Bioorganic & Medicinal Chemistry 17 (2009) 6020–6031

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel indoline-based acyl-CoA: cholesterol acyltransferase inhibitor: Effects of introducing a methanesulfonamide group on physicochemical properties and biological activities

Yoshimichi Shoji [a], Kenji Takahashi [a], Masaru Ohta [a], Masayasu Kasai [a], Kazuyoshi Kunishiro [a], Mamoru Kanda [a], Satoko Yogai [a], Yasuo Takeuchi [b], Hiroaki Shirahase [a,*]

[a] Research Laboratories, Kyoto Pharmaceutical Industries, Ltd 38, Nishinokyo Tsukinowa-cho, Nakagyo-ku, Kyoto 604-8444, Japan
[b] Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 1-1 Tsushima-naka, 1-Chome, Okayama 700-8530, Japan

ARTICLE INFO

Article history:
Received 11 May 2009
Revised 21 June 2009
Accepted 23 June 2009
Available online 27 June 2009

Keywords:
ACAT inhibitor
Methanesulfonamide derivatives
Lipophilicity
Anti-foam cell formation
Pactimibe

ABSTRACT

A novel series of indoline-based acyl-CoA: cholesterol acyltransferase (ACAT) inhibitors with a methanesulfonamide group at the 5-position were synthesized and their lipophilicity and biological activities were evaluated. Hepatic ACAT inhibitory and anti-foam cell formation activity increased dependent on lipophilicity of derivatives with various alkyl chains at the 1-position. The log $D_{7.0}$–biological activity curve of the derivatives with a methanesulfonamide group shifted leftward compared to that of Pactimibe derivatives with a carboxymethyl group, and derivatives with no substituent, suggesting that a methanesulfonamide group plays an important role in the interaction with ACAT protein. Among derivatives, *N*-(1-ethyl-5-methanesulfonylamino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (**1b**) had about twofold lower log $D_{7.0}$ than Pactimibe, while it showed twofold higher hepatic ACAT inhibition than and the same anti-foam cell formation as Pactimibe, respectively. The C_{max} of **1b** (10 mg/kg, po) was higher than that of Pactimibe in rats. The plasma protein binding ratio of **1b** was lower than that of Pactimibe: 64.8% and 97.9%, respectively. Compound **1b** showed greater inhibitory effects on hepatic cholesterol secretion in mice than Pactimibe. In conclusion, the introduction of a methanesulfonamide group is effective to design less lipophilic, more efficacious and more bioavailable indoline-based ACAT inhibitors than previous indoline-based inhibitors.

1. Introduction

Acyl-CoA: cholesterol acyltransferase (ACAT) has been reported to play an important role in hyperlipidemia and atherosclerosis, since esterification of cholesterol with a long chain fatty acid is a key step in the intestinal absorption of dietary cholesterol, hepatic secretion of endogenous cholesterol and accumulation of cholesterol in vascular macrophages.[1–3]

A great number of highly potent but highly lipophilic (thus, less bioavailable) ACAT inhibitors have been reported as hypolipidemic drugs, which inhibit intestinal and hepatic esterification of cholesterol,[4,5] and the target of ACAT inhibitors has shifted from intestinal/hepatic ACAT to macrophage ACAT: hyperlipidemia to atherosclerosis.[6] In atherosclerotic plaque, cholesterol is accumulated via esterification by ACAT in macrophages. There have been some attempts to reduce the lipophilicity of ACAT inhibitors to improve bioavailability;[7–11] however, this is very difficult because lipophilicity is the preferred property for the inhibition of esterification between two lipophilic substrates, free cholesterol and long fatty acid by membrane-bound enzymes such as ACAT.[12–14] We have previously synthesized a new indoline-based ACAT inhibitor Pactimibe with a carboxymethyl group, which is low lipophilic and high water soluble, showed good oral absorption, and exerted excellent pharmacological effects.[15] Pactimibe decreased atherosclerotic areas in apo-E knockout mice and stabilized aortic plaque in WHHL rabbits.[16,17] A clinical study using an intravascular-ultrasonography catheter (ACTIVATE study) did not show plaque retardation at a dose of 100 mg in patients with coronary artery disease (CAD).[18] ACAT inhibitors may stabilize plaque but may not reduce its volume in human atherosclerosis. Alternatively, Pactimibe may not fully inhibit ACAT activity in plaque at the dose used in patients, since it is highly bioavailable but shows a high serum protein binding ratio; therefore, a new ACAT inhibitor, which has physicochemical and biological properties different from those of Pactimibe, would be valuable as a new anti-atherosclerotic drug candidate and as a tool for medical research in atherosclerosis. In the case of Pactimibe, introduction of a carboxymethyl group to the indoline ring resulted in

