Novel Indoline-Based Acyl-CoA:Cholesterol Acyltransferase Inhibitor with Antiperoxidative Activity: Improvement of Physicochemical Properties and Biological Activities by Introduction of Carboxylic Acid

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Received March 7, 2008

A series of novel indoline derivatives with an ionizable moiety were synthesized to find a bioavailable acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor with antiperoxidative activity. [7-(2,2-Dimethylpropanamido)-4,6-dimethyl-1-octylindolin-5-yl]acetic acid hemisulfate (**2**, pactimibe sulfate) with low lipophilicity and high water solubility showed good oral absorption and inhibitory activity against foam cell formation in THP-1 cells exposed to acetyl-LDL after differentiation (IC_{50}: 0.3 μM) and an antiperoxidative effect in LDL of hypercholesterolemic rabbits (IC_{50}: 1.0 μM). **2** inhibited macrophage, hepatic, and intestinal ACAT activity (IC_{50}: 1.9, 0.7, and 0.7 μM, respectively). Maximal plasma concentration after oral administration of **2** at 10 mg/kg was 0.9 μg/mL in rats, 3.0 μg/mL in rabbits, and 11.2 μg/mL in dogs. Repeated administration of **2** lowered plasma LDL/VLDL cholesterol in hypercholesterolemic rabbits at 1 mg/kg/day, rats and dogs at 3 mg/kg/day, and in normocholesterolemic hamsters at 3 mg/kg/day. **2** is a promising candidate for antihyperlipidemic and antiatherosclerotic drugs.

Introduction

Acyl-CoA:cholesterol acyltransferase (ACAT)[a] has been a promising target for hyperlipidemia and atherosclerosis because esterification of cholesterol with a long chain fatty acid is essential for intestinal absorption of dietary cholesterol, incorporation of hepatic cholesterol into very low-density lipoprotein (VLDL) vascular accumulation of cholesterol.[1–3]

Classical ACAT inhibitors have been focused on as hypolipidemic drugs, which inhibit intestinal absorption of cholesterol and the secretion of hepatic cholesterol. A number of highly potent ACAT inhibitors with high lipophilicity and low oral bioavailability have been reported.[4,5] The target of ACAT inhibitors then shifted from intestinal/hepatic ACAT to macrophage ACAT: hyperlipidemia to atherosclerosis.[6] In atherosclerotic plaque, denaturized low-density lipoprotein (LDL), such as oxidized LDL, is taken up by macrophages and cholesterol is accumulated via esterification by ACAT. There have been some attempts to improve bioavailability of ACAT inhibitors by reduction of lipophilicity;[7–11] however, no bioavailable ACAT inhibitors have been successfully developed. It is very difficult to reduce the lipophilicity of ACAT inhibitors while maintaining their activities, because lipophilicity is the preferred property for the inhibition of esterification between two lipophilic substrate molecules (cholesterol and long chain fatty acid) by membrane-bound enzymes such as ACAT.[12,13] Furthermore, ACAT has two isozymes: ACAT-1 expressed in macrophages and adrenal glands, and ACAT-2 expressed in the intestine and liver.[14–16] Thus, adrenotoxicity is a critical problem for systemic bioavailable ACAT inhibitors, which may inhibit adrenal as well as macrophage ACAT. Bioavailable and weak ACAT inhibitors such as 2,6-bis(1-methylethyl)phenyl [[2,4,6-tris(1-methylethyl)phenyl]acetyl]sulfamate (CI-1011, avasimibe) have been reported to show minimal adrenotoxicity;[6,11,17] however, these weak ACAT inhibitors may not effectively prevent cholesterol deposition in atherosclerotic plaques. Therefore, we hypothesized that bioavailable moderate ACAT inhibitors with inhibitory effects on LDL oxidation would be safe and effective antiatherosclerotic drugs, the effects of which may synergistically suppress accumulation of oxidized LDL in macrophages.

An indoline structure is reportedly a pharmacophore responsible for the inhibitory effect of indapamide on lipid peroxidation.[18,19] We synthesized a series of indoline-based ACAT inhibitors and examined their physicochemical properties and biological activities.[20] Among them, *N*-(4,6-dimethyl-1-octylindolin-7-yl)-2,2-dimethylpropanamide hydrochloride (**1**) potently inhibited hepatic ACAT activity and lipid peroxidation. Compound **1** lowered serum cholesterol not only in dietary hypercholesterolemic rats but also in normocholesterolemic hamsters, suggesting that it inhibited hepatic ACAT after oral administration;[20] however, **1** is still highly lipophilic and its bioavailability may not be sufficiently high to inhibit macrophage ACAT and LDL peroxidation via systemic circulation. Therefore, to increase oral bioavailability without reducing ACAT inhibitory and antiperoxidative activities, we attempted to improve lipophilicity and water solubility by introducing an ionizable moiety (a basic or acidic moiety) to the indoline ring of **1**. We found that [7-(2,2-dimethylpropanamido)-4,6-dimethyl-1-octylindolin-5-yl]acetic acid hemisulfate (**2**, pactimibe sulfate) with carboxymethyl moiety at the 5-position of the indoline ring showed

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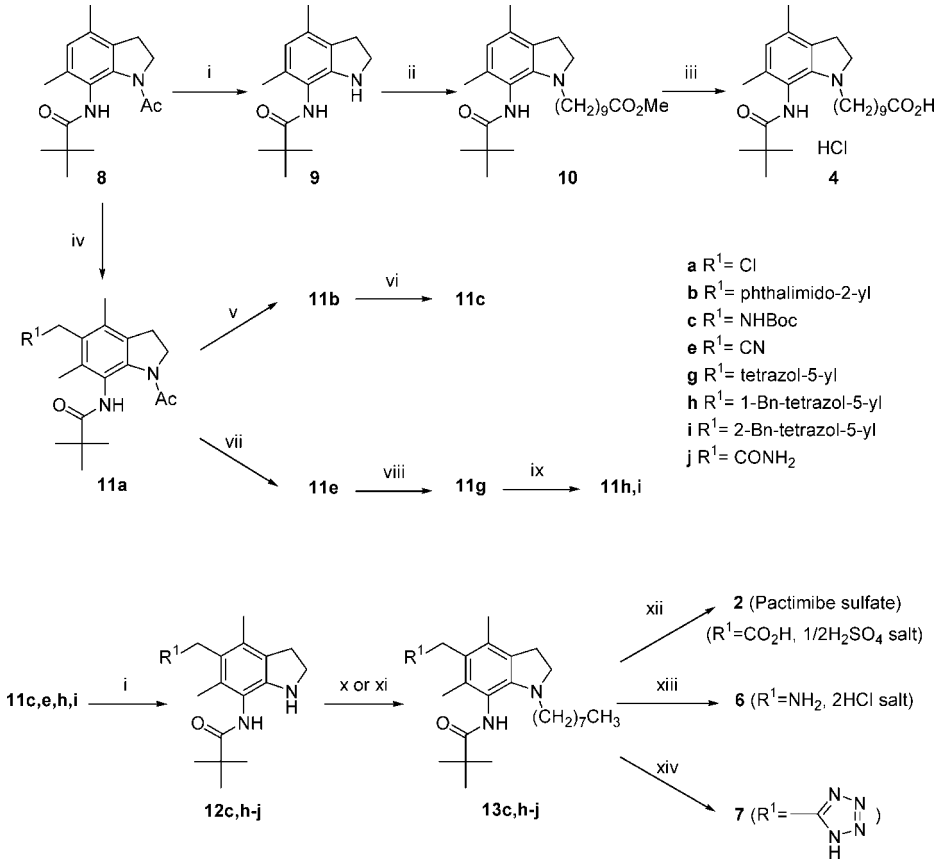
[a] Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; AUC, area under the plasma concentration-time curve; LCAT, lecithin:cholesterol acyltransferase; HDL, high-density lipoprotein; WHHL rabbit, Watanabe heritable hyperlipidemic rabbit; Cmax, maximal concentration; TLC, thin-layer chromatography; IR, infrared spectra; NMR, nuclear magnetic resonance; MS, mass spectra; MDA, malondialdehyde; EC, esterified cholesterol; FBS, fetal bovine serum; PMA, phorbol 12-myristate 13-acetate; KHC rabbit, Kurosawa and Kusanagi hypercholesterolemic rabbit.

10.1021/jm800248r CCC: $40.75
Published on Web 07/12/2008

Scheme 1. Synthesis of Compounds **2**, **4**, **6**, and **7**[a]



[a] Reagents, conditions and yields: (i) NaOH, MeOH-H_2O, 50 °C, 76−94%; (ii) $Br(CH_2)_9CO_2Me$, *i*-Pr_2NEt, KI, DMF, 60 °C, 87%; (iii) NaOH, MeOH-H_2O, rt, then acidified with HCl, 83%; (iv) formalin, HCl (gas), conc. HCl, 50 °C; (v) potassium phthalimide, DMF, rt, 96% (2 steps); (vi) (a) hydrazine monohydrate, MeOH-$CHCl_3$, reflux; (b) Boc_2O, $CHCl_3$, rt, 71% (2 steps); (vii) NaCN, 18-crown-6, MeCN, reflux, 95% (2 steps); (viii) NaN_3, $Me_3NH^+Cl^-$, DMF, 120 °C, 72%; (ix) benzyl chloride, NaH, DMF, rt, 93%; (x) octyl iodide, *i*-Pr_2NEt, DMF, 60 °C, 86−91% (when **13c**,**h**,**i**); (xi) octyl bromide, K_2CO_3, KI, DMF, 40 °C, 76% (when **13j**); (xii) NaOH, *n*-PrOH-H_2O, reflux, then acidified with H_2SO_4, 78%; (xiii) (a) HCl in *i*-PrOH, HCO_2H, rt; (b) HCl in *i*-PrOH, $CHCl_3$, 0 °C, 73% (2 steps); (xiv) H_2, PdO, AcOH, rt, 72%.

low lipophilicity, high water solubility and good oral absorption, and exerted excellent pharmacological effects.

