtained by using Program Ligprep[40] and then performing conformational search of MacroModel[40] with OPLS3 force field. HOMO and LUMO energies of each compound were calculated by Jaguar[40] in Schrödinger using density-functional theory calculation with B3LYP/6-31++G(d,p) basis set.

5.9. Pharmacokinetic evaluation in rats

Each plasma sample was prepared by the screening method referred to above, followed by mixing with water, acetonitrile, and internal standard (IS) acetonitrile solution (20 μM of niflumic acid). For each standard sample, each standard solution of compounds was added to blank plasma and mixed with water, acetonitrile and IS acetonitrile solution. After vortex mixing for 5 to 15 min at room temperature, the mixtures were added to a Captiva plate (Agilent Technologies) and filtrated. The filtrates were subjected to LC-MS/MS analysis using API 4000QTRAP (Applied Biosystems/MDS SCIEX). C_{max} and $AUC_{0\text{-}7h}$ were determined by a non-compartmental model using the BioBook function of E-WorkBook Suite (ID Business Solutions Ltd.).

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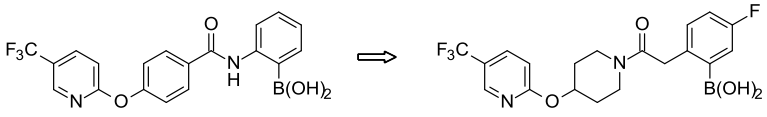
Graphical Abstract



2
HSL enzyme: IC_{50} = 91 nM
Adipocyte: IC_{50} = 470 nM

25a
HSL enzyme: IC_{50} = 68 nM
Adipocyte: IC_{50} = 69 nM
antilipolytic effect in rat: 1 mg/kg