* Corresponding author. Tel.: +81 75 812 2248; fax: +81 75 812 0906.
E-mail address: shirahase@kyoto-pharm.co.jp (H. Shirahase).

0968-0896/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2009.06.047

reduced lipophilicity and increased bioavailability with maintained biological activity. However, the introduction of a tetrazole moiety, a bioisoster of carboxylic acid, markedly reduced biological activity although it maintained relatively high lipophilicity compared to a carboxymethyl group. The present study revealed that the introduction of a methanesulfonamide group slightly increased lipophilicity and greatly enhanced biological activities compared to a carboxymethyl group; thus, some compounds maintaining higher activity were less lipophilic than Pactimibe.

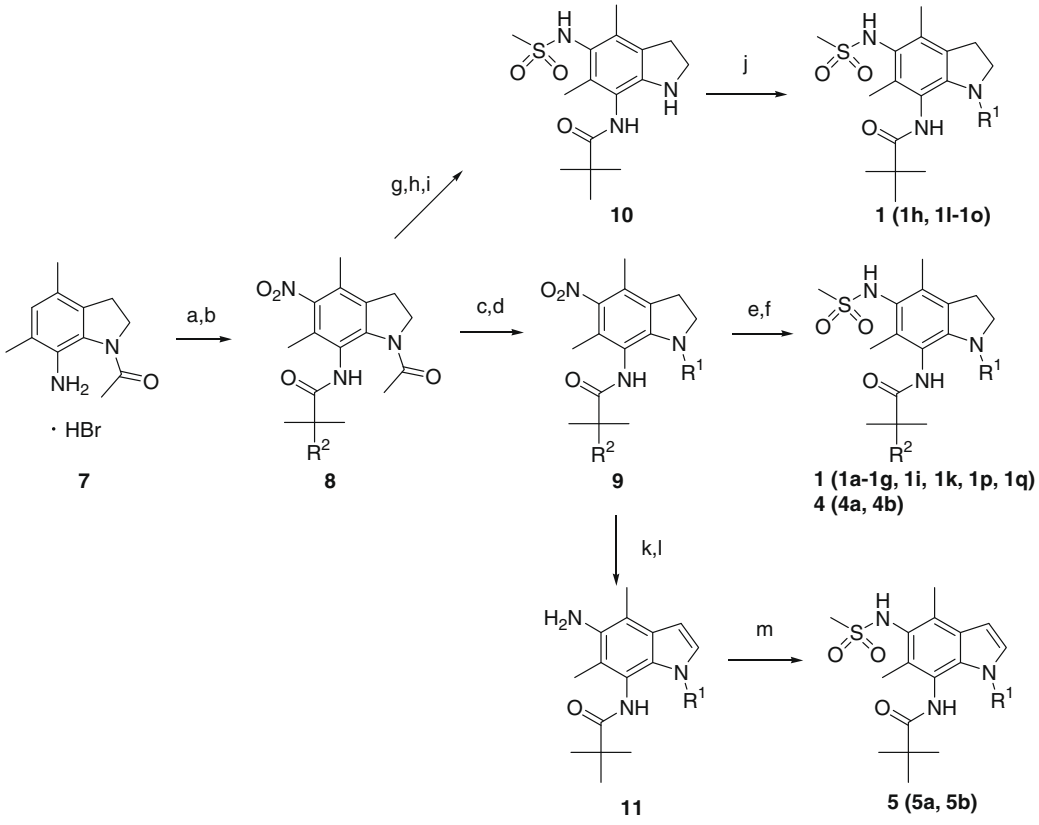
2. Chemistry

The general approach to the synthesis of indoline and indole derivatives with methanesulfonamide (**1**, **4**, **5**) is outlined in Scheme 1. The starting material **7**, which was prepared as described previously,[19] was treated with the corresponding acyl chloride and nitrated to obtain **8**. Deacetylation at indoline 1-position and alkylation with alkyl halide gave the corresponding 1-alkyl indoline derivatives **9**, followed by reduction of a nitro group to afford aniline derivatives. Finally, mesylation of the aniline moiety was carried out to give the target compounds (**1**, **4**). Indoline derivatives with nonsaturated alkylchain **1l** or cyclic alkylchain (**1m**, **1n**, **1o**) were synthesized using different intermediate **10**, which was prepared from **8** in three steps. Indole derivatives (**5a**, **5b**) were prepared from **9** via reduction of a nitro group, oxidation of indoline with Pd–C and mesylation. On the other hand, introduction of a neopentyl group at indoline 1-position was not as successful as in Scheme 1, so compound **1j** was synthesized by a different