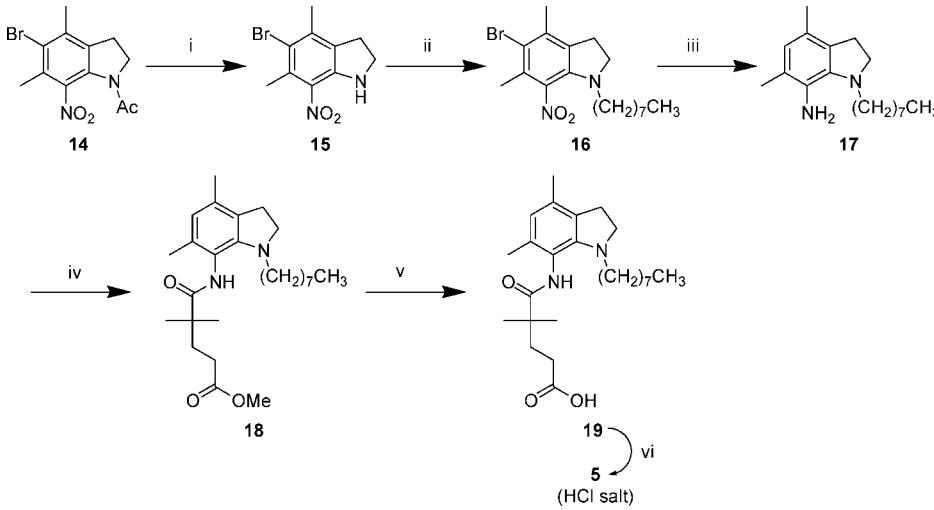
Chemistry

Indoline derivatives with carboxylic acid moiety at 1- or 5-position (**4** or **2**) were synthesized from **8**, which was prepared as described previously (Scheme 1).[20] Derivatives with amine (**6**) and tetrazole (**7**) at 5-position were also synthesized from **8**. For the synthesis of **4**, acetyl moiety of **8** was removed, alkylated with $Br(CH_2)_9CO_2Me$, and then the ester was hydrolyzed to yield **4** as a hydrochloride salt. For the synthesis of **2**, **6**, and **7**, the intermediate **8** was subjected to a chloromethylation to obtain **11a** without purification by column chromatography because of its instability. To prepare **6**, compound **11a** was treated with potassium phthalimide, and it was converted to amine protected with Boc group (**11c**). To prepare **2** and **7**, compound **11a** was substituted with cyanide to give **11e** as an intermediate of **2**, and **11e** was reacted with sodium azide, followed by benzylation, to give a mixture of regioisomers **11h** and **11i** (1:1) as intermediates of **7**, which were used for further reaction without isolating each regioisomer. The intermediates (**11c**,**e**,**h**,**i**) were hydrolyzed to **12c**,**h**−**j** and then were alkylated with octyl iodide at 1-position to afford **13c**,**h**−**j**. The Boc protective group of **13c** was removed with acid, followed by treating with HCl to provide **6** as a dihydrochloride salt. Benzyl groups of the mixture of **13h**,**i** were removed by hydrogenation to afford **7**. Carbamoyl moiety of **13j** was hydrolyzed with NaOH by refluxing, followed by acidification with sulfuric acid to yield **2** as a hemisulfate salt. A derivative with carboxyl moiety at 7-position (**5**) was synthesized from **14** using branched acyl chloride (Scheme 2).[20,21] Scheme 3 describes a method to prepare **3**. Intermediate **26** was synthesized according to the methods reported previously.[20,22] **26** was hydrogenated, pivaloylated, hydrolyzed, and alkylated by reductive amination to **27**, which was treated with HCl to give **3** as a hydrochloride salt.

Results and Discussion

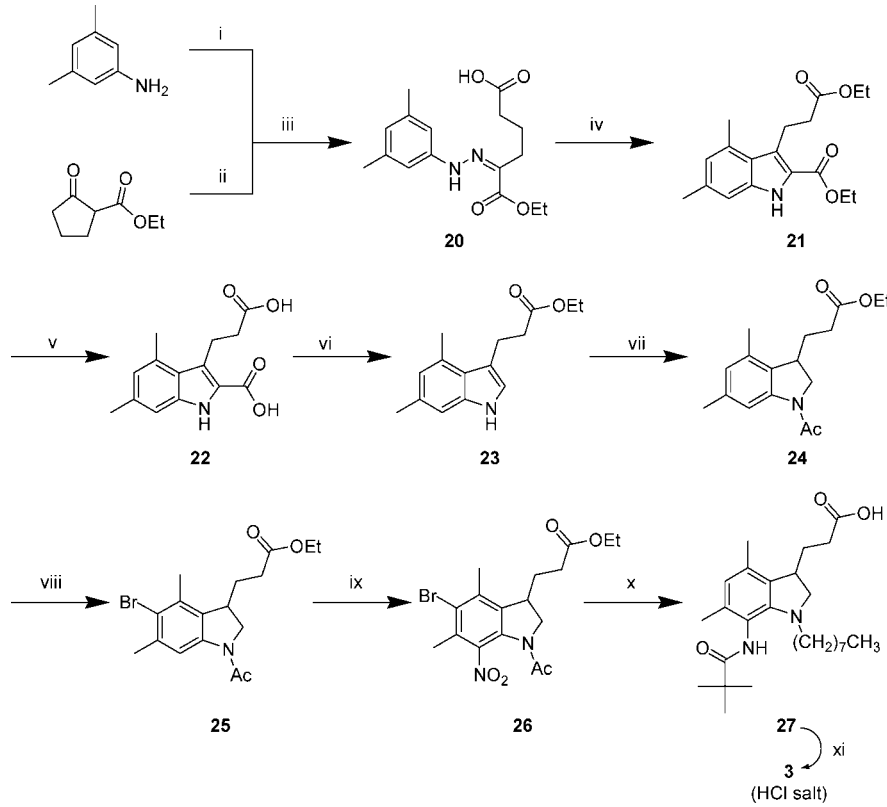
Among the indoline-based ACAT inhibitors previously reported, compound **1** potently inhibited hepatic and intestinal ACAT as well as lipid peroxidation[20] but was expected to show poor oral absorption because of its high lipophilicity and low water solubility. Although the inhibitory effect of **1** on macrophage ACAT has not been reported, the effect of *N*-(4,6-dimethyl-1-pentylindolin-7-yl)-2,2-dimethylpropanamide hydrochloride (KY-455), which is an analogue with a shorter alkyl chain at the 1-position, was approximately 3-fold weaker than that on hepatic ACAT.[23] Thus, it is unlikely that **1** is efficiently absorbed and exerts inhibitory effects on macrophage ACAT and LDL oxidation. In the present study, an ionizable moiety, including carboxylic acid, primary amine, and tetrazole, was introduced at various positions on the indoline ring of **1** to increase oral absorption without marked reduction of inhibitory activities against ACAT and

Scheme 2. Synthesis of Compound **5**[a]



[a] Reagents, conditions and yields: (i) NaOH, MeOH-H_2O, reflux, 97%; (ii) octyl iodide, NaH, DMF, 50 °C, 93%; (iii) H_2, Pd−C, MeOH-THF, 79%; (iv) $ClC(O)C(CH_3)_2CH_2CH_2CO_2Me$,[21] Et_3N, CH_2Cl_2, rt. 88%; (v) NaOH, MeOH-H_2O, rt, 93%; (vi) HCl in *i*-PrOH, AcOEt, rt, 68%.

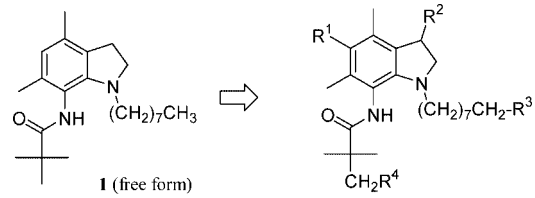
Scheme 3. Synthesis of Compound **3**[a]



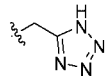
[a] Reagents, conditions and yields: (i) $NaNO_2$, 4 M HCl, 5 °C; (ii) Na, EtOH, rt; (iii) AcONa, H_2O, rt, 79%; (iv) conc HCl, EtOH, reflux, 85%; (v) NaOH, EtOH, reflux, 84%; (vi) (a) 220 °C; (b) HCl in *i*-PrOH, EtOH, reflux, 83% (2 steps); (vii) (a) $NaBH_3CN$, AcOH, 10−15 °C; (b) Ac_2O, $CHCl_3$, rt, 94% (2 steps); (viii) Br_2, AcOH, rt, 91%; (ix) fum.HNO_3, AcOH-conc H_2SO_4, rt, 94%; (x) (a) H_2, Pd−C, MeOH, rt; (b) pivaloyl chloride, Et_3N, CH_2Cl_2, rt; (c) NaOH, MeOH-H_2O, reflux; (d) octyl aldehyde, $NaBH_3CN$, MeOH, rt, 60% (4 steps); (xi) HCl in *i*-PrOH, AcOEt, rt, 73%.

LDL oxidation. Six derivatives were synthesized and their lipophilicity (log $D_{7.0}$) and water solubility were determined in comparison with compound **1** (Table 1). The effects of these derivatives on foam cell formation (esterified cholesterol accumulation by macrophage ACAT), hepatic ACAT activity, lipid peroxidation of rat brain homogenate, and oral absorption (AUC) at 10 mg/kg in rats were examined (Table 2). The effect on foam cell formation was determined as inhibitory activity against esterified cholesterol accumulation in THP-1 cells exposed to acetyl-LDL during differentiation to macrophages. ACAT inhibitory activities were determined using liver microsomes isolated from normocholesterolemic Japanese White rabbits. As shown in Tables 1 and 2, **1** was highly lipophilic and less water soluble, and its AUC was very low in rats. All derivatives with an ionizable moiety showed markedly decreased lipophilicity and increased water solubility. Among them, **5** with a carboxyl group at the end of the 7-amide moiety showed markedly increased AUC in

Table 1. Physicochemical Properties of Indoline Derivatives



1 (free form)

cmpd[a]	R^1	R^2	R^3	R^4	M.W. (free)	log$D_{7.0}$	Sol.[b]
1	H	H	H	H	358.56	6.2	0.08
2	CH_2CO_2H	H	H	H	416.60	2.9	1450
3	H	$(CH_2)_2CO_2H$	H	H	430.62	3.5	355
4	H	H	CH_2CO_2H	H	416.60	2.8	732
5	H	H	H	CH_2CO_2H	416.60	3.0	401
6	CH_2NH_2	H	H	H	387.60	2.3	857
7		H	H	H	440.62	3.7	66

[a] Compounds **1**, **3**−**5**: hydrochloride salt; compound **2**: hemisulfate salt; compound **6**: dihydrochloride salt. [b] Solubility in water at pH 7.0 (μg/mL).

