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A Novel Class of Antihyperlipidemic Agents with Low Density Lipoprotein Receptor Up-Regulation *via* the Adaptor Protein Autosomal Recessive Hypercholesterolemia

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We have previously reported compound **2** as a inhibitor of acyl-coenzyme A:cholesterol *O*-acyltransferase (ACAT) and up-regulator of the low density lipoprotein receptor (LDL-R) expression. In this study we focused on compound **2**, a unique LDL-R up-regulator, and describe the discovery of a novel class of up-regulators of LDL-R. Replacement the methylene urea linker in compound **2** with an acylsulfonamide linker kept a potent LDL-R up-regulatory activity, and subsequent optimization work gave compound **39** as a highly potent LDL-R up-regulator (**39**; EC_{25} = 0.047 μM). Compound **39** showed no ACAT inhibitory activity even at 1 μM. The sodium salts of compound **39** reduced plasma total and LDL cholesterol levels in a dose-dependent manner in an experimental animal model of hyperlipidemia. Moreover, we revealed in this study using RNA interference that autosomal recessive hypercholesterolemia (ARH), an adaptor protein of LDL-R, is essential for compound **39** up-regulation of LDL-R expression.

Introduction

Coronary heart disease (CHD[a]) is one of the leading causes of death in industrialized nations, and its burden on health-care resources is continuously increasing worldwide.[1] Epidemiological evidence indicates that hypercholesterolemia is an important risk factor for CHD;[2] consequently, significant efforts have been undertaken to mitigate this condition. Low density lipoprotein (LDL) is a vehicle lipoprotein that transports cholesterol from the liver to peripheral tissues, and can be retained there by arterial proteoglycans triggering the formation of plaques. Increased LDL levels in the blood are known to be associated with a variety of cardiovascular conditions, including atherosclerosis, stroke, and peripheral vascular diseases. Accordingly, evidence has shown that lowering total cholesterol (TC) and LDL cholesterol (LDL-C) levels can reduce the incidence of CHD.[3] It is known that the LDL receptor (LDL-R) plays a central role in LDL incorporation. The existence of this receptor, which is a ubiquitous cell surface glycoprotein of 839 amino acids, was demonstrated in 1974 by Goldstein and Brown.[4] In subsequent research, LDL-R was purified from bovine adrenal glands in 1982,[5] its human cDNA cloned shortly thereafter,[6] and its gene isolated in 1985.[7]

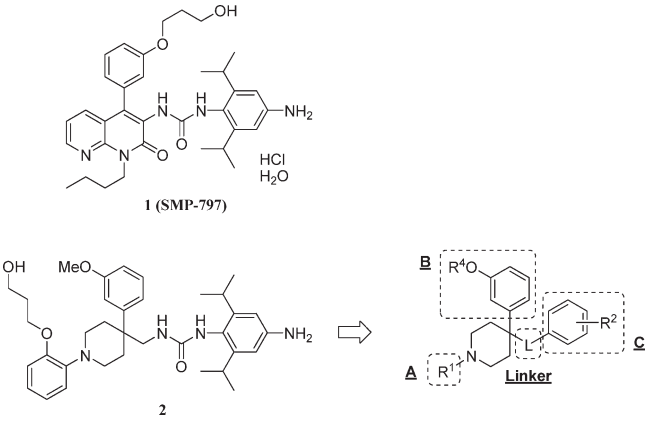

Figure 1

We have previously reported a novel antihyperlipidemic agent **1** (SMP-797)[8] and described 1,4-diarylpiperidine-4-methylurea derivative **2**[9] as its back-up compounds (Figure 1). Compounds **1** and **2** possess dual activity consisting of inhibition of cholesterol *O*-acyltransferase (ACAT) and up-regulation of LDL-R. Although inhibition of ACAT is believed to reduce the absorption of dietary cholesterol in the small intestine,[10,11] thereby lowering serum lipid level,[12] and to prevent the progression of atherosclerotic lesions,[13] numerous clinical trial studies of potent ACAT inhibitors have shown poor efficacy,[14] with no ACAT inhibitor in clinical use so far.[15] Safety concerns have also been reported with some ACAT inhibitors.[16]

On the other hand, numerous clinical trial studies of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase

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[a] Abbreviations: ACAT, acyl-coenzyme A:cholesterol *O*-acyltransferase; CHD, coronary heart disease; TC, total cholesterol; HMG-CoA, 3-hydroxy-3-methylgultryl coenzyme A; BINAP, 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl: TBS, *tert*-butyl dimethylsilyl; SAR, structure−activity relationships; DiI-LDL, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-labeled LDL; C_{max}, maximum drug concentration; AUC, area under the curve; SEM, standard error of the mean; siRNA, short-interfering RNA; HRMS, high-resolution mass spectrometer.

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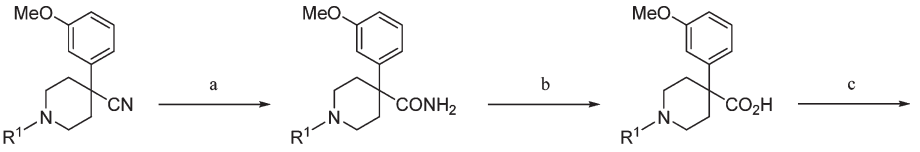
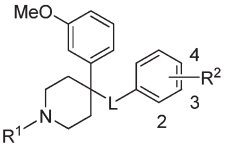
Scheme 1. Synthesis of Compounds **9**—**17**[a]



3 2-OMe-C_6H_4
4 Ph_2CH

5 2-OMe-C_6H_4
6 Ph_2CH

7 2-OMe-C_6H_4
8 Ph_2CH



9	L=CONH	R^1=2-OMe-C_6H_4	R^2=2,6-di^{i}Pr
10	L=CONHSO$_2$NH	R^1=2-OMe-C_6H_4	R^2=2,6-di^{i}Pr
11	L=CONHSO$_3$	R^1=2-OMe-C_6H_4	R^2=H
12	L=CONHSO$_3$	R^1=2-OMe-C_6H_4	R^2=2-Me
13	L=CONHSO$_3$	R^1=2-OMe-C_6H_4	R^2=2-^{n}Pr
14	L=CONHSO$_3$	R^1=2-OMe-C_6H_4	R^2=2,6-^{c}Pentyl
15	L=CONHSO$_3$	R^1=2-OMe-C_6H_4	R^2=2,6-diMe
16	L=CONHSO$_3$	R^1=2-OMe-C_6H_4	R^2=2,6-di^{i}Pr
17	L=CONHSO$_3$	R^1=Ph_2CH	R^2=2,6-di^{i}Pr

[a] Reagents and Conditions: (a) 6 N KOH solution, DMSO; (b) concentrated hydrochloric acid solution; (c) (1) $(COCl)_2$, *N,N*-dimethylformamide, CH_2Cl_2; (2) **19**, NaH, THF or 2,6-diisopropylniline or **26**−**31**, NEt_3, CH_2Cl_2.

inhibitors, known as statins, have shown strong cholesterol-lowering effects; therefore, hypercholesterolemia is presently treated by statins. Statins block the rate-limiting step of cholesterol biosynthesis, up-regulate the LDL-R, and clear LDL particles from the blood.[17] In previous reports, we have shown that up-regulation of LDL-R by **1** or its back-up compounds is independent of the ACAT inhibitory activity of these compounds and that, unlike statins, **1** and its back-up compounds have no effect on cholesterol biosynthesis.

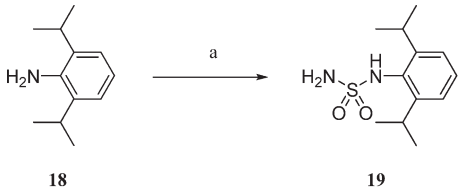
On the basis of this background information, we believe that modification of compound **2** can lead to the discovery of new compounds that possess LDL-R up-regulatory activity without ACAT inhibition. Such compounds are expected to be novel antihyperlipidemic agents without the side effects associated with ACAT inhibition. In this paper, we report the identification and preliminary optimization of a series of piperidine-based compounds as potent LDL-R up-regulators without ACAT inhibition.

Chemistry

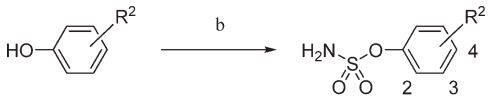
The compounds synthesized in this study and their synthetic methods are shown in Schemes 1−4. The nitriles **3** and **4**[9] were hydrolyzed with aqueous 6 N KOH solution at 130 °C in DMSO to give the carboxamides **5** and **6**, respectively. The carboxamides, thus obtained, were converted to the corresponding carboxylic acids (**7** and **8**) by acidic hydrolysis with concentrated hydrochloric acid solution at 90 °C. Direct hydrolysis of the nitriles to the carboxylic acids in concentrated hydrochloric acid solution did not proceed quickly, leading to generation of byproduct. Conversion of the carboxylic acids **7** and **8** to the corresponding acylchloride using oxalyl chloride, followed by condensation with the appropriate nucleophile, such as 2,6-diisopropylaniline, **19**, or **26**−**31**, afforded the desired amide **9**, acylsulfamide **10**, or acylsulfonamides **11**−**17** (Scheme 1).

Preparation of the sulfamide compound **19** and the sulfamate compounds **26**−**31** used in the above condensation reaction are illustrated in Scheme 2. The 2,6-diisopropylphenylsulfamide **19** was prepared by condensation of 2,6-diisopropylaniline (**18**) with sulfamoyl chloride,[18] which was easily synthesized from chlorosulfonyl isocyanate and formic acid. Phenyl sulfamates **26**−**31** with mono or dialkyl

Scheme 2. Synthesis of Sulfamide Compound **19** and Sulfamate Compounds **26**−**31**[a]



18 **19**



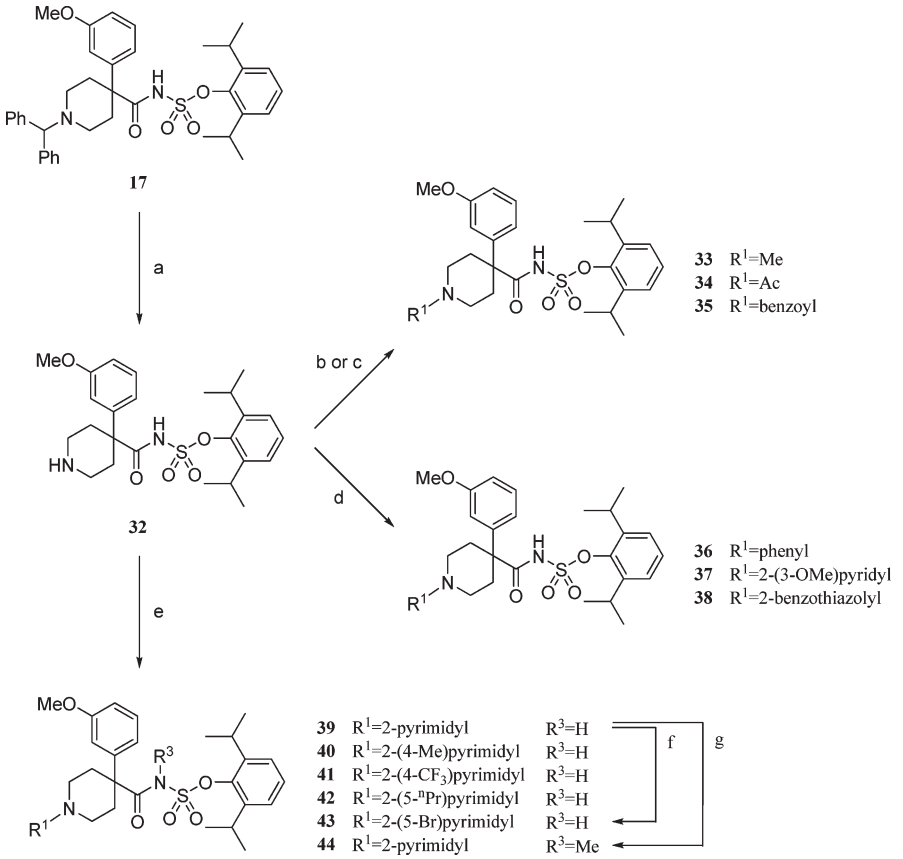
20	R^2=H	**26**	R^2=H
21	R^2=2-Me	**27**	R^2=2-Me
22	R^2=2-^{n}Pr	**28**	R^2=2-^{n}Pr
23	R^2=2-^{c}Pentyl	**29**	R^2=2-^{c}Pentyl
24	R^2=2,6-diMe	**30**	R^2=2,6-diMe
25	R^2=2,6-di^{i}Pr	**31**	R^2=2,6-di^{i}Pr