method from compound **12** (Scheme 2). Namely, pivaloylation and hydrogenation of **12** afforded **13**, followed by reduction with lithium aluminum hydride to afford the desired neopentyl derivative. This led to the free form of **1j** in a similar manner of other indoline derivatives, which was treated with HCl to give **1j** as a HCl salt. Tetrahydroquinoline derivative **6** was synthesized using a procedure similar to the synthesis of indoline derivatives **1** (Scheme 3). 6-Bromo-8-nitro-tetrahydroquinoline **16** was readily prepared by bromination and nitration of **15**, and then, **6** was synthesized by the same method to prepare **1j**. Indoline derivatives with a carboxymethyl group (**2**) and nonsubstituted group (**3**) at 5-position were synthesized according to the methods described in previous reports.[14,15]

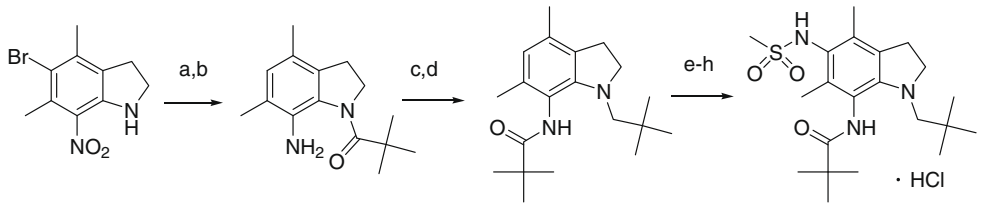
3. Results and discussion

We have reported a series of indoline-based ACAT inhibitors, in which an ionizable carboxyl group was introduced to various positions of an indoline ring to increase bioavailability due to enhanced water solubility.[15] Of these derivatives, Pactimibe with a carboxymethyl group at the 5-position was efficiently absorbed and exerted inhibitory effects on macrophage ACAT and LDL oxidation; however, clinical studies failed to show the plaque-reducing effect of Pactimibe in CAD patients.[18] It remains to be determined whether Pactimibe did not fully inhibit ACAT in the clinical study or whether ACAT inhibition had no reducing effects on human atherosclerosis. To solve this problem, it is necessary to find a new ACAT inhibitor, which is more efficacious and bioavailable, and safer than Pactimibe.

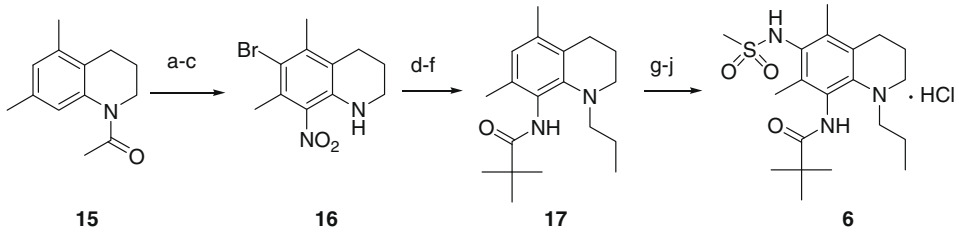
As shown in Pactimibe, a carboxymethyl group improved water solubility and bioavailability with maintained ACAT inhibitory activity, while an amine and tetrazole group increased water solu-