Table 2. In Vitro Activities and Oral Absorption of Indoline Derivatives

cmpd	foam cell formation[a]	liver ACAT[b]	anti-Ox[c]	AUC[d]
1	0.64	0.12	3.1	0.2
2	1.8	1.2	1.3	1.5
3	3.6	5.0	4.2	1.7
4	>10	5.5	48.3	0.1
5	>10	>10	6.6	5.0
6	0.33	1.1	4.2	ND
7	>10	>10	1.7	6.6

[a] IC_{50} (μM) against esterified cholesterol accumulation during differentiation in THP-1 cells, $n = 4$. [b] IC_{50} (μM) against normocholesterolemic rabbit liver ACAT activity. Duplicate assay. [c] IC_{50} (μM) against rat brain homogenate peroxidation. Duplicate assay, $n = 2$. [d] Area under the plasma concentration−time curve (μg·h/mL) for 24 h after oral administration at 10 mg/kg in rats, $n = 3$. ND: Not detected.

Table 3. Inhibitory Effects of **2** on Foam Cell Formation and ACAT Activity[a]

	ACAT IC_{50} (μM)[c]			
foam cell formation[b] IC_{50} (μM)	macrophage	liver	intestine	adrenal
0.30 ± 0.04	1.9	0.69 ± 0.23	0.72 ± 0.24	15.9 ± 5.9

[a] Mean ± SEM. [b] THP-1 cells were exposed to acetyl-LDL after differentiation in the presence of **2**. Duplicate assay, $n = 4$. [c] Microsome fractions of macrophages, liver, intestine, and adrenal gland of hypercholesterolemic rabbits were used as enzymes. Duplicate assay, $n = 4$, macrophage; $n = 1$.

comparison with **1**, while AUC of **4** with carboxyl moiety at the end of the 1-alkyl chain was very low and similar to that of **1**. Compound **4** may have been easily glucuronized or metabolized in the liver. **4** and **5** showed weak inhibitory activity against foam cell formation and hepatic ACAT. We have reported that the introduction of a basic moiety such as imidazole, piperazine, morpholine, and dimethylamine to the end of the 1-alkyl chain markedly decreased hepatic ACAT inhibitory activity.[20] In addition, introduction of a hydroxyl, pyridinyl, and piperidinyl moiety to the end of 7-alkylamide of the 1-hexyl analogue also reduced ACAT inhibitory activity.[20] These results imply that the alkyl chain at 1- or 7-position interacts with the lipophilic pocket of the enzyme, thus an ionizable or polar moiety at these positions may disrupt the interaction. Introduction of carboxymethyl to the 5-position (**2**) and carboxyethyl to the 3-position (**3**) markedly increased oral absorption. Inhibitory activity against foam cell formation of **2** and **3** was about 3-fold and 5-fold weaker than that of **1**, respectively, and hepatic ACAT inhibitory activity was about 10-fold and 40-fold weaker than that of **1**, respectively. The antiperoxidative activity of **2** was about 2-fold stronger, and the activity of **3** was slightly weaker than that of **1**. Compound **3** was weaker than **2** in spite of higher lipophilicity. Compound **3** is racemic, thus an active enantiomer may show effects comparable to those of **2**. These results demonstrated that the 5-position was suitable for the introduction of ionizable moieties. Thus, instead of the carboxymethyl moiety, aminomethyl and tetrazol-5-ylmethyl moieties were introduced to the 5-position (**6** and **7**). Lipophilicity of **6** was similar to that of **2**, but its water solubility was lower. **6** showed 6-fold stronger antifoam cell formation activity and 3-fold weaker antiperoxidative activity than that of **2** and had hepatic ACAT inhibitory activity comparable to that of **2**; however, it was not detected in plasma after oral administration: a primary amine may have been metabolized easily. Compound **7** showed good oral absorption in spite of higher lipophilicity and lower water solubility, and had potent antiperoxidative activity, but no antifoam cell formation or hepatic ACAT inhibitory activity. Although tetrazole is known as a bioisoster of carboxylic acid, its bulky ring structure may have disturbed the interaction between **7** and ACAT protein. Avasimibe, an acyl sulfamate derivative, was also reported to be a bioavailable ACAT inhibitor.[6,11] In our experimental condition, IC_{50} values of avasimibe for antifoam cell formation and hepatic ACAT inhibitory activity were 1.5 and 7.6 μM, respectively. From these results, **2** (pactimibe sulfate) was chosen for further evaluation.

The effects of **2** on foam cell formation when THP-1 cells were exposed to acetyl-LDL after differentiation to macrophages, and on ACAT of intestine, liver, adrenal gland, and peritoneal macrophages isolated from hypercholesterolemic rabbits were examined (Table 3). Compound **2** more potently inhibited foam cell formation after differentiation than during differentiation (Tables 2 and 3), suggesting that it can inhibit foam cell formation in mature macrophages

Table 4. Inhibitory Effects of **2** on Peroxidation of Genetically Hypercholesterolemic Rabbit Serum and LDL[a]

	serum (IC_{50}, μM)[b]	LDL (IC_{50}, μM)[c]
2	4.4 ± 0.81	0.98 ± 0.10
probucol	21.6 ± 28.9	131.9 ± 106.5

[a] Mean ± SEM. [b] Duplicate assay, $n = 2$. [c] Duplicate assay, $n = 4$.

more than in differentiating macrophages. Compound **2** more potently inhibited hepatic ACAT activity in hypercholesterolemia than normocholesterolemia, indicating its high efficacy in hyperlipidemia. It also inhibited macrophage, hepatic, and intestinal ACAT more potently than adrenal ACAT, suggesting its high adrenal safety. Indeed, repeated administration of **2** had no adrenotoxicity at 100 mg/kg/day for 4 days in guinea pigs (unpublished data). ACAT is classified into subtypes: ACAT-1 in macrophages and adrenal glands, and ACAT-2 in the intestine and liver. The present data indicated that **2** is a nonselective ACAT inhibitor and thus is expected to lower plasma cholesterol levels and inhibit foam cell formation in atherosclerotic plaques. Lecithin:cholesterol acyltransferase (LCAT) also mediates esterification of cholesterol and plays an important role in reverse cholesterol transport. Compound **2** did not affect rat LCAT activity up to 10 μM (unpublished). Compound **2** markedly inhibited serum and LDL oxidation (Table 4), suggesting its in vivo inhibitory effects on foam cell formation by inhibition of ACAT activity as well as LDL oxidation. The maximal concentration (Cmax) of a free form of **2** after oral administration at a dose of 10 mg/kg was 0.92 ± 0.02 μg/mL (2.2 μM) in rats, 2.99 ± 0.75 μg/mL (7.2 μM) in rabbits, and 11.18 ± 1.39 μg/mL (26.8 μM) in dogs, which are higher than IC_{50} values for antifoam cell formation, hepatic ACAT inhibitory, and anti-LDL-peroxidative activity. Compound **2** significantly reduced plasma LDL/VLDL cholesterol levels without significantly influencing high-density lipoprotein (HDL) cholesterol levels at 1 or 3 mg/kg in rats, rabbits, and dogs fed a high-cholesterol diet and hamsters fed a regular diet (Table 5). The potent hypolipidemic effect of **2** in normocholesterolemic hamsters indicated that it was efficiently absorbed and reduced hepatic secretion of cholesterol by hepatic ACAT inhibition.

The present study proved that the introduction of carboxymethyl moiety to the 5-position of **1** remarkably improved lipophilicity and water solubility, thus increasing oral bioavailability without marked reduction of ACAT inhibitory activity. To our knowledge, **2** is the first ACAT inhibitor in which carboxylic acid moiety was successfully introduced to increase oral absorption and antihyperlipidemic effects. The antiatherosclerotic effect of **2** was not investigated in the present study; however, it has been reported that **2** reduced the atherosclerotic area in hamsters and apolipoprotein E knockout mice and stabilized plaque in Watanabe heritable hyperlipidemic rabbits (WHHL rabbits).[24–26] A clinical study using an intravascular-ultrasonography catheter failed to show the reduction of plaque volume at a dose of 100 mg in patients with coronary artery disease;[27] however, it remains to be determined whether **2** stabilized the plaque in this study because the catheter used here cannot analyze the plaque structure. Furthermore, **2** could be effective in other patient populations with a relatively high plasma HDL levels or under therapies that increase acceptor particles for reverse cholesterol transport such as HDL and apolipoprotein A-I in the plaque.[28] ACAT is involved in chronic renal failure and Alzheimer disease in addition to hyperlipidemia/atherosclerosis.[29–31] Further studies are needed to determine the efficacy of **2** in hyperlipidemia/atherosclerosis and to find new clinical uses. Compound **2** is still a potential candidate as a novel antihyperlipidemic and antiatherosclerotic drug and is also useful to clarify the pathogenetic mechanism of hyperlipidemia and atherosclerosis and species difference of the pathogenesis and role of ACAT.