[a] Reagents and Conditions: (a) NH_2SO_2Cl, *N*-methyl-2-pyrrolidinone; (b) (1) $ClSO_2NCO$, heptane; (2) H_2O.

substituents on the phenyl ring were readily synthesized from the commercially available phenols by a method described in the literature.[19]

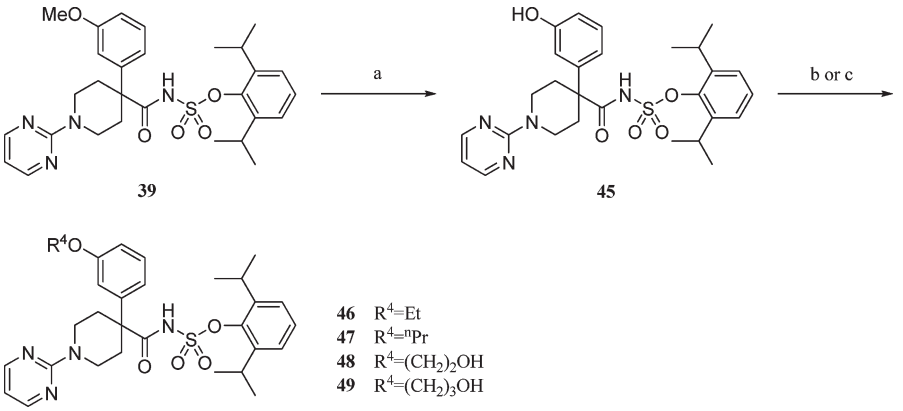
Compounds **32**−**44** containing various substituents on the nitrogen in the piperidine were prepared according to the method described in Scheme 3. Preparation of compound **32** as a key intermediate was carried out by deprotection of compound **17** over 20% palladium hydroxide on carbon under medium hydrogen atmosphere (0.4 MPa). Compound **32** was converted to the *N*-methylpiperidine **33** by reductive amination using formaldehyde and sodium cyanoborohydride. Acylation of **32** with acid chlorides in pyridine gave the *N*-acylated compounds **34** and **35**. Coupling reaction of **32** with aryl bromides, such as bromobenzene, 2-bromo-3-methoxypyridine, or 2-bromobenzothiazole under microwave irradiation in the presence of tris(dibenzylidene acetone)dipalladium(0) and (*S*)-(−)-BINAP gave the corresponding *N*-arylpiperidine compounds **36**−**38**. Nucleophilic addition of **32** to appropriate 2-chloropyrimidines gave **39**−**42** in good yield. Selective bromination of **39** with *N*-bromosuccinimide in THF produced **43**

Scheme 3. Synthesis of Compounds **32**−**44**[a]



[a] Reagents and Conditions: (a) 20% $Pd(OH)_2/C$, H_2, AcOH, *N,N*-dimethylformamide; (b) $NaBH_3CN$, AcOH, 37% HCHO aq, methanol; (c) R^1COCl, 4-(*N,N*-dimethylamino)pyridine, pyridine; (d) R^1Br, sodium *tert*-butoxide, $Pd_2(dba)_3$, (*S*)-(−)-BINAP, toluene; (e) R^1Cl, K_2CO_3, *N,N*-dimethylformamide; (f) *N*-bromosuccinimide, THF; (g) (1) sodium *tert*-butoxide, diethylether; (2) CH_3I, *N,N*-dimethylformamide.

Scheme 4. Synthesis of Compounds **46**−**49**[a]



[a] Reagents and Conditions: (a) BBr_3, CH_2Cl_2; (b) C_2H_5I or C_3H_7I, K_2CO_3, *N,N*-dimethylformamide; (c) (1) $Br(CH_2)_2OTBS$ or $Br(CH_2)_3OTBS$, K_2CO_3, *N,N*-dimethylformamide, KI; (2) tetrabutylammonium fluoride, THF.

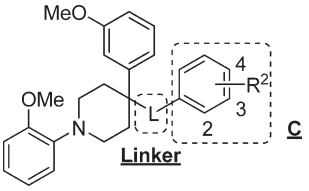
in excellent yield. Treatment of compound **39** with sodium *tert*-butoxide in methanol, followed by *N*-alkylation of the formed sodium salt gave compound **44**. The structure of **44** was determinded to be an *N*-alkyl compound by HMBC experiment and MS/MS analysis (see Supporting Information).

As shown in Scheme 4, demethylation of compound **39** using boron tribromide in CH_2Cl_2 afforded the phenol derivative **45**. Alkylation of compound **45** with commercially available alkyl halides provided various alkoxy derivatives (**46**−**49**). In the case of compound **48** and **49**, the *tert*-butyl dimethylsilyl (TBS) moiety as the protective group was removed by tetrabutylammonium fluoride (TBAF) in THF at the final step. The structure of **46**−**49** was determined to be *O*-alkyl compounds by HMBC experiment (see Supporting Information).

Results and Discussion

As ACAT inhibitors generally contain 2,6-diisopropylphenylurea as a common structure, we hypothesized that modification of this part can be effective in reducing ACAT inhibitory activity. Based on this hypothesis, we changed the

Table 1. Effects of Substitution in the Phenyl Group (C-part) and the Linker Part (L) on Biological Activity



compound	L	R^2	LDL-R up-regulation EC_{25} (μM)[a]	ACAT inhibition IC_{50} (μM)[b]
2			Nt[c]	0.018
9	CONH	2,6-di^{i}Pr	> 10	> 1.0
10	$CONHSO_2NH$	2,6-di^{i}Pr	> 10	> 1.0
11	$CONHSO_3$	H	> 10	> 1.0
12	$CONHSO_3$	2-Me	3.88	> 1.0
13	$CONHSO_3$	2-^{n}Pr	4.07	> 1.0
14	$CONHSO_3$	2-^{c}Pentyl	0.69	> 1.0
15	$CONHSO_3$	2,6-di-Me	> 10	0.41
16	$CONHSO_3$	2,6-di^{i}Pr	0.37	0.33
1			0.49	0.031

[a] Effects of compounds on DiI-LDL uptake in human $HepG_2$ cells. The up-regulatory activity of **1** at 10 μM was assumed as EC_{100}, EC_{25} value means concentration at which LDL-R expression is up-regulated by 25% ($n = 1$). [b] Inhibitory activity for ACAT derived from rat macrophages ($n = 1$). [c] Not tested.

C-part and the linker part (L, Figure 1) of **2** to obtain potent LDL-R up-regulators without ACAT inhibition. The biological activity of compounds **1**, **2**, and **9**−**16** are summarized in Table 1. Replacement of the methylene urea linker in **2** to an amide (**9**) or an acylsulfamide (**10**) gave complete loss of LDL-R up-regulatory activity. On the other hand, this activity remained in the acylsulfonamide **16** with an EC_{25} value comparable to that of **1**. Interestingly, LDL-R up-regulatory activity was enhanced by introduction of sterically hindered substituents at the *ortho*-position on the phenyl moiety in the C-part (compounds **11**−**16**). As expected, ACAT inhibitory activity of compound **16** was about 20-fold weaker than that of compound **2** (**2**; $IC_{50} = 0.018$ μM versus **16**; $IC_{50} = 0.33$ μM). On the basis of these findings, we could confirm that the mechanism of LDL-R up-regulation is independent of ACAT inhibition. As we have previously disclosed the structure−activity relationship (SAR) of A-part (Figure 1) and shown that introduction of 2-methoxyphenyl group in this part provides favorable results,[9] our work in this study was mainly focused on using an alkoxyphenyl or an alkoxypyridyl as substituent. An aromatic group in the A-part is essential for ACAT inhibition. In order to improve the LDL-R up-regulation and to reduce ACAT inhibition of **16**, we examined the SAR of the A-part in **16** using various substituents.

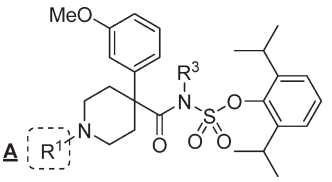
The results are summarized in Table 2. LDL-R up-regulatory activity was diminished in compounds **32**−**35**, but conserved in compound **36**, suggesting that the aryl substituent on the nitrogen in part-A is important. The 2-(3-methoxy)pyridyl **37** showed a loss of LDL-R up-regulatory activity, and this result was similar to the SAR information previously provided for urea derivatives. Surprisingly, the 2-pyrimidyl **39** demonstrated the most potent LDL-R up-regulatory activity with an EC_{25} value about 10-fold greater than that of **16** (**16**; $EC_{25} = 0.37$ μM versus **39**; $EC_{25} = 0.047$ μM). In addition, compound **39** did not inhibit ACAT enzyme even at 1 μM, selectivity (LDL-R up-regulation/ACAT inhibition) of **39** compared to compound **16**. The 2-benzothiazolyl **38** showed an LDL-R up-regulatory activity comparable to that of **16**. These findings suggest that two hetero atoms in the aromatic ring might be essential for LDL-R up-regulation. Next, we introduced several substituents onto the pyrimidine ring. As shown in Table 2, compounds **40**−**43** gave diminished LDL-R up-regulatory activity, a finding that could not be explained by electronic effect or substituent size. Additionally, we examined the acylsulfonamide part of the linker (L, Figure 1) and found that compound **44** with *N*-Me had no LDL-R up-regulatory activity. These findings indicate that the hydrogen atom in the linker is necessary to keep the activity.