Scheme 1. Synthesis of compounds **1**, **4** and **5**. Reagents, conditions and yields: (a) acyl chloride, Et_3N, $CHCl_3$, rt, 81–97%; (b) concd HNO_3, concd H_2SO_4, AcOH, rt, or concd HNO_3, AcOH, 60 °C; (c) 3.5–5.5 M NaOH, MeOH, 50–60 °C, 66–94%; (d) alkyl halide, NaH, DMF, rt, 19–76%, or alkyl halide, Et_3N, DMF, 70 °C, 29–65%; (e) H_2, Pd–C, MeOH–toluene, rt; (f) MsCl, Et_3N, $CHCl_3$, rt, 29–54%; (g) H_2, Pd–C, MeOH, rt, 93%; (h) MsCl, pyridine, CH_2Cl_2, rt, quant.; (i) 9.0 M NaOH, EtOH, reflux, 91%; (j) alkyl halide, Et_3N, 80 °C, 11–62%; (k) H_2, Pd–C, MeOH–toluene, rt; (l) Pd–C, toluene, reflux, 46–55%; (m) MsCl, Et_3N, $CHCl_3$, rt, 33–64%.



Scheme 2. Synthesis of compound **1j**. Reagents, conditions and yields: (a) pivaloyl chloride, Et_3N, $CHCl_3$, reflux, 99%; (b) H_2, Pd–C, MeOH, rt, 83%; (c) $LiAlH_4$, Et_2O, 0 °C; (d) pivaloyl chloride, Et_3N, $CHCl_3$, 0 °C, 42%; (e) concd HNO_3, toluene, rt, 36%; (f) H_2, Pd–C, MeOH, rt; (g) MsCl, Et_3N, $CHCl_3$, 0 °C; (h) 8.0 M HCl in *i*-PrOH, CH_2Cl_2–MeOH, rt, 67%.



Scheme 3. Synthesis of compound **6**. Reagents, conditions and yields: (a) Br_2, AcOH, rt, 52%; (b) concd HNO_3, Ac_2O, 0 °C, 93%; (c) 2.0 M NaOH, EtOH, reflux, 80%; (d) H_2, Pd–C, MeOH, 40 °C; (e) pivaloyl chloride, Et_3N, $CHCl_3$, rt, 78%; (f) *n*-propyl iodide, Et_3N, DMF, 80 °C, 74%; (g) fum. HNO_3, Ac_2O, 5 °C, 43%; (h) H_2, Pd–C, MeOH, 40 °C; (i) MsCl, Et_3N, $CHCl_3$, 0 °C; (j) 8.0 M HCl in *i*-PrOH, MeOH, 0 °C, 36%.