Experimental Section

General Procedures. Chemicals were obtained from commercial sources and used without purification. Intermediates **8** and **14** were synthesized according to the method previously reported.[20] Reactions were monitored by thin-layer chromatography (TLC) on Merck precoated silica gel 60 F_{254} (0.25 mm) plates. Column chromatography was performed on silica gel (Daiso no. 1001W, Daiso, Osaka, Japan). Melting points were measured on a melting point apparatus (MP-21, Yamato Scientific, Tokyo, Japan) and are uncorrected. Infrared spectra (IR) were obtained with an infrared spectrometer (FT-720, HORIBA, Kyoto, Japan). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 400 MHz on a nuclear magnetic resonance spectrometer (JNM-AL400, JEOL, Tokyo, Japan) using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on a QTRAP LC/MS/MS system (API2000, Applied Biosystems, Lincoln Centre Drive Foster, CA).

***N*-(4,6-Dimethylindolin-7-yl)-2,2-dimethylpropanamide (9).** To a solution of **8** (60.0 g, 208 mmol) in MeOH (600 mL) was added 10.5 M NaOH solution (200 mL, 2.1 mol), followed by stirring at 50 °C for 2 h under nitrogen atmosphere. After evaporation of the solvent under reduced pressure, the product was extracted with $CHCl_3$ (3 × 500 mL) and the extracts were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The solid residue was rinsed with *i*-Pr_2O to give **9** (48.4 g, 94% yield). ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 9H), 2.16 (s, 3H), 2.16 (s, 3H), 2.95 (t, J = 8.4 Hz, 2H), 3.59 (t, J = 8.4 Hz, 2H), 6.40 (s, 1H), 7.05 (s, 1H). MS *m/z*: 247 $[M + H]^+$.

Methyl 10-[7-(2,2-Dimethylpropanamido)-4,6-dimethylindolin-1-yl]decanoate (10). To a solution of **9** (4.35 g, 17.7 mmol) in DMF (20 mL) were added $Br(CH_2)_9CO_2Me$ (5.5 mL, 24 mmol), diisopropylethylamine (4.6 mL, 26 mmol), and KI (2.93 g, 21.2 mmol), followed by stirring at 60 °C for 4 h. After 10% citric acid solution (100 mL) was added, the reaction mixture was extracted with AcOEt (300 mL), washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (10:1 to 3:1) to give **10** (6.58 g, 87% yield). ^{1}H NMR ($CDCl_3$): δ 1.25−1.29 (m, 8H), 1.33 (s, 9H), 1.45−1.65 (m, 6H), 2.06 (s, 3H), 2.29 (s, 3H), 2.29 (t, J = 7.5 Hz, 2H), 2.82 (t, J = 8.4 Hz, 2H), 3.13 (t, J = 7.8 Hz, 2H), 3.41 (t, J = 8.4 Hz, 2H), 3.66 (s, 3H), 6.40 (s, 1H), 6.73 (s, 1H). MS *m/z*: 431 $[M + H]^+$.

10-[7-(2,2-Dimethylpropanamido)-4,6-dimethylindolin-1-yl]decanoic Acid Hydrochloride (4). To a solution of **10** (6.33 g, 14.7 mmol) in MeOH (50 mL) was added 3.6 M NaOH solution (20 mL, 72 mmol), followed by stirring at room temperature for 2.5 h under nitrogen atmosphere. After water (50 mL) was added, the solvent was evaporated under reduced pressure, and the residue was extracted with AcOEt (2 × 250 mL). The extracts were combined, and the organic layer was extracted with H_2O (3 × 500 mL). The aqueous layers were combined, neutralized with 6 M HCl solution (13 mL), stirred for 10 min, and extracted with $CHCl_3$ (2 × 500 mL). The extracts were combined, dried over Na_2SO_4, and evaporated under reduced pressure to give **4** (5.54 g, 83% yield). The analytically pure sample was obtained by recrystallization from EtOH-*i*-Pr_2O (1:2), and then CH_2Cl_2-*i*-Pr_2O (1:2); mp 179 °C (CH_2Cl_2-*i*-Pr_2O). IR (ATR): 3254, 1738, 1674 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.25−1.33 (m, 10H), 1.42 (s, 9H), 1.56−1.73 (m, 3H), 1.96−2.10 (m, 1H), 2.15 (s, 3H), 2.26 (s, 3H), 2.32 (t, J = 7.3 Hz, 2H), 3.03−3.27 (m, 4H), 3.62−4.14 (m, 2H), 7.12 (s, 1H), 9.20 (s, 1H), 13.80−14.00 (br, 1H). MS *m/z*: 417 $[M + H]^+$. Anal. ($C_{25}H_{40}N_2O_3 \cdot HCl \cdot 0.1H_2O$) C, H, N.

Table 5. Cholesterol-Lowering Effects of **2** in Rats, Rabbits, Dogs, and Hamsters[a]

		rat[b]	rabbit[c]	dog[d]	hamster[e]
LDL/VLDL (mg/dL)	control	72.9 ± 8.3	606.4 ± 4.5	103.6 ± 22.2	115.6 ± 4.8
	2	9.7 ± 2.7[f]	119.5 ± 39.1[f]	31.6 ± 8.4[f]	70.3 ± 4.5[f]
HDL (mg/dL)	control	30.4 ± 0.5	21.4 ± 5.3	150.4 ± 6.5	66.3 ± 3.1
	2	29.4 ± 1.1	15.4 ± 3.1	141.3 ± 9.3	58.3 ± 4.4

[a] Mean ± SEM. [b] 3 mg/kg/day for 3 days, $n = 5$. [c] 1 mg/kg/day for 7 days, $n = 5$. [d] 3 mg/kg/day for 7 days, $n = 3$. [e] 3 mg/kg/day for 3 weeks, $n = 5$. [f] $p < 0.01$, vs control, Student's *t*-test. LDL/VLDL: LDL/VLDL cholesterol. HDL: HDL cholesterol.

***N*-[1-Acetyl-5-(phthalimido-2-ylmethyl)-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (11b).** To a stirred solution of **8** (20.0 g, 69.4 mmol) in concentrated hydrochloric acid (100 mL) was added 35% formalin (8.2 mL, 104 mmol), and the reaction mixture was bubbled with HCl gas for 30 min, followed by stirring at 50 °C for 1.5 h. The reaction mixture was poured into ice water and extracted with $CHCl_3$ (3 × 1 L). The extracts were combined, washed with water and brine, dried over Na_2SO_4, and evaporated to give crude **11a** (21.6 g). To a stirred suspension of the obtained **11a** (8.0 g) in DMF (60 mL) was added potassium phthalimide (4.82 g, 26.0 mmol), followed by stirring at room temperature for 16 h under nitrogen atmosphere. After addition of water, the reaction mixture was extracted with AcOEt (300 mL) and the extract was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with $CHCl_3$:MeOH (20:1) to give **11b** (10.7 g, 96% yield). ^{1}H NMR ($CDCl_3$): δ 1.25 (s, 9H), 2.24 (s, 3H), 2.29 (s, 3H), 2.34 (s, 3H), 2.80−3.23 (m, 2H), 3.95−4.30 (m, 2H), 4.80−5.05 (m, 2H), 7.66−7.78 (m, 4H), 9.14 (s, 1H). MS *m*/*z*: 448 $[M + H]^+$.

***N*-(1-Acetyl-5-*tert*-butoxycarbonylaminomethyl-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (11c).** A solution of **11b** (5.97 g, 13.3 mmol) and hydrazine monohydrate (1.0 mL, 20.6 mmol) in MeOH (50 mL) and $CHCl_3$ (25 mL) was refluxed for 1 h. After the solvent was evaporated under reduced pressure, the residue was diluted with $CHCl_3$ (200 mL), washed with a saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated. To a solution of the obtained residue in $CHCl_3$ (50 mL) was added Boc_2O (2.99 g, 13.7 mmol), followed by stirring at room temperature for 1 h. The reaction mixture was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with $CHCl_3$:MeOH (50:1) to give **11c** (4.05 g, 71% yield). ^{1}H NMR ($CDCl_3$): δ 1.28 (s, 9H), 1.44 (s, 9H), 2.19 (s, 3H), 2.24 (s, 3H), 2.31 (s, 3H), 2.82−3.22 (m, 2H), 3.95−4.48 (m, 5H), 9.14 (s, 1H). MS *m*/*z*: 416 $[M - H]^-$.

***N*-(1-Acetyl-5-cyanomethyl-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (11e).** A suspension of crude **11a** (8.0 g), NaCN (3.57 g, 71.8 mmol) and 18-crown-6 (330 mg, 1.25 mmol) in MeCN (50 mL) was refluxed under nitrogen atmosphere for 3.5 h. After the solvent was evaporated under reduced pressure, the residue was diluted with $CHCl_3$ (300 mL), washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with $CHCl_3$ to give **11e** (7.70 g, 95% yield). ^{1}H NMR ($CDCl_3$): δ 1.28 (s, 9H), 2.21 (s, 3H), 2.27 (s, 3H), 2.32 (s, 3H), 2.79−3.28 (m, 2H), 3.62−3.70 (m, 2H), 3.96−4.27 (m, 2H), 9.20 (s, 1H). MS *m*/*z*: 328 $[M + H]^+$.

***N*-[1-Acetyl-4,6-dimethyl-5-(1*H*-tetrazol-5-ylmethyl)indolin-7-yl]-2,2-dimethylpropanamide (11g).** To a stirred suspension of **11e** (2.00 g, 6.11 mmol) in DMF (40 mL) were added $Me_3NH^+Cl^-$ (5.83 g, 61.0 mmol) and NaN_3 (3.96 g, 60.9 mmol), followed by stirring at 120 °C for 3 h under nitrogen atmosphere. After cooling, 1 M hydrochloric acid (100 mL) was added and the reaction mixture was extracted with AcOEt (2 × 150 mL), the extracts were combined, washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with $CHCl_3$:MeOH (100:1 to 10:1) to give **11g** (1.70 g, 72% yield). ^{1}H NMR (DMSO-d_6): δ 1.15 (s, 9H), 1.99 (s, 3H), 2.19 (s, 3H), 2.28 (s, 3H), 2.80−3.20 (m, 2H), 3.80−4.10 (m, 1H), 4.20−4.45 (m, 2H), 9.22 (s, 1H). MS *m*/*z*: 371 $[M + H]^+$.