In addition to the SAR of the A-part, we investigated in this study the effect of an alkoxy substituent on the phenyl moiety in the B-part. The results are summarized in Table 3. Compound **45** with a phenolic hydroxyl group showed no activity, and replacement of the methoxy group with an ethoxy or propoxy group (**46** or **47**), or a hydroxyalkoxy group (**48** or **49**) was also unfavorable, giving decreased activity due to elongation of the alkyl chain. These findings suggested that a methoxy group could be the most suitable substituent at this position, a result which does not agree with our previous work.[9]

Before examining cholesterol-lowering effect of the selected compounds **16** and **39**, we confirmed the oral bioavalability of these compounds. As compound **16** oral bioavailability in male hamsters was poor (maximum drug concentration (C_{max}) = 0.013 μg/mL at 10 mg/kg), hence we prepared the hydrochloride and sodium salt of this compound. As shown in Figure 2, both salts showed improved oral bioavailability compared to the free base, in particular the sodium salt was better than the hydrochloride as indicated by C_{max} and area under the curve $(AUC)_{0-360}$ at 10 mg/kg (hydrochloride; $C_{max} = 0.30$ μg/mL, $AUC_{0-360} = 0.69$ μg·h/mL versus sodium salt; $C_{max} = 0.42$ μg/mL, $AUC_{0-360} = 1.16$ μg·h/mL). We therefore used the sodium salts of **16** and **39** for *in vivo* evaluation. Comparing to compound **16**, compound **39** has approximately 8-fold potent activity in terms of *in vitro* LDL-R up-regulation, and **39** showed 3−10-fold potent lipid lowering effect. Additionally, pharmacokinetics properties of compounds **16** and **39** were similar, thus these results will be helpful for a better understanding of the relationship of a unique LDL-R up-regulatory activity (*in vitro*) and cholesterol-lowering effect in plasma (*in vivo*) (sodium salts of **39**; $C_{max} = 0.29$ μg/mL, $AUC_{0-360} = 1.09$ μg·h/mL).

For further *in vivo* characterization, the cholesterol-lowering effect of the sodium salts of **16** and **39** was examined in male hamster fed a cholesterol-rich diet. The test-compounds were orally given at 1, 3, 10, or 30 mg/kg once a day for 7 consecutive

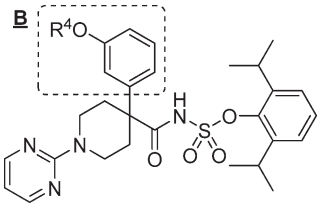
Table 2. Effects of Substitution in the Nitrogen of the Piperidine (A-part) on Biological Activity



compound	R^1	R^3	LDL-R up-regulation EC_{25}, (μM)[a]	ACAT inhibition IC_{50}, (μM)[b]	solubility at pH = 7.4 (mg/mL)[c]
2			Nt[e]	0.018	0.022
32	H	H	> 10	> 1.0	Nt[e]
33	Me	H	> 10	> 1.0	Nt[e]
34	Ac	H	> 10	> 1.0	Nt[e]
35	benzoyl	H	> 10	> 1.0	Nt[e]
36	phenyl	H	1.52	> 1.0	0.18
16	2-OMe-C_6H_4	H	0.37	0.33	0.010
37	2-(3-OMe)pyridyl	H	> 10	> 1.0	Nt[e]
38	2-benzothiazolyl	H	0.26	> 1.0	0.003
39	2-pyrimidyl	H	0.047	> 1.0	0.23[d]
40	2-(4-Me)pyrimidyl	H	0.50	> 1.0	0.20
41	2-(4-CF_3)pyrimidyl	H	> 10	> 1.0	Nt[e]
42	2-(5-^{n}Pr)pyrimidyl	H	> 10	> 1.0	Nt[e]
43	2-(5-Br)pyrimidyl	H	0.61	> 1.0	< 0.0001
44	2-pyrimidyl	Me	> 10	> 1.0	< 0.0003
1			0.49	0.031	0.010

[a] Effects of compounds on DiI-LDL uptake in human $HepG_2$ cells (n = 1). [b] Inhibitory activity for ACAT in rat macrophages (n = 1). [c] Solubility in phosphate buffer solution at room temperature was determined by HPLC analysis. [d] Sodium salt. [e] Not tested.

Table 3. Effects of Substitution in the 4-Phenyl Moiety (B-part) on Biological Activity



compound	R^4	LDL-R up-regulation EC_{25}, (μM)[a]	solubility at pH = 7.4 (mg/mL)[b]
45	H	> 10	0.20
39	Me	0.047	0.23[c]
46	Et	3.1	0.069
47	^{n}Pr	> 10	< 0.0003
48	$(CH_2)_2OH$	1.5	> 0.20
49	$(CH_2)_3OH$	> 10	> 0.20
1		0.49	0.010

[a] Effects of compounds on DiI-LDL uptake in human $HepG_2$ cells (n = 1). [b] Solubility in phosphate buffer solution at room temperature was determined by HPLC analysis. [c] Sodium salts.

days based on cholesterol plasma levels reached in a preliminary *in vivo* pharmacokinetics study. Both compounds reduced plasma TC and LDL-C levels in a dose-dependent manner (Figure 3). Particularly, the lipids-lowering effect of compound **39** was approximately 10-fold more potent than that of compound **16**. *In vitro* experiments also supported this result. Statistically significant TC and LDL-C lowering effects were seen at a dose over 3 mg/kg/day for **39**, and pharmacokinetics data indicated that **39** plasma concentration could keep over EC_{25} for several hours following administration of this compound at by 10 mg/kg. These findings suggest that the EC_{25} value is sufficient potency for lipids-lowering efficacy. Interestingly, cholesterol-lowering effect of **39** was almost the same as that of **1**, which possesses both ACAT inhibitory activity and LDL-R up-regulatory activity. These findings were encouraging as they supported our hypothesis; i.e. compounds that can up-regulate LDL-R without inhibiting ACAT may be effective in lowering TC and LDL-C *in vivo*.

Finally, we confirmed that compound **39** even at a concentration of 1 μM had no effect on cholesterol synthesis. This concentration is much higher than that needed for LDL-R up-regulation in $HepG_2$ cells, where up-regulation of LDL-R expression by acylsulfonamide compounds is not due to transcriptional modulation of LDL-R gene. Hence, this mechanism of action is completely different from that of HMG-CoA reductase inhibitors, which up-regulate LDL-R by indirect action, resulting from cholesterol starvation. Recently, it has been reported that LDL-R expression and activity are regulated not only by the transcriptional modulation of LDL-R gene but also by other mechanisms, such as stability of LDL-R mRNA,[20] LDL-R adaptor protein,[21] and degradation of LDL-R protein.[22,23] Therefore, we speculate that acylsulfonamide compounds may regulate LDL-R expression by a mechanism other than transcriptional regulation of LDL-R gene.

Identification of the molecular defect responsible for the recessive form of hypercholesterolemia that clinically resembles familial hypercholesterolemia has provided new insights into LDL-R physiology. Autosomal recessive hypercholesterolemia (ARH) is a rare Mendelian dyslipidemia characterized by markedly elevated plasma LDL levels. ARH patients show increased cell-surface LDL binding, and impaired LDL degradation, consistent with a defect in LDL-R internalization. Recently, the disorder was shown to be caused by mutation in a phosphotyrosine binding domain protein, ARH, which is required for internalization of LDL in the liver.[24] M. Arca et al. reported that transient loss of the adaptor protein, ARH, by short-interfering RNA (siRNA) results in failure of LDL endocytosis and almost complete absence of LDL/LDL-R

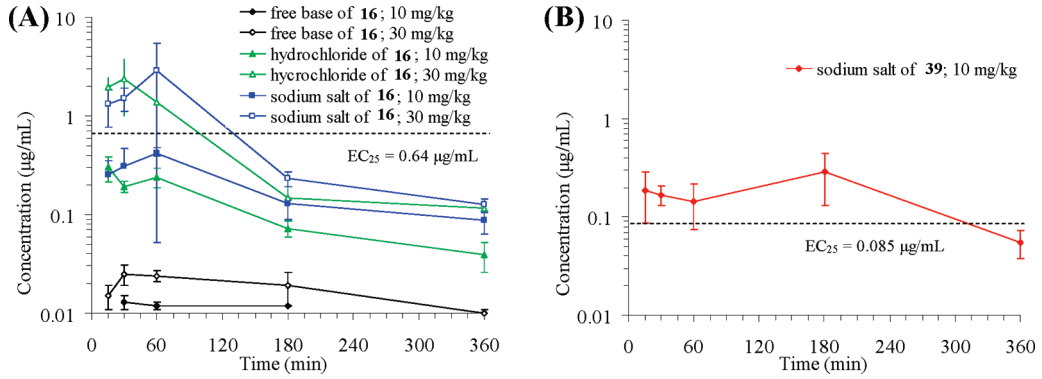


Figure 2. (A) Serum concentration of free base, hydrochloride, and sodium salt of compound **16** after oral administration (10 or 30 mg/kg) to male hamsters. (B) Serum concentration of sodium salt of compound **39** after oral administration (10 mg/kg) to male hamsters. Each point with vertical bar represents the mean ± standard error of the mean (SEM) (n = 3).

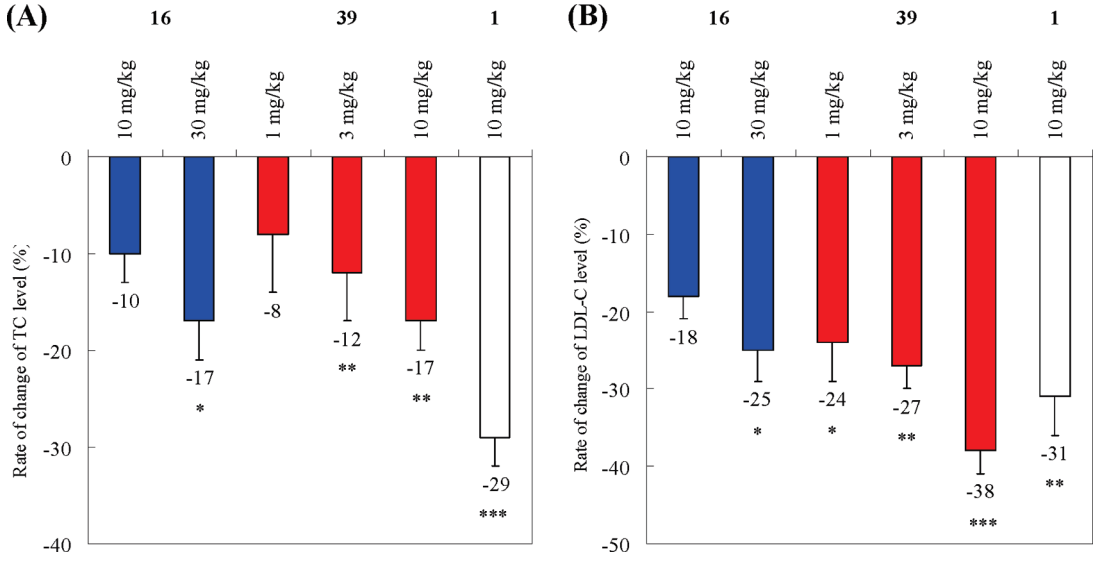


Figure 3. (A) Effects of compound **16** (blue column), **39** (red column), and **1** (white column) on plasma TC levels in hamster fed a rich diet for 1 week. (B) Effects of compounds **16** (blue column), **39** (red column), and **1** (white column) on plasma LDL-C levels in hamster fed a rich diet for 1 week. All compounds were orally administered to hamster by gavage once a day for 1 week. Each column with vertical bar represents the mean ± SEM (compound **16** and **39**; n = 5, **1**; n = 12). Significantly different from control using unpaired two-tailed t-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

complex in the internal compartment of hepatocytes.[25] Additionally, immunofluorescence analysis revealed that most LDL resides on the cell surface in hepatocytes lacking ARH. These findings suggest that ARH is required to promote internalization of LDL/LDL-R complex into hepatocytes. In order to clarify the mechanism of LDL-R up-regulation by compound **39**, we evaluated the activity of **39** on ARH protein expression in $HepG_2$ cells. The expression of ARH was suppressed by addition of ARH-specific siRNA. Under these conditions, compound **39** did not up-regulate LDL-R even at a concentration of 1 μM, while under the same condition, Atrovastatin, an HMG-CoA reductase inhibitor, up-regulated LDL-R. ARH could therefore be the target molecule of compound **39** for LDL-R up-regulation, indicating that the mechanism by which compound **39** acts on LDL-R expression is different from that of HMG-CoA reductase inhibitors.