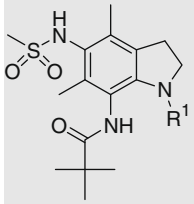
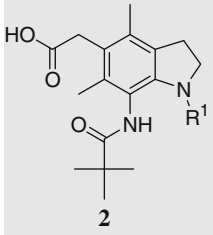
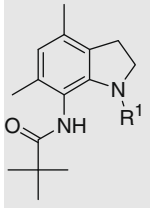
bility, but markedly reduced bioavailability and activity, respectively.[15] In the present study, a methanesulfonamide group, which is a bioisoster of carboxylic acid and is hardly ionized around neutral pH, was introduced to the 5-position of indoline-based ACAT inhibitors with various side chains at the 1-position. Table 1 shows the chemical structures, log $D_{7.0}$ values, hepatic ACAT inhibitory and anti-foam cell formation activities of the derivatives with a methanesulfonamide group, a carboxymethyl group and no substituents. Hepatic ACAT inhibitory activities were determined using liver microsomes isolated from normocholesterolemic rabbits. The effect on foam cell formation was determined as inhibitory activity against esterified cholesterol accumulation in THP-1 cell-derived macrophages exposed to hypercholesterolemic rabbit serum. In all three groups of derivatives, both activities increased according to the length of alkyl chains at the 1-position, and thus lipophilicity. As reported previously, the in vitro activity of ACAT inhibitors is dependent on their lipophilicity because they are concentrated in the lipophilic reaction phase consisting of membrane-bound enzyme ACAT, cholesterol and long chain fatty acid.[12–14] The activities of **1g** with a methanesulfonamide were higher than those of **2c** (Pactimibe) with a carboxymethyl group and **3h** with no substituents, while its lipophilicity was slightly higher than Pactimibe and lower than **3h**. As shown in Figure 1, log $D_{7.0}$–activity curves for the inhibition of hepatic ACAT activity and foam cell formation of 5-methanesulfonamide derivatives positioned at lower log $D_{7.0}$ range than those of 5-carboxymethyl and 5-unsubstituted derivatives, indicating that a methanesulfonamide moiety plays a key role in interaction between an inhibitor and ACAT protein. Indeed, a sulfonamide moiety is known to interact with various proteins, including ACAT: avasimibe has an acylsulfonamide structure as a pharmacophore.[20] In the present derivatives, in addition to an amide structure as a pharmacophore at the 7-position, a sulfonamide structure at the 5-position may also interact with the enzyme; however, we have not yet found a strong ACAT inhibitor with a sulfonamide moiety instead of an amide moiety on an indoline ring. On the other hand, introduction of a nitro and hydroxy group also increased activity with reduced bioavailability (unpublished data). Further study is needed to clarify the mode of interaction between ACAT protein and unionized substituent containing hetero atoms at the 5-position of indoline derivatives.

In 5-carboxymethyl derivatives, the effect of lipophilicity on ACAT inhibitory activity was limited, thus the maximal activity level in the log $D_{7.0}$–activity curve was much lower than that of 5-methanesulfonamide and unsubstituted derivatives, suggesting that a carboxymethyl group may disturb the interaction between an inhibitor and enzyme. Alternatively, an ionizable carboxylic acid moiety may limit inhibitor concentration in the lipophilic reaction phase. For anti-foam cell formation activity, the effect of lipophilicity on activity was smaller not only in 5-carboxymethyl derivatives but also in 5-unsubstituted derivatives than 5-methanesulfonamide derivatives. 5-Methanesulfonamide derivatives showed high activity at relatively low lipophilicity, and thus may have freely penetrated the cell membrane, while 5-unsubstituted and 5-carboxymethyl derivatives showed their activity at relatively high lipophilicity, and thus may have been trapped in the lipid bi-layer of the cell membrane.

Indoline-based methanesulfonamide derivatives with branched and cyclic side chains at the 1-position, and indole- and tetrahydroquinoline-based methanesulfonamide derivatives were synthesized and their activities are shown in Table 2. Indoline-based derivatives with branched and cyclic side chains showed comparable or weaker activities than those with normal alkyl chains. The same tendency was observed in indoline-based ACAT inhibitors with no substituents at the 5-position: reduction of lipophilicity may relate to weaker activities in branched or cyclic chains.[14] Indole, but not tetrahydroquinoline-based derivatives, showed weaker activities than those of the indoline-based derivative: direction of the alkyl chain may be most preferably fixed for interaction with the enzyme in the indoline and tetrahydroquinoline than the indole (aromatic ring) structure.

Compound **1b** was chosen for further evaluation, since its activities were most close to those of Pactimibe among methanesulfonamide derivatives. Compound **1b** showed twofold more potent hepatic ACAT inhibition and similar anti-foam cell formation activities to Pactimibe, while it was much less lipophilic than Pactimibe (log $D_{7.0}$: 1.4 vs 3.0). Compound **1b** was expected to be more highly

Table 1
Chemical structures, molecular weights, log $D_{7.0}$ and inhibitory effects of indoline derivatives on hepatic ACAT and foam cell formation in THP-1 cells