***N*-[1-Acetyl-5-(1-benzyltetrazol-5-ylmethyl)-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (11h) and *N*-[1-Acetyl-5-(2-benzyltetrazol-5-ylmethyl)-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (11i).** To a stirred suspension of **11g** (8.70 g, 23.5 mmol) in DMF (55 mL) was added a 60% suspension of NaH in mineral oil (1.17 g, 29 mmol) portionwise in an ice bath. After stirring at room temperature for 30 min, benzyl chloride (3.9 mL, 34 mmol) was added, followed by stirring for 17 h. After addition of water (200 mL), the reaction mixture was extracted with AcOEt (3 × 500 mL) and the extracts were combined, washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with AcOEt to give a mixture of **11h** and **11i** (10.1 g, 93% yield). ^{1}H NMR ($CDCl_3$): δ **11h**: 1.27 (s, 9H), 1.80 (s, 3H), 1.91 (s, 3H), 2.31 (s, 3H), 2.65−3.25 (m, 2H), 3.95−4.20 (m, 4H), 5.38−5.53 (m, 2H), 7.26−7.35 (m, 5H), 9.27 (s, 1H); **11i**: 1.27 (s, 9H), 2.20 (s, 3H), 2.25 (s, 3H), 2.29 (s, 3H), 2.87−3.16 (m, 2H), 3.95−4.20 (m, 2H), 4.20−4.30 (m, 2H), 5.65 (s, 2H), 7.25−7.40 (m, 5H), 9.17 (s, 1H). MS *m*/*z*: 461 $[M + H]^+$.

***N*-(5-*tert*-Butoxycarbonylaminomethyl-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (12c).** To a solution of **11c** (3.58 g, 8.57 mmol) in MeOH (60 mL) was added a 2.5 M NaOH solution (18 mL, 45 mmol), followed by stirring at 50 °C for 22 h under nitrogen atmosphere. After addition of water (50 mL), the solvent was evaporated under reduced pressure, and the residue was extracted with $CHCl_3$ (3 × 200 mL). The extracts were combined, washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give **12c** (2.88 g, 89% yield). ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 9H), 1.44 (s, 9H), 2.19 (s, 3H), 2.21 (s, 3H), 3.00 (t, $J = 8.3$ Hz, 2H), 3.58 (t, $J = 8.3$ Hz, 2H), 4.20−4.40 (m, 4H), 7.05 (s, 1H). MS *m*/*z*: 374 $[M - H]^-$.

***N*-[5-(1-Benzyltetrazol-5-ylmethyl)-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (12h) and *N*-[5-(2-Benzyltetrazol-5-ylmethyl)-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (12i).** A mixture of **12h** and **12i** was prepared from the mixture of **11h** and **11i** using the same procedure as for **12c** (88% yield). ^{1}H NMR ($CDCl_3$): δ **12h**: 1.33 (s, 9H), 1.80 (s, 3H), 1.86 (s, 3H), 2.92−3.00 (m, 2H), 3.58 (t, $J = 8.0$ Hz, 2H), 4.06 (s, 2H), 5.36 (s, 2H), 6.92 (s, 1H), 7.29−7.38 (m, 5H); **12i**: 1.34 (s, 9H), 2.22 (s, 3H), 2.26 (s, 3H), 3.00 (t, $J = 8.3$ Hz, 2H), 3.56 (t, $J = 8.3$ Hz, 2H), 4.15 (s, 2H), 5.66 (s, 2H), 7.06 (s, 1H), 7.32−7.38 (m, 5H). MS *m*/*z*: 419 $[M + H]^+$.

***N*-(5-Carbamoylmethyl-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (12j).** Compound **12j** was prepared from **11e** using the same procedure as for **12c** (76% yield). ^{1}H NMR (DMSO-d_6): δ 1.22 (s, 9H), 1.96 (s, 3H), 2.08 (s, 3H), 2.82−2.98 (m, 2H), 3.30−3.45 (m, 4H), 4.43 (s, 1H), 6.80 (s, 1H), 7.07 (s, 1H), 8.63 (s, 1H). MS *m*/*z*: 304 $[M + H]^+$.

***N*-(5-*tert*-Butoxycarbonylaminomethyl-4,6-dimethyl-1-octylindolin-7-yl)-2,2-dimethylpropanamide (13c).** A solution of **12c** (2.20 g, 5.86 mmol), octyl iodide (1.27 mL, 7.03 mmol), and diisopropylethylamine (1.53 mL, 8.78 mmol) in DMF (19 mL) was stirred at 60 °C for 60 h under nitrogen atmosphere. After addition of 10% citric acid solution (50 mL), the reaction mixture was extracted with AcOEt (3 × 150 mL) and the extracts were combined, washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (5:1) to give **13c** (2.47 g, 86% yield). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, $J = 6.8$ Hz, 3H), 1.22−1.32 (m, 10H), 1.34 (s, 9H), 1.43 (s, 9H), 1.58 (s, 2H), 2.09 (s, 3H), 2.17 (s, 3H), 2.86−2.92 (m, 2H), 3.13 (t, $J = 7.8$ Hz, 2H),

3.38−3.46 (m, 2H), 4.23−4.29 (m, 2H), 4.35 (s, 1H), 6.77 (s, 1H). MS *m/z*: 488 $[M + H]^+$.

***N*-[5-(1-Benzyltetrazol-5-ylmethyl)-4,6-dimethyl-1-octylindolin-7-yl]-2,2-dimethylpropanamide (13h) and *N*-[5-(2-Benzyltetrazol-5-ylmethyl)-4,6-dimethyl-1-octylindolin-7-yl]-2,2-dimethylpropanamide (13i).** A mixture of **13h** and **13i** was prepared from the mixture of **12h** and **12i** using the same procedure as for **13c** (91% yield). ^{1}H NMR ($CDCl_3$): δ **13h**: 0.85−0.90 (m, 3H), 1.22−1.30 (m, 10H), 1.40−1.55 (m, 2H), 1.74 (s, 3H), 1.76 (s, 3H), 2.74−2.82 (m, 2H), 3.05−3.20 (m, 2H), 3.35−3.43 (m, 2H), 4.02 (s, 2H), 5.39 (s, 2H), 6.75 (s, 1H), 7.27−7.37 (m, 5H); **13i**: 0.87 (t, $J = 6.6$ Hz, 3H), 1.22−1.30 (m, 10H), 1.33 (s, 9H), 1.40−1.58 (m, 2H), 2.11 (s, 3H), 2.20 (s, 3H), 2.88 (t, $J = 8.6$ Hz, 2H), 3.10 (t, $J = 7.6$ Hz, 2H), 3.39 (t, $J = 8.6$ Hz, 2H), 4.12 (s, 2H), 5.66 (s, 2H), 6.78 (s, 1H), 7.30−7.40 (m, 5H). MS *m/z*: 538 $[M + H]^+$.

***N*-(5-Carbamoylmethyl-4,6-dimethyl-1-octylindolin-7-yl)-2,2-dimethylpropanamide (13j).** To a suspension of **12j** (5.00 g, 16.5 mmol) in DMF (25 mL) were added octyl bromide (6.1 g, 32 mmol), K_2CO_3 (4.3 g, 31 mmol), and KI (0.52 g, 3.1 mmol), followed by stirring at 40 °C for 23 h. After addition of water (150 mL), the reaction mixture was extracted with AcOEt (150 and 80 mL), the extracts were combined, washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was suspended in *n*-hexane, stirred at 60 °C for 10 min, and then cooled. After the precipitate was collected by filtration, the product was dissolved in AcOEt (15 mL) by heating, and to the solution hot *n*-hexane (24 mL) was added and allowed to standing at room temperature for 14 h. The precipitate was collected by filtration to give **13j** (5.20 g, 76% yield). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 6.9$ Hz, 3H), 1.20−1.29 (m, 12H), 1.28 (s, 9H), 2.03 (s, 3H), 2.12 (s, 3H), 2.90 (t, $J = 8.6$ Hz, 2H), 3.10−3.25 (m, 2H), 3.43 (t, $J = 8.6$ Hz, 2H), 3.53 (s, 2H), 5.29 (s, 1H), 5.54 (s, 1H), 6.86 (s, 1H). MS *m/z*: 416 $[M + H]^+$.

[7-(2,2-Dimethylpropanamido)-4,6-dimethyl-1-octylindolin-5-yl]acetic Acid Hemisulfate (2, pactimibe sulfate). To a suspension of **13j** (4.50 g, 10.8 mmol) in *n*-PrOH and H_2O (3:1) (25 mL) was added 2.3 M NaOH solution (9.0 mL, 21 mmol), followed by refluxing for 23 h under nitrogen atmosphere. After evaporation of the solution under reduced pressure, the residue was dissolved in AcOEt (60 mL), washed with 1 M NaOH solution (3 × 60 mL), and extracted with water (120 mL). The aqueous extract was washed with AcOEt (30 mL), acidified with 4 M H_2SO_4 solution (15 mL) to pH 1−2, stirred for 2 h, and allowed to stand for 1 h at room temperature. The formed precipitate was collected by filtration to give **2** (3.90 g, 78% yield). The analytically pure sample was obtained by recrystallization of **2** (3.7 g) from 50% EtOH (60 mL) containing H_2SO_4 (0.8 g) and hot water (130 mL); mp 167−169 °C (EtOH-H_2O). IR (ATR): 1736, 1682, 1670, 1498 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.82−0.90 (m, 3H), 1.20−1.35 (m, 19H), 1.49−1.60 (m, 2H), 1.98 (s, 3H), 2.14 (s, 3H), 2.95−3.10 (m, 2H), 3.12−3.20 (m, 2H), 3.53−3.67 (m, 4H), 8.83−8.97 (br, 1H). Anal. ($C_{25}H_{40}N_2O_3 \cdot {}^1/_2H_2SO_4 \cdot 0.2H_2O$) C, H, N.