Compound **39** not only up-regulated LDL-R expression in $HepG_2$ cells, but also increased degradation of [I^{125}]-labeled LDL cholesterol in a concentration-dependent manner (119% @ 0.01 μM, 136% @ 0.1 μM, 140% @ 1 μM). Accordingly, we speculate that compound **39** up-regulatory activity for LDL-R, which is via ARH protein, is due to acceleration of endocytosis of LDL/LDL-R complex or recycling of LDL-R by quick degradation of the LDL/LDL-R complex taken into cells. As far as we know, no report has investigated such a unique mechanism of antihyperlipidemic drugs. To reduce plasma LDL, combination therapy with compound **39** and an HMG-CoA reductase inhibitor may be useful, because the mechanism of action for LDL-R up-regulation is different between these two compounds.

Conclusion

Based on our previous work we tried in this study to find compounds that possess LDL-R up-regulating activity without ACAT inhibition. We started this approach by modification of 1,4-diarylpiperidine-4-methylurea **2**. Replacement of the methyleneurea linker (**2**) with the acylsulfonamide (**16**) was effective in keeping the up-regulatory activity for LDL-R expression and reducing ACAT inhibitory activity. Particularly, conversion of the 2-methoxyphenyl group (**16**), which is essential for ACAT inhibition, into a 2-pyrimidyl group (**39**) enhanced LDL-R up-regulatory activity and abolished ACAT inhibitory activity. Additionally, the sodium salt of

the selected compounds **16** and **39** showed good oral pharmacokinetics properties in hamsters, and reduced plasma TC and LDL-C levels in a dose-dependent manner in an experimental animal model of hyperlipidemia. These results indicate that LDL-R up-regulation, but not ACAT inhibition, is important for plasma lipids reduction. Finally, we clarified the mechanism of action of **39** toward LDL-R up-regulation using ARH-specific RNA interference, and revealed that ARH, an adaptor protein of LDL-R, is a potential target for LDL-R up-regulation. The results of this study indicate that compound **39** with its unique mechanism of action might replace **1** or **2** as a novel antihyperlipidemic agent.

Experimental Section

Melting points were determined on an electrothermal apparatus without correction. IR spectra were recorded on a JEOL JIR-SPX60 spectrometer as ATR. NMR spectra were recorded on a JEOL JNM-LA300 spectrometer or on a Varian Mercury-vx spectrometer. Chemical shifts (σ) are given in parts per million, and tetramethylsilane was used as internal standard for spectra obtained in DMSO-d_6 and $CDCl_3$. All J values are given in Hz. Mass spectra were recorded on a Bruker Daltonics esquire 3000plus and high-resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific LTQ orbitrap Discovery MS equipment. Elemental analysis was performed on a CE Instrument EA1110 and a Yokokawa analytical system IC7000. Reagents and solvents were used as obtained from commercial suppliers without further purification. Column chromatography was carried out using a Yamazen W-prep system and performed using prepacked silica-gel or amino silica-gel columns. Reaction progress was determined by TLC analysis on a silica-gel or an amino silica-gel coated glass plate. Visualization was done with UV light (254 nm) or iodine. All reactions were carried out under a nitrogen atmosphere unless otherwise mentioned. HPLC was performed using a Shimadzu Corporation system. The sample was dissolved in methanol/0.1%-TFA solution, applied on a Myghtysil RP-18 GP column (4.6 mm × 150 mm, 5 μM), and eluted at 1 mL/min with a 30 min gradient (from 60% B to 90% B), where solvent A is water (0.1% TFA solution) and solvent B is methanol (Method A) or acetonitrile (Method B). The purity of test compounds was determined by HPLC and was ≥95%.

1-(2-Methoxyphenyl)-4-(3-methoxyphenyl)piperidine-4-carboxamide (5). To a solution of **3** (3.22 g, 0.01 mol) in DMSO (15 mL) was added 6 N KOH solution (15 mL), and the mixture was stirred at 120 °C for 6 h. The reaction was quenched by adding water (60 mL) at room temperature, and the mixture was neutralized with hydrochloric acid solution. The resulting solid was filtered, and the solid was washed with diethylether to give **5** (3.03 g, 89%) as a white solid. Mp 139−141 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.91 (2H, m), 2.53 (2H, m), 2.74 (2H, m), 3.17 (2H, m), 3.74 (3H, s), 3.76 (3H, s), 6.79−6.90 (5H, m), 6.95 (3H, m), 7.17 (1H, s), 7.25 (1H, dd, J = 8.0, 8.0 Hz); IR (ATR) 3426, 3197, 1664 cm^{-1}; HRMS (ESI) m/z Calcd for $C_{20}H_{25}N_2O_3$ 341.1860; Found 341.1854 (Δ = −1.57 ppm).

1-(Diphenylmethyl)-4-(3-methoxyphenyl)piperidine-4-carboxamide (6). Compound **6** was prepared from **4** in a manner similar to that described for compound **5** with a yield of 100% as a white solid. Mp 78−80 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.77 (2H, m), 2.03 (2H, m), 2.35 (2H, m), 2.52 (2H, m), 3.68 (3H, s), 4.17 (1H, s), 6.76 (1H, m), 6.83−6.90 (3H, m), 7.00 (1H, s), 7.12 (2H, m), 7.17−7.25 (5H, m), 7.34 (4H, m); IR (ATR) 3475, 3213, 1680 cm^{-1}; HRMS (ESI) m/z Calcd for $C_{26}H_{29}N_2O_2$ 401.2224; Found 401.2213 (Δ = −2.54 ppm).

1-(2-Methoxyphenyl)-4-(3-methoxyphenyl)piperidine-4-carboxylic acid (7). To compound **5** (1.70 g, 5.0 mmol) was added concentrated hydrochloric acid solution (17 mL), and the mixture was stirred at 100 °C for 2 days. The mixture was concentrated, and then a dilution was made of the residue with toluene. The mixture was extracted with 1 N sodium hydroxide solution (2×), and then the aqueous layer was neutralized with hydrochloric acid solution. The mixture was extracted with ethyl acetate (2×), washed by brine, and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica-gel column chromatography to give **7** (1.31 g, 77%) as a white solid. Mp 156−158 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.93 (2H, m), 2.49 (2H, m), 2.69 (2H, m), 3.32 (2H, m), 3.75 (3H, s), 3.76 (3H, s), 6.81−6.94 (6H, m), 7.00 (1H, d, J = 7.9 Hz), 7.28 (1H, dd, J = 8.1, 8.1 Hz), 12.57 (1H, s); IR (ATR) 1707, 1599, 1589, 1497 cm^{-1}; HRMS (ESI) m/z Calcd for $C_{20}H_{24}NO_4$ 342.1700; Found 342.1694 (Δ = −1.75 ppm).

1-(Diphenylmethyl)-4-(3-methoxyphenyl)piperidine-4-carboxylic acid (8). Compound **8** was prepared from **6** in a manner similar to that described for compound **7** with a yield of 67% as a white solid. Mp 215−217 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.86 (2H, m), 2.05 (2H, m), 2.35 (2H, m), 2.64 (2H, m), 3.73 (3H, s), 4.27 (1H, s), 6.83 (1H, dd, J = 8.2, 2.5 Hz), 6.88 (1H, d, J = 2.5 Hz), 6.97 (1H, d, J = 8.2 Hz), 7.17 (2H, m), 7.26 (5H, m), 7.40 (4H, m), 12.44 (1H, s); IR (ATR) 1701, 1581, 1489 cm^{-1}; HRMS (ESI) m/z Calcd for $C_{26}H_{28}NO_3$ 402.2064; Found 402.2056 (Δ = −1.86 ppm).

***N*-(2,6-Diisopropylphenyl)-1-(2-methoxyphenyl)-4-(3-methoxyphenyl)piperidine-4-carboxamide (9).** To a solution of compound **7** (171 mg, 0.50 mmol) in CH_2Cl_2 (3.0 mL) was added *N*,*N*-dimethylformamide (5.0 μL) and oxaryl chloride (89.9 μL, 1.00 mmol) at 0 °C, and then the mixture was stirred at room temperature for 3 h. The solvent was removed in vacuo, and then the residue was azeotropied with toluene (2x). To a solution of the acid chloride thus obtained in CH_2Cl_2 (3.0 mL) was added 2,6-diisopropylaniline (104 μL, 0.55 mmol) and triethylamine (279 μL, 2.00 mmol) at room temperature, and then the mixture was stirred for 6 h. The reaction was quenched by adding water, and then the mixture was extracted with chloroform. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography. The solvent was removed in vacuo, and the residue obtained was triturated with diethylether/methanol to give **9** (181 mg, 72%) as a white solid. Mp 144−146 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.91 (12H, m), 2.08 (2H, m), 2.68 (2H, m), 2.87 (2H, m), 2.91 (2H, m), 3.26 (2H, m), 3.78 (3H, s), 3.79 (3H, s), 6.89 (3H, m), 6.94 (2H, m), 7.07 (2H, m), 7.13−7.21 (3H, m), 7.31 (1H, dd, J = 8.1, 8.1 Hz), 8.87 (1H, s); IR (ATR) 3365, 1643, 1599, 1497 cm^{-1}; MS (ESI) m/z 501 (M + 1); Anal. Calcd for $C_{32}H_{40}N_2O_3$: C, 76.77; H, 8.05; N, 5.60. Found: C, 76.81; H, 8.10; N, 5.58; LC: 97.6% pure, t_R = 9.01 min (Method A).

***N*-{[(2,6-Diisopropylphenyl)amino]sulfonyl}-1-(2-methoxyphenyl)-4-(3-methoxyphenyl)piperidine-4-carboxamide (10).** To a solution of compound **7** (171 mg, 0.50 mmol) in CH_2Cl_2 (3.0 mL) was added *N*,*N*-dimethylformamide (5.0 μL) and oxaryl chloride (89.9 μL, 1.00 mmol) at 0 °C, and then the mixture was stirred at room temperature for 3 h. The solvent was removed in vacuo, and then the residue was azeotropied with toluene (2x). To a solution of *N*-(2,6-diisopropylphenyl)sulfamide **19** (385 mg, 1.50 mmol) in THF (3.0 mL) was added 55% sodium hydride with mineral oil (65.6 mg, 1.50 mmol) at 0 °C, and then the mixture was stirred. After 15 min, to the mixture was added THF (4.0 mL) solution of the acid chloride obtained above at 0 °C, and then the mixture was stirred for 16 h at room temperature. The reaction was quenched by adding water, and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography. The solvent was removed in vacuo, and the residue obtained was triturated with diethylether/hexane to give **10** (181 mg, 62%) as a white solid. Mp 164−166 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.98 (12H, d, J = 6.8 Hz), 2.09 (2H, m), 2.62 (2H, m), 2.86 (2H, m), 3.22 (4H, m), 3.77 (3H, s), 3.78 (3H, s), 6.84−6.92 (5H, m), 7.06 (4H, m), 7.20 (1H, m), 7.32 (1H, dd, J = 7.8, 7.8 Hz), 9.28 (1H, s), 11.18 (1H, s); IR (ATR) 3230, 1684,

1597, 1495, 1338, 1242 cm^{-1}; MS (ESI) m/z 580 (M + 1); Anal. Calcd for $C_{32}H_{41}N_3O_5S$: C, 66.29; H, 7.13; N, 7.25. Found: C, 66.46; H, 7.17; N, 7.26; LC: 98.1% pure, t_R = 10.73 min (Method A).