	Compound[a]	R^1	M.W. (free)	Log $D_{7.0}$	Hepatic ACAT[b]	THP-1[c]
 1	**1a**	$-CH_3$	353.48	0.9	8.8	7.5
	1b	$-CH_2CH_3$	367.51	1.4	1.2	2.0
	1c	$-CH_2CH_2CH_3$	381.53	1.9	0.24	0.30
	1d	$-CH_2(CH_2)_2CH_3$	395.56	2.4	0.069	0.058
	1e	$-CH_2(CH_2)_3CH_3$	409.59	2.9	0.049	0.034
	1f	$-CH_2(CH_2)_4CH_3$	423.61	3.5	0.042	0.0092
	1g	$-CH_2(CH_2)_6CH_3$	451.67	4.7	0.013	0.0021
 2	**2a**	$-CH_2(CH_2)_4CH_3$	388.54	2.0	4.3	5.0
	2b	$-CH_2(CH_2)_5CH_3$	402.57	2.5	5.8	4.9
	2c	$-CH_2(CH_2)_6CH_3$	416.60	3.0	2.3	2.0
	2d	$-CH_2(CH_2)_7CH_3$	430.62	3.5	1.2	0.53
	2e	$-CH_2(CH_2)_8CH_3$	444.65	4.0	0.91	0.98
	2f	$-CH_2(CH_2)_9CH_3$	458.68	4.5	0.96	0.85
	2g	$-CH_2(CH_2)_{10}CH_3$	472.70	5.0	1.1	1.1
 3	**3a**	$-CH_3$	260.37	2.0	31	43
	3b	$-CH_2CH_3$	274.40	2.4	4.0	20
	3c	$-CH_2CH_2CH_3$	288.43	3.0	0.57	6.9
	3d	$-CH_2(CH_2)_2CH_3$	302.45	3.5	0.22	1.1
	3e	$-CH_2(CH_2)_3CH_3$	316.48	3.8	0.11	0.51
	3f	$-CH_2(CH_2)_4CH_3$	330.51	4.2	0.067	0.47
	3g	$-CH_2(CH_2)_5CH_3$	344.53	4.7	0.030	0.38
	3h	$-CH_2(CH_2)_6CH_3$	358.56	5.1	0.024	0.29
	3i	$-CH_2(CH_2)_8CH_3$	386.61	6.8	0.027	0.29
	3j	$-CH_2(CH_2)_{10}CH_3$	414.67	7.3	0.058	0.29

[a] Compounds **2b**–**g**: hemisulfate salt.
[b] IC_{50} values (μM) for inhibitory effects on ACAT activity in rabbit liver microsome. $n = 2$.
[c] IC_{50} values (μM) for inhibitory effects on accumulation of esterified cholesterol in THP-1 cells, $n = 2$. Compound **2c**: Pactimibe.

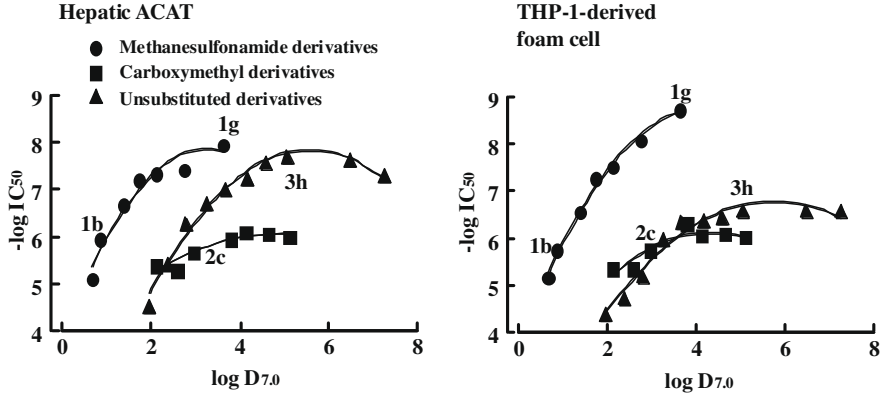
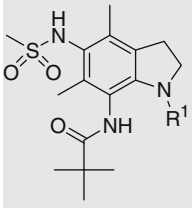
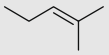
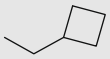
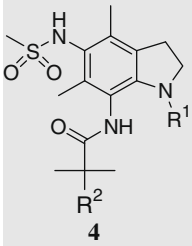
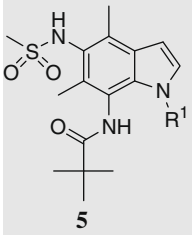
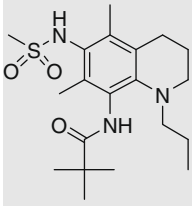