***N*-(5-Aminomethyl-4,6-dimethyl-1-octylindolin-7-yl)-2,2-dimethylpropanamide Dihydrochloride (6).** To a solution of **13c** (5.47 g, 11.2 mmol) in HCO_2H (20 mL) was added 9.25 M HCl in *i*-PrOH (3.6 mL, 33 mmol) in an ice bath, followed by stirring at the same temperature for 1 h. *n*-Hexane (150 mL) was mixed, and the supernatant was removed by decantation. After the manipulation was performed 3 times, the residue was diluted in $CHCl_3$ (100 mL), washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the residue in $CHCl_3$ (20 mL) was added 9.25 M HCl in *i*-PrOH (2.4 mL, 22 mmol) in an ice bath, followed by stirring at the same temperature for 10 min. After *i*-Pr_2O was added to the reaction solution, the formed precipitate was collected by filtration to give **6** (4.0 g, 73% yield). The analytically pure sample was obtained by recrystallization from EtOH-AcOEt (1:5); mp 198−200 °C (EtOH-AcOEt). IR (ATR): 3342, 1687 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, $J = 6.8$ Hz, 3H), 1.18−1.36 (m, 10H), 1.41 (s, 9H), 1.64−1.80 (m, 1H), 1.94−2.08 (m, 1H), 2.26 (s, 3H), 2.39 (s, 3H), 2.98−3.44 (m, 4H), 3.64−4.08 (m, 2H), 4.08−4.26 (m, 2H), 8.28−8.46 (m, 4H), 9.39 (s, 1H). MS *m/z*: 388 $[M + H]^+$. Anal. ($C_{24}H_{41}N_3O \cdot 2HCl \cdot 0.1H_2O$) C, H, N.

***N*-[4,6-Dimethyl-1-octyl-5-(1*H*-tetrazol-5-ylmethyl)indolin-7-yl]-2,2-dimethylpropanamide (7).** A solution of the mixture of **13h** and **13i** (4.03 g, 7.59 mmol) in AcOH (80 mL) was hydrogenated at 0.4 MPa in the presence of PdO (1.0 g) at room temperature for 4 days. After removal of the catalyst by filtration, water (200 mL) was added and the mixture was neutralized with K_2CO_3 and extracted with AcOEt (2 × 400 mL). The extracts were combined, washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (5:1 to 1:1) to give **7** as a foam (2.40 g, 72% yield). The analytically pure sample was obtained by recrystallization from AcOEt; mp 181 °C (AcOEt). IR (ATR): 3236, 1641, 1601 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.22−1.34 (m, 10H), 1.36 (s, 9H), 1.45−1.56 (m, 2H), 1.97 (s, 3H), 2.02 (s, 3H), 2.80 (t, $J = 8.6$ Hz, 2H), 3.14 (t, $J = 7.7$ Hz, 2H), 3.39 (t, $J = 8.6$ Hz, 2H), 4.08 (s, 2H), 7.16 (s, 1H). MS *m/z*: 441 $[M + H]^+$. Anal. ($C_{25}H_{40}N_6O \cdot 0.1H_2O$) C, H, N.

5-Bromo-4,6-dimethyl-7-nitroindoline (15). To a stirred suspension of **14** (6.63 g, 21.2 mmol) in MeOH (66 mL) was added 5.0 M NaOH solution (21 mL, 0.11 mol), followed by refluxing for 30 min under nitrogen atmosphere. After cooling, water was added and a precipitate was collected by filtration to give **15** (5.57 g, 97% yield). ^{1}H NMR ($CDCl_3$): δ 2.30 (s, 3H), 2.66 (s, 3H), 3.11 (t, $J = 8.5$ Hz, 2H), 3.82 (t, $J = 8.5$ Hz, 2H), 6.41 (s, 1H). MS *m/z*: 268, 270 $[M - H]^-$.

5-Bromo-4,6-dimethyl-7-nitro-1-octylindoline (16). To a solution of **15** (8.00 g, 29.5 mmol) and octyl iodide (8.1 mL, 45 mmol) in DMF (80 mL) was added a 60% suspension of NaH in mineral oil (2.84 g, 71 mmol) at 0 °C, followed by stirring at 50 °C for 14 h under nitrogen atmosphere. Water was added and the mixture was extracted with AcOEt (320 mL), washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (20:1) to give **16** (10.5 g, 93% yield). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 7.1$ Hz, 3H), 1.20−1.35 (m, 10H), 1.40−1.50 (m, 2H), 2.25 (s, 3H), 2.29 (s, 3H), 2.90−3.00 (m, 4H), 3.57 (t, $J = 8.6$ Hz, 2H). MS *m/z*: 383, 385 $[M + H]^+$.

7-Amino-4,6-dimethyl-1-octylindoline (17). A solution of **16** (13.3 g, 34.7 mmol) in MeOH and THF (3:1) (130 mL) was hydrogenated at 0.3 MPa in the presence of 10% Pd−C (1.33 g) at room temperature for 15 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was diluted with AcOEt (500 mL), washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (8:1 to 1:1) to give **17** (7.52 g, 79% yield). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.20−1.40 (m, 10H), 1.50−1.60 (m, 2H), 2.12 (s, 3H), 2.13 (s, 3H), 2.88 (t, $J = 8.3$ Hz, 2H), 2.95−3.02 (m, 2H), 3.25−3.40 (m, 2H), 3.42 (t, $J = 8.3$ Hz, 2H), 6.48 (s, 1H). MS *m/z*: 275 $[M + H]^+$.

Methyl 4-(4,6-Dimethyl-1-octylindolin-7-yl)carbamoyl-4-methylpentanoate (18). To a stirred solution of **17** (2.60 g, 9.47 mmol) and Et_3N (3.2 mL, 23 mmol) in CH_2Cl_2 (26 mL) was added $ClC(O)C(CH_3)_2CH_2CH_2CO_2Me$[21] (2.20 g, 11.4 mmol) at 0 °C, followed by stirring at room temperature for 9 h. The solution was washed with 5% citric acid solution, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (4:1) to give **18** (3.60 g, 88% yield). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.20−1.30 (m, 10H), 1.33 (s, 6H), 1.45−1.55 (m, 2H), 1.95−2.05 (m, 2H), 2.07 (s, 3H), 2.11 (s, 3H), 2.40−2.50 (m, 2H), 2.83 (t, $J = 8.5$ Hz, 2H), 3.08−3.14 (m, 2H), 3.41 (t, $J = 8.5$ Hz, 2H), 3.66 (s, 3H), 6.41 (s, 1H), 6.79 (s, 1H). MS *m/z*: 431 $[M + H]^+$.

4-(4,6-Dimethyl-1-octylindolin-7-yl)carbamoyl-4-methylpentanoic Acid (19). To a stirred solution of **18** (500 mg, 1.16 mmol) in MeOH (5 mL) was added 3.6 M NaOH solution (2.3 mL, 8.3 mmol) at room temperature, followed by stirring at the same temperature for 6 h. After the solvent was evaporated under reduced pressure, the reaction mixture was extracted with AcOEt (50 mL), washed with 5% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with $CHCl_3$:MeOH (1:1) to give **19** (449 mg, 93% yield). ^{1}H NMR ($CDCl_3$): δ 0.85 (t, J = 7.1 Hz, 3H), 1.15−1.25 (m, 10H), 1.27 (s, 6H), 1.50−1.60 (m, 2H), 2.00−2.10 (m, 2H), 2.11 (s, 3H), 2.16 (s, 3H), 2.35−2.45 (m, 2H), 2.91 (t, J = 8.3 Hz, 2H), 3.00−3.10 (m, 2H), 3.42 (t, J = 8.3 Hz, 2H), 6.71 (s, 1H), 8.62 (s, 1H).

4-(4,6-Dimethyl-1-octylindolin-7-yl)carbamoyl-4-methylpentanoic Acid Hydrochloride (5). To a stirred solution of **19** (3.30 g, 7.92 mmol) in AcOEt (5 mL) was added 9.25 M HCl in *i*-PrOH (1.0 mL, 9.3 mmol) in an ice bath, followed by stirring at the same temperature for 30 min. The precipitate was collected by filtration and washed with AcOEt to give **5** as a crystalline prduct (2.40 g, 68% yield); mp 136−139 °C. IR (ATR): 1726, 1651 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.84 (t, J = 7.1 Hz, 3H), 1.15−1.25 (m, 10H), 1.27 (s, 6H), 1.55−1.75 (m, 2H), 1.80−1.90 (m, 2H), 2.07 (s, 3H), 2.21 (s, 3H), 2.15−2.25 (m, 2H), 3.00−3.20 (m, 4H), 3.70−3.85 (m, 2H), 7.08 (s, 1H), 9.30 (s, 1H). MS m/z: 417 [M + H]$^+$. Anal. ($C_{25}H_{40}N_2O_3 \cdot HCl \cdot 0.4H_2O$) C, H, N.

1-Ethyl 2-[(3,5-Dimethylphenyl)hydrazono]hexanedioate (20). To a suspension of 3,5-dimethylaniline (25.0 g, 206 mmol) in 4 M hydrochloric acid (200 mL) was added an aqueous solution of $NaNO_2$ (14.2 g, 206 mmol/50 mL) below 5 °C, followed by stirring at room temperature for 30 min. On the other hand, to a solution of ethyl cyclopentanone-2-carboxylate (32.2 g, 206 mmol) in EtOH (150 mL) was added Na (4.74 g, 206 mmol) in an ice bath, followed by stirring at room temperature for 2 h. To an aqueous solution of AcONa (25.4 g, 309 mmol/150 mL) was added the two prepared reaction mixtures simultaneously in an ice bath, followed by stirring at the same temperature for 3 h. The reaction mixture was neutralized with 5% $NaHCO_3$ solution, extracted with AcOEt (3 × 150 mL), the organic layers were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (10:1) to give **20** (49.7 g, 79% yield). ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 7.1 Hz, 3H), 1.85−2.00 (m, 1H), 2.00−2.15 (m, 1H), 2.26 (s, 1H), 2.36 (s, 6H), 2.36−2.55 (m, 2H), 2.60−2.80 (m, 2H), 4.25−4.35 (m, 2H), 7.10 (s, 1H), 7.33 (s, 2H). MS m/z: 305 [M − H]$^-$.