Phenyl {[1-(2-Methoxyphenyl)-4-(3-methoxyphenyl)piperidin-4-yl]carbonyl}sulfamate (11). Compound **11** was prepared from **7** in a manner similar to that described for compound **9** with a yield of 37% as a white solid (methanol). Mp 185−187 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 2.12 (2H, m), 2.65 (2H, m), 3.18 (2H, m), 3.36 (2H, m), 3.75 (3H, s), 3.85 (3H, s), 6.88 (1H, m), 6.96−7.31 (12H, m); IR (ATR) 1570, 1490, 1327, 1255 cm^{-1}; MS (ESI) m/z 497 (M + 1); Anal. Calcd for $C_{26}H_{28}N_2O_6S$: C, 62.89; H, 5.68; N, 5.64. Found: C, 62.81; H, 5.68; N, 5.61; LC: 99.5% pure, t_R = 3.50 min (condition A).

2-Methylphenyl {[1-(2-Methoxyphenyl)-4-(3-methoxyphenyl)-piperidin-4-yl]carbonyl}sulfamate (12). Compound **12** was prepared from **7** in a manner similar to that described for compound **9** with a yield of 24% as a white solid (methanol). Mp 191−193 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 2.10 (2H, m), 2.23 (3H, s), 2.64 (2H, m), 3.10 (2H, m), 3.34 (2H, m), 3.76 (3H, s), 3.84 (3H, s), 6.89 (1H, m), 6.97−7.31 (11H, m); IR (ATR) 1574, 1491, 1321, 1255 cm^{-1}; MS (ESI) m/z 511 (M + 1); Anal. Calcd for $C_{27}H_{30}N_2O_6S$: C, 63.51; H, 5.92; N, 5.49. Found: C, 63.68; H, 6.00; N, 5.46; LC: 99.8% pure, t_R = 4.77 min (Method A).

2-Propylphenyl {[1-(2-Methoxyphenyl)-4-(3-methoxyphenyl)-piperidin-4-yl]carbonyl}sulfamate (13). Compound **13** was prepared from **7** in a manner similar to that described for compound **9** with a yield of 44% as a white solid (methanol). Mp 187−188 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 0.85 (3H, t, J = 7.3 Hz), 1.52 (2H, m), 2.10 (2H, m), 2.59 (2H, m), 2.64 (2H, m), 3.10 (2H, m), 3.38 (2H, m), 3.76 (3H, s), 3.84 (3H, s), 6.89 (1H, m), 6.97−7.20 (10H, m), 7.29 (1H, dd, J = 8.1, 8.1 Hz); IR (ATR) 1570, 1485, 1302, 1255 cm^{-1}; MS (ESI) m/z 539 (M + 1); Anal. Calcd for $C_{29}H_{34}N_2O_6S$: C, 64.66; H, 6.36; N, 5.20. Found: C, 64.42; H, 6.42; N, 5.15; LC: 99.9% pure, t_R = 9.68 min (Method A).

2-Cyclopentylphenyl {[1-(2-Methoxyphenyl)-4-(3-methoxyphenyl)-piperidin-4-yl]carbonyl}sulfamate (14). Compound **14** was prepared from **7** in a manner similar to that described for compound **9** with a yield of 36% as a white solid (methanol). Mp 192−194 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 1.48 (2H, m), 1.60 (2H, m), 1.73 (2H, m), 1.95 (2H, m), 2.10 (2H, m), 2.49 (1H, m), 2.64 (2H, m), 3.10 (2H, m), 3.40 (2H, m), 3.76 (3H, s), 3.84 (3H, s), 6.89 (1H, m), 6.98−7.32 (11H, m); IR (ATR) 1568, 1331, 1255 cm^{-1}; MS (ESI) m/z 565 (M + 1); Anal. Calcd for $C_{31}H_{36}N_2O_6S$: C, 65.94; H, 6.43; N, 4.96. Found: C, 65.84; H, 6.44; N, 4.99; LC: 99.5% pure, t_R = 12.57 min (Method A).

2,6-Dimethylphenyl {[1-(2-Methoxyphenyl)-4-(3-methoxyphenyl)-piperidin-4-yl]carbonyl}sulfamate (15). Compound **15** was prepared from **7** in a manner similar to that described for compound **9** with a yield of 32% as a white solid (methanol). Mp 188−189 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 2.13 (2H, m), 2.18 (6H, s), 2.64 (2H, m), 3.36 (4H, m), 3.77 (3H, s), 3.85 (3H, s), 6.87 (1H, m), 6.99−7.09 (9H, m), 7.28 (1H, dd, J = 8.1, 8.1 Hz); IR (ATR) 1564, 1481, 1321, 1255 cm^{-1}; MS (ESI) m/z 525 (M + 1); Anal. Calcd for $C_{28}H_{32}N_2O_6S$: C, 64.10; H, 6.15; N, 5.34. Found: C, 63.93; H, 6.16; N, 5.35; LC: 99.3% pure, t_R = 6.74 min (Method A).

2,6-Diisopropylphenyl {[1-(2-Methoxyphenyl)-4-(3-methoxyphenyl)piperidin-4-yl]carbonyl}sulfamate (16). Compound **16** was prepared from **7** in a manner similar to that described for compound **9** with a yield of 45% as a white solid (methanol). Mp 181−183 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 1.05 (12H, d, J = 6.8 Hz), 2.17 (2H, m), 2.69 (2H, m), 3.48 (4H, m), 3.77 (3H, s), 3.86 (3H, s), 6.88 (1H, m), 6.97−7.19 (9H, m), 7.30 (1H, m); IR (ATR) 1566, 1464, 1317, 1255 cm^{-1}; MS (ESI) m/z 581 (M + 1); Anal. Calcd for $C_{32}H_{40}N_2O_6S \cdot 0.25H_2O$: C, 65.67; H, 6.98; N, 4.79. Found: C, 65.62; H, 6.98; N, 4.89; LC: 97.4% pure, t_R = 14.63 min (Method A).

Hydrochloride of Compound 16. To a suspension of **16** (116 mg, 0.20 mmol) in methanol (2 mL) was added 1 N hydrochloride diethylether solution (220 μL, 0.22 mmol) at room temperature, and the mixture was stirred for 1 h. The solvent was removed in vacuo, and the residue was triturated with diethylether. The resulting solid was filtered, and washed by diethylether to give hydrochloride (115 mg, 93%) as a white solid. Mp 165−167 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 1.05 (12H, d, J = 6.8 Hz), 2.48 (2H, m), 2.75 (2H, m), 3.40 (2H, m), 3.55 (2H, m), 3.65 (2H, m), 3.78 (3H, s), 3.86 (3H, s), 6.92 (1H, m), 7.04 (3H, m), 7.12−7.19 (4H, m), 7.20 (2H, m) 7.66 (1H, m); IR (ATR) 2972, 1608, 1492, 1454, 1382, 1292 cm^{-1}; HRMS (ESI) m/z Calcd for $C_{32}H_{39}N_2O_6S$ 579.2534; Found 579.2534 (Δ = 0.02 ppm); Anal. Calcd for $C_{32}H_{41}ClN_2O_6S \cdot 1.25H_2O$: C, 60.08; H, 6.85; N, 4.38. Found: C, 59.93; H, 6.75; N, 4.49; LC: 96.6% pure, t_R = 14.72 min (Method A).

Sodium Salt of Compound 16. To a suspension of **16** (116 mg, 0.20 mmol) in methanol (3 mL) was added sodium *tert*-butoxide (18.8 mg, 0.196 mmol) at room temperature, and the mixture was stirred for 30 min. The solvent was removed in vacuo, and the residue was triturated with diethylether. The resulting solid was filtered, and washed by diethylether to give sodium salt (115 mg, 96%) as a white solid. Mp 256−258 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.01 (12H, d, J = 6.8 Hz), 1.78 (2H, m), 2.52 (2H, m), 2.81 (2H, m), 3.17 (2H, m), 3.61 (2H, m), 3.72 (3H, s), 3.76 (3H, s), 6.74 (1H, m), 6.82−6.89 (4H, m), 6.99 (5H, m), 7.18 (1H, dd, J = 8.1, 8.1 Hz); IR (ATR) 1597, 1498, 1439, 1290 cm^{-1}; HRMS (ESI) m/z Calcd for $C_{32}H_{39}N_2O_6S$ 579.2534; Found 579.2536 (Δ = 0.25 ppm); Anal. Calcd for $C_{32}H_{39}N_2NaO_6S \cdot 2H_2O$: C, 60.17; H, 6.79; N, 4.39. Found: C, 59.75; H, 6.23; N, 4.44; LC: 96.7% pure, t_R = 14.94 min (Method A).

2,6-Diisopropylphenyl {[1-(Diphenylmethyl)-4-(3-methoxyphenyl)-piperidin-4-yl]carbonyl}sulfamate (17). Compound **17** was prepared from **8** in a manner similar to that described for compound **9** with a yield of 65% as a white solid (hexane/ethanol). Mp 212−214 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.99 (12H, d, J = 6.8 Hz), 1.95 (1.4H, m), 2.41 (0.6H, m), 2.63 (1.4H, m), 2.85 (0.6H, m), 3.03 (1.4H, m), 3.22 (2H, m), 3.33 (0.6H, m), 3.50 (0.6H, m), 3.62 (1.4H, m), 3.70 (3H, s), 5.65 (0.7H, m), 5.81 (0.3H, m), 6.79 (0.7H, m), 6.85 (0.3H, m), 6.92 (2H, m), 7.02 (3H, m), 7.18−7.50 (7H, m), 7.63−7.71 (4H, m), 9.71 (0.7H, s), 9.95 (0.3H, s); IR (ATR) 1556, 1423, 1321, 1255 cm^{-1}; MS (ESI) m/z 641 (M + 1); *Anal.* Calcd for $C_{38}H_{44}N_2O_5S$: C, 71.22; H, 6.92; N, 4.37. Found: C, 70.88; H, 6.98; N, 4.48.