Ethyl 3-(2-Ethoxycarbonylethyl)-4,6-dimethylindole-2-carboxylate (21). To a solution of **20** (38.6 g, 126 mmol) in EtOH (386 mL) was added concentrated hydrochloric acid (17.2 mL) at room temperature, followed by refluxing for 17 h. After cooling, the reaction solution was neutralized with saturated $NaHCO_3$ solution, concentrated under reduced pressure, and the reaction mixture was extracted with $CHCl_3$ (1 L). The extract was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (10:1 to 5:1) to give **21** (34.1 g, 85% yield). ^{1}H NMR ($CDCl_3$): δ 1.25 (t, J = 7.1 Hz, 3H), 1.42 (t, J = 7.3 Hz, 3H), 2.40 (s, 3H), 2.69 (s, 3H), 2.60−2.65 (m, 2H), 3.50−3.60 (m, 2H), 4.15 (t, J = 7.1 Hz, 2H), 4.40 (t, J = 7.3 Hz, 2H), 6.71 (s, 1H), 6.98 (s, 1H), 8.63 (s, 1H). MS m/z: 316 [M − H]$^-$.

3-(2-Carboxyethyl)-4,6-dimethylindole-2-carboxylic Acid (22). To a solution of **21** (39.5 g, 124 mmol) in EtOH (390 mL) was added NaOH (24.8 g, 620 mmol) at room temperature, followed by refluxing for 4 h. After cooling, the precipitate was collected by filtration and dissolved in H_2O (300 mL). The aqueous solution was acidified with 10% citric acid solution and the precipitate was collected to give **22** (27.3 g, 84% yield). ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 3H), 2.60 (s, 3H), 2.40−2.50 (m, 2H), 3.35−3.45 (m, 2H), 6.60 (s, 1H), 7.00 (s, 1H), 11.28 (s, 1H), 12.30−12.60 (br, 2H). MS m/z: 260 [M − H]$^-$.

Ethyl 3-(4,6-Dimethylindol-3-yl)propionate (23). 22 (27.3 g, 104 mmol) was melted by heating at 220 °C for 10 min. After cooling, the residue was dissolved with EtOH (500 mL), and 9.25 M HCl in *i*-PrOH (16.9 mL, 156 mmol) was added, followed by refluxing for 30 min. After cooling, the reaction solution was neutralized with saturated $NaHCO_3$ solution and brine, evaporated under reduced pressure, and the residue was extracted with AcOEt (1 L). The extract was washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. The solid residue was stirred in *i*-Pr_2O (100 mL) for 30 min and collected by filtration to give **23** (21.3 g, 83% yield). ^{1}H NMR (DMSO-d_6): δ 1.17 (t, J = 7.1 Hz, 3H), 2.30 (s, 3H), 2.55 (s, 3H), 2.62 (t, J = 7.6 Hz, 2H), 3.09 (t, J = 7.6 Hz, 2H), 4.06 (t, J = 7.1 Hz, 2H), 6.52 (s, 1H), 6.90−6.93 (m, 2H), 10.57 (s, 1H). MS m/z: 246 [M + H]$^+$.

Ethyl 3-(1-Acetyl-4,6-dimethylindolin-3-yl)propionate (24). To a suspension of **23** (19.3 g, 78.7 mmol) in AcOH (190 mL) was added $NaBH_3CN$ (11.0 g, 175 mmol) between 10−15 °C, followed by stirring at the same temperature for 1.5 h. The reaction solution was neutralized with saturated $NaHCO_3$ solution and extracted with AcOEt (500 mL). The extract was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the residue in $CHCl_3$ (290 mL) was added Ac_2O (8.9 mL, 94 mmol) in an ice bath, followed by stirring for 2 h at room temperature. The reaction solution was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (2:1) to give **24** (21.4 g, 94% yield). ^{1}H NMR ($CDCl_3$): δ 1.25 (t, J = 7.1 Hz, 3H), 1.75−1.85 (m, 1H), 1.95−2.10 (m, 1H), 2.22 (s, 3H), 2.29 (s, 3H), 2.30 (s, 3H), 2.25−2.35 (m, 2H), 3.30−3.40 (m, 1H), 3.77 (dd, J = 10.2, 2.2 Hz, 1H), 4.04 (t, J = 10.2 Hz, 1H), 4.11 (q, J = 7.1 Hz, 2H), 6.68 (s, 1H), 7.90 (s, 1H). MS m/z: 290 [M + H]$^+$.

Ethyl 3-(1-Acetyl-5-bromo-4,6-dimethylindolin-3-yl)propionate (25). To a solution of **24** (15.0 g, 51.8 mmol) in AcOH (150 mL) was added Br_2 (3.3 mL, 65 mmol) in an ice bath, followed by stirring at room temperature for 1 h. Then 10% $NaHSO_3$ solution was added and the precipitate was collected by filtration. The obtained solid was dissolved with $CHCl_3$ (500 mL), washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The solid residue was rinsed with *i*-Pr_2O to give **25** (17.4 g, 91% yield). ^{1}H NMR ($CDCl_3$): δ 1.25 (t, J = 7.1 Hz, 3H), 1.70−1.85 (m, 1H), 1.90−2.05 (m, 1H), 2.22 (s, 3H), 2.30 (t, J = 7.3 Hz, 2H), 2.36 (s, 3H), 2.40 (s, 3H), 3.35−3.45 (m, 1H), 3.79 (dd, J = 10.2, 1.7 Hz, 1H), 4.04 (t, J = 10.2 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 8.01 (s, 1H). MS m/z: 368, 370 [M + H]$^+$.

Ethyl 3-(1-Acetyl-5-bromo-4,6-dimethyl-7-nitroindolin-3-yl)propionate (26). To a solution of fuming HNO_3 (3.2 mL, 71 mmol) in AcOH (120 mL) and concentrated H_2SO_4 (60 mL) was added **25** (17.4 g, 47.2 mmol) in an ice bath, followed by stirring at the same temperature for 6.5 h. Ice water was poured into the reaction mixture, and the precipitate was collected by filtration. The obtained solid was dissolved in $CHCl_3$ (500 mL), washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with $CHCl_3$:MeOH (10:1) to give **26** (18.4 g, 94% yield). ^{1}H NMR ($CDCl_3$): δ 1.27 (t, J = 7.1 Hz, 3H), 1.66−1.78 (m, 1H), 1.92−2.02 (m, 1H), 2.24 (s, 3H), 2.30−2.40 (m, 2H), 2.45 (s, 3H), 2.48 (s, 3H), 3.25−3.35 (m, 1H), 3.90−4.00 (m, 1H), 4.10−4.20 (m, 3H). MS m/z: 448 [M + H]$^+$.

3-[4,6-Dimethyl-7-(2,2-dimethylpropanamido)-1-octylindolin-3-yl]propionic Acid (27). A solution of **26** (19.8 g, 47.9 mmol) in MeOH (400 mL) was hydrogenated at 0.3 MPa in the presence of 10% Pd−C (1.98 g) at room temperature for 14 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (300 mL), washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the residue and Et_3N (7.3 mL, 52 mmol) in $CHCl_3$ (200 mL) was added pivaloyl chloride (5.8 mL, 48 mmol) in an ice bath, followed by stirring at room temperature for 30 min. The mixture was washed with 5% citric acid solution and brine, dried over Na_2SO_4, and evaporated under

reduced pressure. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (1:1) to give a mixture of a methyl ester derivative and an ethyl ester one (17.5 g). To a solution of the obtained mixture (5.94 g) in MeOH (50 mL) was added 5.0 M NaOH solution (15.5 mL, 78 mmol), followed by refluxing for 3.5 h. After cooling, water (50 mL) was added to the reaction solution and the solvent was evaporated under reduced pressure. The residue was acidified with citric acid until pH 4, extracted with AcOEt (200 mL), washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the residue and octyl aldehyde (1.99 g, 15.5 mmol) in MeOH (15 mL) was added $NaBH_3CN$ (1.08 g, 15.5 mmol) at room temperature, followed by stirring for 1.5 h at the same temperature. After addition of water (20 mL), the solvent was evaporated under reduced pressure and the reaction mixture was extracted with AcOEt (200 mL) the extract was washed with water and brine. The residue was purified by column chromatography, eluting with *n*-hexane:AcOEt (2:1) to give **27** as a foam (4.23 g, 60% yield). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 6.8 Hz, 3H), 1.20−1.50 (m, 21H), 1.80−1.95 (m, 2H), 2.08 (s, 3H), 2.17 (s, 3H), 2.20−2.35 (m, 2H), 2.85−2.95 (m, 1H), 3.20−3.35 (m, 4H), 6.46 (s, 1H), 7.28 (s, 1H).

3-[4,6-Dimethyl-7-(2,2-dimethylpropanamido)-1-octylindolin-3-yl]propionic Acid Hydrochloride (3). To a solution of **27** (3.77 g, 8.75 mmol) in AcOEt (35 mL) was added 9.25 M HCl in *i*-PrOH (1.1 mL, 10 mmol) in an ice bath, followed by stirring at the same temperature for 30 min. The precipitate was collected by filtration to give **3** as a crystalline product (2.99 g, 73% yield); mp 178−182 °C. IR (ATR): 1713, 1686 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 0.80−0.90 (m, 3H), 1.15−1.30 (m, 19H), 1.50−1.75 (m, 3H), 1.95−2.05 (m, 1H), 2.04 (s, 3H), 2.27 (s, 3H), 2.30−2.40 (m, 2H), 3.00−3.30 (m, 2H), 3.40−3.80 (m, 3H), 6.94 (s, 1H), 9.15 (s, 1H). MS *m*/*z*: 431 [M + H]$^+$. Anal. ($C_{26}H_{42}N_2O_3 \cdot HCl \cdot 0.2H_2O$) C, H, N.