***N*-(2,6-Diisopropylphenyl)Sulfamide (19).** To a solution of sulfamoyl chloride[18] (1.15 g, 0.01 mol) in *N*-methyl-2-pyrrolidinone (10 mL) was added 2,6-diisopropylaniline (942 μL, 5.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 6 h. The mixture was poured into water, and then was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and residue was purified by silica gel column chromatography to give **19** (981 mg, 77%) as a white solid. Mp 130−132 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.11 (12H, d, J = 6.8 Hz), 3.59 (2H, m), 6.72 (2H, s), 7.13 (2H, d, J = 7.3 Hz), 7.20 (1H, m), 8.36 (1H, s); IR (ATR) 3365, 3334, 3273, 1373, 1155 cm^{-1}; HRMS (ESI) m/z Calcd for $C_{12}H_{21}N_2O_2S$ 257.1318; Found 257.1315 (Δ = −1.37 ppm).

Phenyl Sulfamate (26). To a solution of phenol (0.44 mL, 5.0 mmol) in heptane (20 mL) was added chlorosulfonylisocyanate (0.48 mL, 5.5 mmol) at room temperature, and the mixture was stirred at reflux for 10 h. The mixture was added water (0.7 mL) at room temperature, and stirred at reflux for 2 h. The reaction was quenched by adding water, and then the mixture was extracted with ethyl acetate. The organic layer was washed twice with water, and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and residue was triturated with hexane to give **26** (536 mg, 62%) as a white solid. Mp 77−79 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.26−7.34 (3H, m), 7.46 (2H, m), 7.98 (2H, s); IR (ATR) 3417, 3304, 1360, 1142 cm^{-1}; HRMS (APCI) m/z Calcd for $C_6H_6NO_3S$ 172.0074; Found 172.0068 (Δ = −3.18 ppm).

2-Methylphenyl Sulfamate (27). Compound **27** was prepared from 2-methylphenol in a manner similar to that described for compound **26** with a yield of 82% as a white solid (hexane). Mp 36−38 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.28 (3H, s), 7.16−7.31 (4H, m), 8.01 (2H, s); IR (ATR) 3379, 3275, 1342, 1153 cm^{-1}; HRMS (APCI) m/z Calcd for $C_7H_8NO_3S$ 186.0230; Found 186.0224 (Δ = −3.59 ppm).

2-Propylphenyl Sulfamate (28). Compound **28** was prepared from 2-propylphenol in a manner similar to that described for compound **26** with a yield of 61% as a white solid (hexane). Mp 56−58 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.91 (3H, t, J = 7.3 Hz), 1.58 (2H, tq, J = 7.7, 7.3 Hz), 2.64 (2H, t, J = 7.7 Hz), 7.19−7.34 (4H, m), 8.04 (2H, s); IR (ATR) 3367, 3280, 1373, 1153 cm^{-1}; HRMS (APCI) m/z Calcd for $C_9H_{12}NO_3S$ 214.0543; Found 214.0535 (Δ = −4.00 ppm).

2-Cyclopentylphenyl Sulfamate (29). Compound **29** was prepared from 2-cyclopentylphenol in a manner similar to that described for compound **26** with a yield of 38% as a white solid (hexane). Mp 103−105 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.51 (2H, m), 1.64 (2H, m), 1.78 (2H, m), 1.99 (2H, m), 3.40 (1H, m), 7.21−7.32 (3H, m), 7.40 (1H, m), 8.04 (2H, s); IR (ATR) 3365, 3261, 1352, 1155 cm^{-1}; HRMS (APCI) m/z Calcd for $C_{11}H_{14}NO_3S$ 240.0700; Found 240.0689 (Δ = −4.35 ppm).

2,6-Dimethylphenyl Sulfamate (30). Compound **30** was prepared from 2,6-dimethylphenol in a manner similar to that described for compound **26** with a yield of 61% as a white solid (hexane). Mp 102−104 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.31 (6H, s), 7.09 (3H, m), 8.04 (2H, s); IR (ATR) 3352, 3267, 1338, 1144 cm^{-1}; HRMS (APCI) m/z Calcd for $C_8H_{10}NO_3S$ 200.0387; Found 200.0378 (Δ = −4.55 ppm).

2,6-Diisopropylphenyl Sulfamate (31). Compound **31** was prepared from 2,6-diisopropylphenol in a manner similar to that described for compound **26** with a yield of 78% as a white solid (hexane). Mp 101−103 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.13 (12H, d, J = 7.0 Hz), 3.49 (2H, m) 7.17−7.21 (3H, m), 8.07 (2H, s); IR (ATR) 3380, 3278, 1371, 1190 cm^{-1}; HRMS (APCI) m/z Calcd for $C_{12}H_{18}NO_3S$ 256.1013; Found 256.1002 (Δ = −4.06 ppm).

2,6-Diisopropylphenyl {[4-(3-Methoxyphenyl)Piperidin-4-yl]-carbonyl}sulfamate (32). To a solution of compound **17** (3.84 g, 6.0 mmol) in *N*,*N*-dimethylformamide (60 mL) was added 20% palladium hydroxide on carbon (50% wet, 600 mg), and the mixture was stirred under hydrogen atmosphere at room temperature for 1 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated. To a solution of the residue in 1,4-dioxane (50 mL) was added activated carbon (100 mg), and then the mixture was stirred for 30 min. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was purified by recrystallization with diethylether to give **32** (2.73 g, 96%) as a white solid. Mp 219−221 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.01 (12H, d, J = 6.8 Hz), 1.80 (2H, m), 2.53 (2H, m), 2.93 (2H, m), 3.22 (2H, m), 3.56 (2H, m), 3.72 (3H, s), 6.81 (1H, m), 6.93 (2H, m), 7.04 (3H, m), 7.22 (1H, dd, J = 7.7, 7.7 Hz), 8.31 (2H, s); IR (ATR) 3076, 1608, 1599, 1552, 1330, 1257 cm^{-1}; HRMS (ESI) m/z Calcd for $C_{25}H_{35}N_3O_5S$ 475.2261; found 475.2246 (Δ = −3.13 ppm); Anal. Calcd for $C_{25}H_{34}N_2O_5S\cdot 0.5H_2O$: C, 62.09; H, 7.29; N, 5.79. Found: C, 61.86; H, 7.21; N, 5.82; LC: 97.2% pure, t_R = 12.48 min (Method A).

2,6-Diisopropylphenyl {[4-(3-Methoxyphenyl)-1-methylpiperidin-4-yl]carbonyl}sulfamate (33). To a suspension of compound **32** (95 mg, 0.20 mmol) in methanol (2.0 mL) was added 35% formaldehyde solution (47.6 μL, 0.60 mmol), acetic acid (45.8 μL, 0.80 mmol), and sodium cyanoborohydride (50.3 mg, 0.80 mmol) at room temperature, and the mixture was stirred for 1 day. The reaction was quenched by adding brine, and then the mixture was extracted with chloroform (2×). The organic layer was dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by recrystallization with methanol to give **33** (29.9 mg, 31%) as a white solid. Mp 222−224 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.04 (12H, d, J = 6.8 Hz), 1.80 (2H, m), 2.70 (2H, m), 2.75 (3H, s), 2.92 (2H, m), 3.44 (2H, m), 3.65 (2H, m), 3.76 (3H, s), 6.81 (1H, m), 6.95 (2H, m), 7.04 (3H, m), 7.22 (1H, dd, J = 8.0, 8.0 Hz), 9.08 (1H, s); IR (ATR) 1549, 1302, 1161 cm^{-1}; MS (ESI) m/z 489 (M + 1); Anal. Calcd for $C_{26}H_{36}N_2O_5S\cdot 0.5H_2O$: C, 62.75; H, 7.49; N, 5.63. Found: C, 62.45; H, 7.30; N, 5.68; LC: 96.6% pure, t_R = 12.67 min (Method A).

2,6-Diisopropylphenyl {[1-Acetyl-4-(3-methoxyphenyl)piperidin-4-yl]carbonyl}sulfamate (34). To a solution of compound **32** (95 mg, 0.20 mmol) in pyridine (1.0 mL) was added acetyl chloride (28.5 μL, 0.40 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (2.4 mg, 0.020 mmol) at room temperature, and the mixture was stirred for 6 h. The solvent was removed in vacuo, the residue was distilled with ethyl acetate, and the mixture was washed with saturated ammonium chloride solution, brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography. The solvent was removed in vacuo, and the residue was triturated with hexane/diethylether to give **34** (62.3 mg, 60%) as a white solid. Mp 168−170 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.04 (12H, d, J = 6.8 Hz), 1.75 (1H, m), 1.90 (1H, m), 2.00 (3H, s), 2.49 (2H, m), 2.86 (1H, m), 3.23 (1H, m), 3.31 (2H, m), 3.72 (1H, m), 3.75 (3H, s), 4.13 (1H, m), 6.87−6.95 (3H, m), 7.18 (3H, m), 7.31 (1H, m); IR (ATR) 1718, 1605, 1369 cm^{-1}; MS (ESI) m/z 517 (M + 1); Anal. Calcd for $C_{27}H_{36}N_2O_6S\cdot 1.25H_2O$: C, 60.15; H, 7.20; N, 5.20. Found: C, 59.99; H, 6.85; N, 5.31; LC: 96.7% pure, t_R = 16.72 min (Method A).

2,6-Diisopropylphenyl {[1-Benzoyl-4-(3-methoxyphenyl)piperidin-4-yl]carbonyl}sulfamate (35). Compound **35** was prepared with benzoyl chloride in a manner similar to that described for compound **34** with a yield of 65% as a white solid. Mp 150−152 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 1.05 (12H, d, J = 7.0 Hz), 1.78 (2H, m), 2.48 (2H, m), 3.21 (2H, m), 3.52 (2H, m), 3.74 (3H, s), 3.90 (2H, br), 6.82 (1H, m), 6.98 (2H, m), 7.12 (3H, m), 7.24 (1H, dd, J = 8.3, 8.3 Hz), 7.35−7.44 (5H, m); IR (ATR) 1718, 1632, 1448, 1250 cm^{-1}; MS (ESI) m/z 579 (M + 1); Anal. Calcd for $C_{32}H_{38}N_2O_6S\cdot 0.75H_2O$: C, 64.90; H, 6.72; N, 4.73. Found: C, 64.96; H, 6.39; N, 4.76; LC: 97.1% pure, t_R = 19.92 min (condition A).

2,6-Diisopropylphenyl {[4-(3-Methoxyphenyl)-1-phenylpiperidin-4-yl]carbonyl}sulfamate (36). To a solution of compound **32** (95 mg, 0.20 mmol) in 1,4-dioxane (1.0 mL) was added bromobenzene (42.1 μL, 0.40 mmol), (*S*)-(−)-BINAP (7.5 mg, 0.012 mmol), tris(dibenzylideneacetone)dipalladium(0) (5.5 mg, 0.006 mmol), and sodium *tert*-butoxide (57.7 mg, 0.60 mmol) at room temperature, and the mixture was stirred for 2 h at 120 °C under microwave irradiation. The reaction was quenched by adding saturated ammonium chloride solution, and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography. The solvent was removed in vacuo, and the residue was triturated with methanol to give **36** (95 mg, 86%) as a pale-yellow solid. Mp 165−167 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 1.05 (12H, d, J = 6.8 Hz), 2.07 (2H, m), 2.67 (2H, m), 3.01 (2H, m), 3.38 (2H, m), 3.58 (2H, m), 3.77 (3H, s), 6.89 (2H, m), 7.00 (4H, m), 7.13−7.27 (5H, m), 7.31 (1H, dd, J = 8.1, 8.1 Hz); IR (ATR) 1560, 1489, 1263 cm^{-1}; MS (ESI) m/z 551 (M + 1); Anal. Calcd for $C_{31}H_{38}N_2O_5S\cdot 1.5H_2O$: C, 64.45; H, 7.15; N, 4.85. Found: C, 64.19; H, 7.11; N, 5.22; LC: 97.2% pure, t_R = 15.43 min (Method A).