Partition Coefficient at pH 7.0. Octanol−water partition coefficients at pH 7.0 were determined and log $D_{7.0}$ values were calculated as an index of lipophilicity. First, 5 mg of compounds were weighed and dissolved in 5 mL of octanol and mixed with 5 mL of phosphate buffer (1/15 M K_2HPO_4/KH_2PO_4, pH 7.0). The mixed solution was vigorously shaken at room temperature for 30 min and then centrifuged at 3000 rpm for 10 min at room temperature. Concentrations in the octanol fraction and buffer fraction were determined using HPLC. The HPLC equipment consisted of a pump (PU-980, JASCO, Tokyo, Japan), a UV detector (Shodex EC-1, SHOWA DENKO, Tokyo, Japan), an autoinjector (AS-950, JASCO), and a Develosil-ODS-UG-5 column (5 μm, 4.6 mm × 150 mm, NOMURA Chemical, Seto, Japan). The octanol−water partition coefficent was calculated as the ratio of concentration in the octanol fraction to that in the buffer fraction. The log $D_{7.0}$ value was determined as the logarithm of a partition coefficient.

Water Solubility. First, 6 mg of compounds were placed in 3 mL phosphate buffer (1/15 M K_2HPO_4/KH_2PO_4, pH 7.0), vigorously shaken for 30 min at room temperature, and then centrifuged at 3000 rpm for 10 min at room temperature. The supernatant was filtrated using DISMIC-13HP (0.45 μm, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). Concentrations of compounds in the filtrate were determined using HPLC as described above.

Lipid Peroxidation in Rat Brain Homogenate. The production of lipid peroxide was determined as malondialdehyde (MDA) in the rat brain homogenate, as reported previously.[18,20] Briefly, the cerebral cortex isolated from male SD rats (6 weeks old, Japan SLC, Shizuoka, Japan) was homogenized in ice-cold 50 mM phosphate-buffered saline (pH 7.4). The homogenate was centrifuged at 1300*g* for 10 min at 4 °C. The supernatant was incubated at 37 °C for 30 min in the presence of vehicle or test compounds. The reaction was terminated by addition of 20% trichloroacetic acid, and the mixture was immediately centrifuged at 2000*g* for 15 min at 4 °C. The supernatant was heated at 100 °C for 15 min with thiobarbituric acid solution at pH 7.0. The optical absorbance of MDA was measured at 532 nm. The MDA level in medium before the assay was determined, and the production of MDA during the incubation period in the presence of vehicle or test compounds was determined. Inhibition (%) of lipid peroxidation by various concentrations of test compounds was determined, and IC_{50} values were calculated.

Esterified Cholesterol (EC) Accumulation in THP-1 Cell-Derived Macrophages. Effects of test compounds on EC accumulation in THP-1 cells were determined during differentiation and foam cell formation. THP-1 cells were grown in RPMI-1640 medium containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin. To differentiate into macrophages and to form foam cells, the cells were suspended in RPMI-1640 medium containing 10% FBS, phorbol 12-myristate 13-acetate (PMA, 200 nM), and acetyl-LDL (400 μg/mL), which was prepared from the plasma of Kurosawa and Kusanagi hypercholesterolemic (KHC) rabbits (Japan Laboratory Animals, Tokyo, Japan) and acetylated with acetic anhydride. Then, they were incubated at 4 × 10^5 cells/well in a humidified atmosphere of 95% air, 5% CO_2 at 37 °C for 3 days in the presence or absence of test compounds. In separate experiments, the effects of **2** on EC accumulation in THP-1 cell-derived macrophages after differentiation were determined. The cells were differentiated into macrophages by culture in RPMI-1640 medium containing 10% FBS, PMA (200 nM), and 2-mercaptoethanol (50 μM) for 3 days. Then, the cells were washed with RPMI-1640 medium and cultured in RPMI-1640 medium containing 2-mercaptoethanol (50 μM), acetyl-LDL (400 μg/mL), and **2** for 2 days. Cellular cholesterol was extracted by hexane/isopropanol (3:2) and determined by enzymatic methods. EC content was calculated by subtracting the amount of free cholesterol from the total amount of cholesterol. Cellular protein was measured by Lowry's method.

Plasma Levels of Compounds after Oral Administration in Rats, Rabbits, and Dogs. Compounds were suspended in 5% arabic gum and administered orally at 10 mg/kg to male SD rats (n = 3, 6−7 weeks old, Japan SLC). SD rats were fasted overnight before administration. Compound **2** was suspended in 5% arabic gum and administered orally at 10 mg/kg to male Japanese white rabbits (n = 4, 2.7−3.0 kg, Japan SLC) and male beagles (n = 4, 9.0−11.6 kg, HRP Inc., Kalamazoo, MI). Blood samples were drawn using a heparinized syringe from the jugular vein at 0.5, 1, 3, 5, 8, and 24 h after administration in rats and at 0.25, 0.5, 1, 2, 3, 5, 8, and 12 h in beagles, and from the ear vein at 0.25, 0.5, 1, 2, 3, 5, 8, and 24 h in rabbits. Blood was centrifuged at 3000 rpm for 10 min at room temperature. Concentrations of a free form of compounds in the plasma were determined using HPLC, as described above.

In Vitro ACAT Activity. Male Japanese White rabbits (2.5 kg, Japan SLC) were anesthetized with sodium pentobarbital (30 mg/kg, iv) and exsanguinated from the common carotid artery, and then the liver was isolated. Hyperlipidemic male Japanese White rabbits (2−3 kg) fed a high cholesterol diet (1% cholesterol CR-3, Clea Japan, Osaka, Japan) for one month were anesthetized and exsanguinated from the common carotid artery, and then the intestinal mucosa, liver, and adrenal were isolated. Rabbits fed a high cholesterol diet were separately injected with thioglycolate medium (200 mL/animal, ip), and then 4 days later peritoneal macrophages were isolated under deep anesthesia. Microsomes were prepared according to the method of Field and Mathur.[32] Briefly, each sample was homogenized in a buffered sucrose solution (250 mM sucrose, 5 mM K_2HPO_4/KH_2PO_4, 1 mM EDTA, and 1 mM dithioerythritol, pH 7.4) using a Teflon-glass homogenizer. The homogenate was centrifuged at 12000*g* for 15 min at 4 °C. The resulting supernatant was centrifuged at 105000*g* for 30 min at 4 °C. The microsomal fraction was used as the ACAT preparation. ACAT activity was determined according to the method described by Heider et al.[33] The microsomes were incubated in 154 mM phosphate buffer (pH 7.4) containing bovine serum albumin. Test compounds were applied and preincubated at 37 °C for 5 min, then 30 nmol of [1-^{14}C]oleoyl-CoA (PerkinElmer, Waltham, MA) was added. The reaction mixture was incubated at 37 °C for 7.5 min for the intestine, 20 min for the liver, 30 min for macrophages, and 10 min for the adrenal gland. EC was extracted with chloroform/methanol (2:1) and separated by thin-layer chromatog-

raphy. The EC produced in microsomes treated with vehicle and test compounds were determined. IC_{50} values were calculated using data from duplicate assay tubes at the concentrations of test compounds in each experiment, and the mean value was calculated for 3−5 experiments.

Effect on in Vitro Plasma Lipid and LDL Peroxidation. Blood was taken from the ear artery using a heparinized syringe in KHC rabbits (2.4−2.7 kg, Japan Laboratory Animals), and plasma was isolated and incubated with $CuSO_4$ (2 mM) at 37 °C for 4 h in the presence of the vehicle or test compounds. Lipid peroxide was determined as MDA using a commercial kit (Wako Pure Chemical Industries, Ltd.). The effects of test compounds on LDL oxidation were determined according to the method reported previouly.[23,34] Briefly, the LDL fraction was isolated from the plasma of male KHC rabbits and incubated with $CuSO_4$ (5 μM) at 37 °C for 1 h in the presence of vehicle or test compounds. Oxidized LDL was determined as MDA. IC_{50} values were calculated using data from duplicate assay tubes at each concentration of test compounds.

Effect on Serum Cholesterol Level. Male Wistar rats (8−9 weeks old, Japan SLC) were fed a high-cholesterol diet (1% cholesterol, 0.5% cholic acid, and 10% coconut oil) for 3 days, during which period **2** suspended in a 5% arabic gum solution and the vehicle were orally administered at a dose of 3 mg/kg once a day (n = 5/group). The animals were fasted for 5 h after the final administration, and blood samples were collected from the abdominal aorta under anesthesia with sodium pentobarbital (50 mg/kg, ip). Male Japanese white rabbits (2.5−3.0 kg, Japan SLC) were fed a high-cholesterol diet (1% cholesterol) for 7 days, during which period **2** suspended in a 5% arabic gum solution and the vehicle were orally administered at a dose of 1 mg/kg once a day (n = 5/group). The animals were fasted overnight after the final administration, and blood samples were collected from the ear artery. Male beagles (8.8−12.0 kg, HRP Inc.) were fed a Western diet including meat, eggs, and butter for 7 days, during which period **2** suspended in a 5% arabic gum solution and the vehicle were orally administered at a dose of 3 mg/kg once a day (n = 3/group). Blood samples were collected 24 h after the final administration from the carotid vein. Male Syrian hamsters (9 weeks old, Japan SLC) were fed a regular diet for 3 weeks, during which period **2** suspended in a 5% arabic gum solution and the vehicle were orally administered at a dose of 3 mg/kg once a day (n = 5/group). Blood samples were collected 24 h after the final administration from the abdominal aorta under anesthesia with sodium pentobarbital (50 mg/kg, ip). Serum total cholesterol levels and HDL cholesterol level were measured by an enzymatic method using a commercial assay kit (Wako Pure Chemicals Industries, Ltd., Osaka, Japan). Non-HDL (LDL/VLDL) cholesterol was calculated.

Supporting Information Available: Elemental analysis data of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM800248R