2,6-Diisopropylphenyl {[4-(3-Methoxyphenyl)-1-(3-methoxypyridin-2-yl)piperidin-4-yl]carbonyl}sulfamate (37). Compound **37** was prepared with 2-bromo-3-methoxypyridine in a manner similar to that described for compound **36** with a yield of 72% as a white solid. Mp 195−197 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 1.06 (12H, d, J = 6.8 Hz), 2.02 (2H, m), 2.59 (2H, m), 3.18 (2H, m), 3.39 (2H, m), 3.76 (3H, s), 3.80 (2H, m), 3.83 (3H, s), 6.90 (2H, m), 7.02 (2H, m), 7.15 (3H, m), 7.27 (2H, m), 7.74 (1H, d, J = 5.1 Hz); IR (ATR) 1618, 1540, 1460, 1325, 1246 cm^{-1}; MS (ESI) m/z 582 (M + 1); Anal. Calcd for $C_{31}H_{39}N_3O_6S\cdot 2H_2O$: C,

60.27; H, 7.02; N, 6.80. Found: C, 59.89; H, 6.75; N, 6.75; LC: 97.8% pure, t_R = 13.99 min (Method A).

2,6-Diisopropylphenyl {[1-(1,3-Benzothiazol-2-yl)-4-(3-methoxyphenyl)piperidin-4-yl]carbonyl}sulfamate (38). Compound **38** was prepared with 2-bromobenzothiazole in a manner similar to that described for compound **36** with a yield of 65% as a pale-yellow solid. Mp 181−183 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 1.07 (12H, d, J = 7.0 Hz), 2.08 (2H, m), 2.62 (2H, m), 3.39 (2H, m), 3.42 (2H, m), 3.77 (3H, s), 3.91 (2H, m), 6.90 (1H, m), 7.02 (2H, m), 7.07 (1H, m), 7.18 (3H, m), 7.30 (2H, m), 7.46 (1H, d, J = 8.1 Hz), 7.75 (1H, d, J = 7.9 Hz); IR (ATR) 1618, 1576, 1454, 1259 cm^{-1}; MS (ESI) m/z 608 (M + 1); Anal. Calcd for $C_{32}H_{37}N_3O_5S_2 \cdot 2H_2O$: C, 59.70; H, 6.42; N, 6.53. Found: C, 59.56; H, 6.09; N, 6.61; LC: 99.3% pure, t_R = 11.18 min (Method B).

2,6-Diisopropylphenyl {[4-(3-Methoxyphenyl)-1-pyrimidin-2-ylpiperidin-4-yl]carbonyl}sulfamate (39). To a solution of compound **32** (950 mg, 2.0 mmol) in *N*,*N*-dimethylformamide (10 mL) was added 2-chloropyrimidine (275 mg, 2.40 mmol) and potassium carbonate (553 mg, 4.0 mmol) at room temperature, and the mixture was stirred at 60 °C for 3 h. The reaction was quenched by adding water, and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography. The solvent was removed in vacuo, and the residue was washed with diethylether/hexane to give **39** (1.05 g, 95%) as a white solid. Mp 201−203 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.01 (12H, d, J = 6.8 Hz), 1.60 (2H, m), 2.50 (2H, m), 3.20 (2H, m), 3.58 (2H, m), 3.71 (3H, s), 4.42 (2H, m), 6.55 (1H, dd, J = 4.8, 4.8 Hz), 6.76 (1H, m), 6.96 (2H, m), 7.05 (3H, m), 7.18 (1H, dd, J = 8.2, 8.2 Hz), 8.32 (2H, d, J = 4.8 Hz); IR (ATR) 1585, 1286 cm^{-1}; MS (ESI) m/z 553 (M + 1); Anal. Calcd for $C_{29}H_{36}N_4O_5S \cdot 1.1H_2O$: C, 60.84; H, 6.73; N, 9.79. Found: C, 60.55; H, 6.33; N, 9.71; LC: 99.4% pure, t_R = 18.99 min (Method A).

Sodium Salt of Compound 39. Sodium salts of compound **39** was prepared in a manner similar to that described for sodium salt of compound **16** with a yield of 95% as a white solid. Mp >280 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.01 (12H, d, J = 7.0 Hz), 1.55 (2H, m), 2.50 (2H, m), 3.19 (2H, m), 3.62 (2H, m), 3.70 (3H, s), 4.41 (2H, m), 6.54 (1H, dd, J = 4.8, 4.8 Hz), 6.74 (1H, dd, J = 8.1, 2.2 Hz), 6.96 (2H, m), 7.02 (3H, s), 7.16 (1H, dd, J = 8.1, 8.1 Hz), 8.31 (1H, d, J = 4.8 Hz); IR (ATR) 1585, 1282 cm^{-1}; HRMS (ESI) m/z Calcd for $C_{29}H_{35}N_4O_5S$ 551.2334; Found 551.2335 (Δ = 0.27 ppm); Anal. Calcd for $C_{29}H_{35}N_4NaO_5S \cdot 0.75H_2O$: C, 59.22; H, 6.25; N, 9.53. Found: C, 58.90; H, 6.24; N, 9.29; LC: 99.1% pure, t_R = 19.03 min (Method A).

2,6-Diisopropylphenyl {[4-(3-Methoxyphenyl)-1-(4-methylpyrimidin-2-yl)piperidin-4-yl]carbonyl}sulfamate (40). Compound **40** was prepared with 2-chloro-4-methylpyrimidine in a manner similar to that described for compound **39** with a yield of 72% as a white solid. Mp 164−166 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 1.06 (12H, d, J = 6.8 Hz), 1.87 (2H, m), 2.28 (3H, s), 2.54 (2H, m), 3.28 (2H, m), 3.41 (2H, m), 3.76 (3H, s), 4.37 (2H, m), 6.49 (1H, d, J = 5.0 Hz), 6.88 (1H, m), 6.98 (2H, m), 7.17 (3H, m), 7.28 (1H, dd, J = 8.1, 8.1 Hz), 8.20 (1H, d, J = 5.0 Hz); IR (ATR) 3292, 1728, 1578, 1417, 1257 cm^{-1}; MS (ESI) m/z 567 (M + 1); Anal. Calcd for $C_{30}H_{38}N_4O_5S \cdot 0.25H_2O$: C, 63.08; H, 6.79; N, 9.81. Found: C, 62.86; H, 6.82; N, 9.65; LC: 99.3% pure, t_R = 17.61 min (Method A).

2,6-Diisopropylphenyl ({4-(3-Methoxyphenyl)-1-[4-(trifluoromethyl)pyrimidin-2-yl]piperidin-4-yl}carbonyl)sulfamate (41). Compound **41** was prepared with 2-chloro-4-trifuluoromethylpyrimidine in a manner similar to that described for compound **39** with a yield of 90% as a white solid. Mp 174−176 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 1.05 (12H, d, J = 6.8 Hz), 1.89 (2H, m), 2.58 (2H, m), 3.36 (2H, m), 3.46 (2H, m), 3.75 (3H, s), 4.37 (2H, m), 6.86 (1H, m), 6.96 (1H, d, J = 5.0 Hz), 6.99 (2H, m), 7.14 (3H, m), 7.27 (1H, dd, J = 8.0, 8.0 Hz), 8.66 (1H, d, J = 5.0 Hz); IR (ATR) 3311, 1722, 1589, 1448, 1327 cm^{-1}; MS (ESI) m/z 621 (M + 1); Anal. Calcd for $C_{30}H_{35}F_3N_4NaO_5S$: C, 58.05; H, 5.68; N, 9.03. Found: C, 57.76; H, 5.63; N, 8.96; LC: 99.6% pure, t_R = 19.25 min (Method B).

2,6-Diisopropylphenyl {[4-(3-Methoxyphenyl)-1-(5-propylpyrimidin-2-yl)piperidin-4-yl]carbonyl}sulfamate (42). Compound **42** was prepared with 2-chloro-5-propylpyrimidine in a manner similar to that described for compound **39** with a yield of 41% as a white solid. Mp 89−91 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 0.87 (3H, t, J = 7.3 Hz), 1.05 (12H, d, J = 6.8 Hz), 1.52 (2H, m), 1.83 (2H, m), 2.37 (2H, t, J = 7.4 Hz), 2.50 (2H, m), 3.26 (2H, m), 3.48 (2H, m), 3.74 (3H, s), 4.33 (2H, m), 6.83 (1H, m), 6.98 (2H, m), 7.12 (3H, m), 7.25 (1H, dd, J = 8.2, 8.2 Hz), 8.21 (2H, s); IR (ATR) 1585, 1464, 1363, 1248 cm^{-1}; MS (ESI) m/z 595 (M + 1); Anal. Calcd for $C_{32}H_{42}N_4O_5S \cdot 1H_2O$: C, 62.72; H, 7.24; N, 9.14. Found: C, 62.74; H, 7.06; N, 9.09; LC: 97.7% pure, t_R = 13.56 min (Method B).

2,6-Diisopropylphenyl {[1-(5-Bromopyrimidin-2-yl)-4-(3-methoxyphenyl)piperidin-4-yl]carbonyl}sulfamate (43). To a solution of compound **39** (100 mg, 0.181 mmol) in THF (2.0 mL) was added *N*-bromosuccinimide (32.2 mg, 0.181 mmol) at 0 °C, and the mixture was stirred for 1 h. The reaction was quenched by adding saturated sodium hydrogen carbonate solution, and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography. The solvent was removed in vacuo, and the residue was triturated with diethylether to give **43** (95.6 mg, 84%) as a white solid. Mp 106−108 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.01 (12H, d, J = 6.8 Hz), 1.58 (2H, m), 2.50 (2H, m), 3.20 (2H, m), 3.62 (2H, m), 3.70 (3H, s), 4.35 (2H, m), 6.75 (1H, d, 8.1 Hz), 6.96 (2H, m), 7.03 (3H, s), 7.17 (1H, dd, J = 8.1, 8.1 Hz), 8.41 (2H, s); IR (ATR) 1578, 1362, 1250 cm^{-1}; MS (ESI) m/z 631 (M + 1); Anal. Calcd for $C_{29}H_{35}BrN_4O_5S \cdot 2.5H_2O$: C, 51.48; H, 5.96; N, 8.28. Found: C, 51.33; H, 5.59; N, 8.19; LC: 99.8% pure, t_R = 19.66 min (Method B).

2,6-Diisopropylphenyl {[4-(3-Methoxyphenyl)-1-pyrimidin-2-ylpiperidin-4-yl]carbonyl}methylsulfamate (44). To a solution of sodium salt of compound **39** (100 mg, 0.174 mmol) in *N*,*N*-dimethylformamide (2.0 mL) was added methyl iodide (217 μL, 3.48 mmol) at room temperature, and the mixture was stirred at 60 °C for 20 h. The reaction was quenched by adding water, and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography to give **44** (43.6 mg, 44%) as a white amorphous. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.11 (12H, d, J = 6.8 Hz), 1.97 (2H, m), 2.46 (2H, m), 2.92 (3H, s), 3.16 (4H, m), 3.77 (3H, s), 4.58 (2H, m), 6.63 (1H, dd, J = 4.6, 4.6 Hz), 6.87−6.97 (3H, m), 7.25 (3H, m), 7.41 (1H, dd, J = 8.0, 8.0 Hz), 8.36 (2H, d, J = 4.6 Hz); IR (ATR) 1705, 1583, 1365, 1253 cm^{-1}; MS (ESI) m/z 567 (M + 1); Anal. Calcd for $C_{30}H_{38}N_4O_5S \cdot 0.25H_2O$: C, 63.08; H, 6.79; N, 9.81. Found: C, 62.83; H, 6.71; N, 9.76; LC: 97.6% pure, t_R = 13.82 min (Method B).

2,6-Diisopropylphenyl {[4-(3-Hydroxyphenyl)-1-pyrimidin-2-ylpiperidin-4-yl]carbonyl}sulfamate (45). To a solution of compound **39** (1.0 g, 1.81 mmol) in CH_2Cl_2 (15 mL) was added 1 M boron tribromide dichloromethane solution (5.43 mL, 5.42 mmol) at 0 °C, and the mixture was stirred at room temperature for 20 h. The reaction mixture was poured into ice water, and the mixture was extracted with chloroform (2x). The organic layer was dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography. The mixture obtained was washed by methanol to give **45** (850 mg, 87%) as a white solid. Mp 193−195 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ; 1.05 (12H, d, J = 6.8 Hz), 1.85 (2H, m), 2.54 (2H, m), 3.28 (4H, m), 4.38 (2H, m), 6.63 (1H, dd, J = 4.8, 4.8 Hz), 6.70 (1H, m), 6.80 (2H, m), 7.19 (4H, m), 8.36 (2H, d, J = 4.8 Hz), 9.46 (1H, s); IR

(ATR) 3491, 3300, 1714, 1585, 1414, 1369, 1215 cm^{-1}; HRMS (ESI) *m/z* Calcd for $C_{28}H_{35}N_4O_5S$ 539.2323; Found 539.2304 ($\Delta = -3.52$ ppm). Anal. Calcd for $C_{28}H_{34}N_4O_5S \cdot 0.25H_2O$: C, 61.92; H, 6.40; N, 10.31. Found: C, 61.93; H, 6.26; N, 10.35; LC: 98.4% pure, t_R = 15.40 min (Method A).

2,6-Diisopropylphenyl {[4-(3-Ethoxyphenyl)-1-pyrimidin-2-ylpiperidin-4-yl]carbonyl}sulfamate (46). To a solution of compound **45** (108 mg, 0.20 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added ethyl iodide (32.0 μL, 0.40 mmol) and cesium carbonate (196 mg, 0.60 mmol) at room temperature, and the mixture was stirred at 60 °C for 4 h. The reaction was quenched by adding water, and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by preparative TLC. The crude was washed with diethylether to give **46** (88.7 mg, 78%) as a white solid. Mp 173−175 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 1.04 (12H, d, J = 6.8 Hz), 1.31 (3H, t, J = 6.9 Hz), 1.69 (2H, m), 2.52 (2H, m), 3.27 (2H, m), 3.58 (2H, m), 4.00 (2H, q, J = 6.9 Hz), 4.38 (2H, m), 6.54 (1H, dd, J = 4.8, 4.8 Hz), 6.76 (1H, m), 6.97 (2H, m), 7.07 (3H, m), 7.19 (1H, dd, J = 8.2, 8.2 Hz), 8.32 (2H, d, J = 4.8 Hz); IR (ATR) 1587, 1367, 1281 cm^{-1}; MS (ESI) *m/z* 567 (M + 1); Anal. Calcd for $C_{30}H_{38}N_4O_5S \cdot 0.75H_2O$: C, 62.10; H, 6.86; N, 9.66. Found: C, 61.70; H, 6.44; N, 9.59; LC: 96.6% pure, t_R = 11.49 min (Method B).

2,6-Diisopropylphenyl {[4-(3-Propoxyphenyl)-1-pyrimidin-2-ylpiperidin-4-yl]carbonyl}sulfamate (47). Compound **47** was prepared with 1-propyl iodide in a manner similar to that described for compound **46** with a yield of 60% as a white solid. Mp 188−190 °C; ^{1}H NMR (DMSO-d_6, 60 °C, 300 MHz) δ 0.96 (3H, t, J = 7.4 Hz), 1.03 (12H, d, J = 6.8 Hz), 1.60 (2H, m), 1.72 (2H, qt, J = 7.4, 6.5 Hz), 2.50 (2H, m), 3.25 (2H, m), 3.67 (2H, m), 3.88 (2H, t, J = 6.5 Hz), 4.38 (2H, m), 6.52 (1H, dd, J = 4.7, 4.7 Hz), 6.73 (1H, m), 6.97 (2H, m), 7.03 (3H, m), 7.14 (1H, dd, J = 7.9, 7.9 Hz), 8.31 (2H, d, J = 4.7 Hz); IR (ATR) 1585, 1464, 1363, 1248 cm^{-1}; MS (ESI) *m/z* 581 (M + 1); Anal. Calcd for $C_{31}H_{40}N_4O_5S \cdot 2H_2O$: C, 60.37; H, 7.19; N, 9.08. Found: C, 60.07; H, 6.88; N, 9.06; LC: 99.0% pure, t_R = 13.97 min (Method B).

2,6-Diisopropylphenyl ({4-[3-(2-hydroxyethoxy)phenyl]-1-pyrimidin-2-ylpiperidin-4-yl}carbonyl)sulfamate (48). To a solution of compound **45** (108 mg, 0.20 mmol) in *N*,*N*-dimethylformamide (1.0 mL) was added 2-bromoethyl *tert*-butyldimethylsilyl ether (86.1 μL, 0.40 mmol) and cesium carbonate (196 mg, 0.60 mmol) at room temperature, and the mixture was stirred at 60 °C for 4 h. The reaction was quenched by adding water, and then the mixture was extracted with ethyl acetate. The organic layer was washed with brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by preparative TLC to give *tert*-butyldimethylsilyl ether derivative (125 mg) as a white solid. To a solution of the *tert*-butyldimethylsilyl ether derivative (125 mg, 0.179 mmol) in THF (2.5 mL) was added 1 M tetrabutylammonium fluoride (540 μL, 0.538 mmol) at room temperature, the mixture was stirred for 2 h. The reaction was quenched by adding saturated ammonium chloride solution, and then the mixture was extracted with ethyl acetate. The organic layer was washed with 0.2 N hydrochloric acid solution (2x), brine and dried over anhydrous magnesium sulfate. After filtration, the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography. The solvent was removed in vacuo, and the residue was triturated with diethylether/hexane to give **48** (67.0 mg, 57%) as a white solid. Mp 143−145 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.03 (12H, d, J = 6.8 Hz), 1.87 (2H, m), 2.56 (2H, m), 3.25 (2H, m), 3.34 (2H, m), 3.69 (2H, t, J = 5.1 Hz), 3.99 (2H, t, J = 5.1 Hz), 4.38 (2H, m), 6.61 (1H, dd, J = 4.7, 4.7 Hz), 6.88 (1H, m), 6.96 (2H, m), 7.19 (3H, m), 7.29 (1H, dd, J = 8.3, 8.3 Hz), 8.35 (2H, d, J = 4.7 Hz); IR (ATR) 3510, 1703, 1589, 1495, 1255 cm^{-1}; MS (ESI) *m/z* 583 (M + 1); Anal. Calcd for $C_{30}H_{38}N_4O_6S \cdot 0.25H_2O$: C, 61.36; H, 6.61; N, 9.54. Found: C, 61.21; H, 6.56; N, 9.47; LC: 99.0% pure, t_R = 15.81 min (Method A).

2,6-Diisopropylphenyl ({4-[3-(3-Hydroxypropoxy)Phenyl]-1-pyrimidin-2-ylpiperidin-4-yl}carbonyl)sulfamate (49). Compound **49** was prepared with 3-bromopropyl *tert*-butyldimethylsilyl ether in a manner similar to that described for compound **48** with a yield of 89% as a white solid. Mp 112−114 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.01 (12H, d, J = 7.0 Hz), 1.59 (2H, m), 1.83 (2H, m), 3.21 (2H, m), 3.30 (2H, m), 3.54 (2H, t, J = 6.2 Hz), 3.60 (2H, m), 3.98 (2H, t, J = 6.4 Hz), 4.40 (2H, m), 6.54 (1H, dd, J = 4.7, 4.7 Hz), 6.73 (1H, d, J = 7.5 Hz), 6.95 (2H, m), 7.04 (3H, m), 7.16 (1H, m), 8.32 (2H, d, J = 4.7 Hz); IR (ATR) 1583, 1458, 1363, 1242 cm^{-1}; MS (ESI) *m/z* 597 (M + 1); Anal. Calcd for $C_{31}H_{40}N_4O_6S \cdot 0.5H_2O$: C, 61.47; H, 6.82; N, 9.25. Found: C, 61.38; H, 6.84; N, 9.02; LC: 98.6% pure, t_R = 16.69 min (Method A).

Effects of Compounds on LDL-R Activity in Human Hepatoma Cell Line. Preparation of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI)-labeled LDL (DiI-LDL) was carried out as follows. DiI (invitrogen, U.S.A) was mixed with numan LDL (CHEMICON, U.S.), and the mixture was kept for 8 h at 37 °C. Next, a specific density solution (d = 1.182) was added and mixed, and the whole was centrifuged (32 krpm, 14 h; using Beckman Ti55.2 rotator). After centrifugation, the DiI-labeled LDL fraction was collected and dialyzed against saline. The effect of each compound on LDL-R activity was determined as the amount of DiI-LDL up-taken into $HepG_2$ cells used as indicator. Namely hepatoma cell line $HepG_2$ used for the experiment. $HepG_2$ cells were plated on a 96 well plate and incubated with Dulbecco's modified Eagle/F-12 medium (DMEM/F-12) containing 10% fetal bovine serum and antibiotics for 2−3 days at 37 °C in a CO_2 incubator. After washing the cells, DMEM/F-12 media containing the test compound, 10% delipoprotein serum, and antibiotics was added thereto, and the cells were incubated for 19 h at 37 °C. DiI-LDL was next added, and the cells were incubated for an additional 5 h at 37 °C in a CO_2 incubator. For measurement of the amount of nonspecific DiI-LDL uptake, 30−50 times the nonlabeled LDL was added to each well. After washing the cells with Dulbecco's phosphate buffer (SIGMA, U.S.), Dulbecco's phosphate buffer was added to each well, and the fluorescence was measured with a fluorescence plate reader to determine the amount of DiI-LDL up-taken into the cells. After measuring the fluorescence, the Dulbecco's phosphate buffer was removed. Cells were then dissolved in 1 N sodium hydroxide solution. Using part of the solution, protein amount was measured. The effect of each compound on LDL-R was estimated on the basis of 100% of the control group, using the value given by subtracting the nonspecifically uptaken amount from the calculated fluorescence value/protein amount.

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Supporting Information Available: NMR spectroscopic data for compounds **44** and **46**, and MS/MS analytical data for compound **44**. This material is available free of charge via the Internet at http://pubs.acs.org.

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