Figure 1. Log $D_{7.0}$–activity relationships for inhibition of hepatic ACAT and foam cell formation in indoline-based ACAT inhibitors. Compound **2c**: Pactimibe.

dissolved in gastro-intestinal fluid and more efficiently absorbed than Pactimibe. Indeed, C_{max} and $AUC_{0\text{-}24}$ of **1b** after oral administration at 10 mg/kg were about 3.6-fold higher than those of Pactimibe (Table 3). Compound **1b** also showed a lower rat plasma protein binding ratio than Pactimibe (64.8 ± 0.80% vs 97.9 ± 0.15%, $n = 3$), meaning that its effective plasma concentration (free form concentration) could be higher than Pactimibe. The anti-foam cell formation activity of Pactimibe was much more markedly reduced by the presence of albumin than **1b**: 10-fold and twofold, respectively. Another set of experiments demonstrated that **1b** more strongly inhibited hepatic cholesterol secretion than Pactimibe in Triton WR-1339-injected mice (% inhibition: 41% vs

Table 2
Chemical structures, molecular weights and inhibitory effects of indoline, indole an d tetrahydroquinoline derivatives on hepatic ACAT and foam cell formation in THP-1 cells

	Compound[a]	R^1	R^2	M.W. (free)	HepaticACAT[b]	THP-1[c]
	1h	$-CH(CH_3)_2$	–	381.53	3.7	0.67
	1i	$-CH_2CH(CH_3)_2$	–	395.56	0.071	0.082
	1j	$-CH_2C(CH_3)_3$	–	409.59	0.061	0.022
	1k	$-CH_2CH_2CH(CH_3)_2$	–	409.59	0.040	0.060
 **1**	**1l**		–	407.57	0.068	0.13
	1m		–	393.54	0.16	0.65
	1n		–	407.57	0.036	0.055
	1o		–	407.57	0.095	0.089
	1p	$-CH_2CH_2OCH_3$	–	397.53	0.27	1.6
	1q	$-CH_2CH_2OCH_2CH_3$	–	411.56	0.31	0.82
 **4**	**4a**	$-CH_2(CH_2)_2CH_3$	–H	381.53	3.1	1.9
	4b	$-CH_2(CH_2)_2CH_3$	$-CH_2CH_3$	409.59	0.053	0.045
 **5**	**5a**	$-CH_2(CH_2)_2CH_3$	–	393.54	0.24	0.54
	5b	$-CH_2CH(CH_3)_2$	–	393.54	0.48	1.4
	**6**	–	–	395.56	0.13	0.29

[a] Compounds **1j** and **6**: HCl salt.
[b] IC_{50} values (μM) for inhibitory effects on ACAT activity in rabbit liver microsome. $n = 2$.
[c] IC_{50} values (μM) for inhibitory effects on accumulation of esterified cholesterol in THP-1 cells, $n = 2$.

28%). Furthermore, nonspecific chemical toxicities tend to increase dependent on lipophilicity,[21] suggesting that **1b** is safer than Pactimibe.

In conclusion, the introduction of methanesulfonamide in indoline-based ACAT inhibitors is useful to enhance activities and improve oral absorption. Of these compounds, **1b**, showing less lipophilicity and stronger activity than Pactimibe, would be a candidate for a new efficacious and safe anti-atherosclerotic drug, and would be a useful tool to clarify the role of ACAT in humans and experimental atherosclerosis.

Table 3
Pharmacokinetic parameters of **1b** and Pactimibe (10 mg/kg, po) in male SD rats

	C_{max} (μg/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{0-24} (μg h/mL)
1b	2.90 ± 0.28	0.50 ± 0.00	2.59 ± 0.60	10.75 ± 1.78
Pactimibe	0.80 ± 0.22	0.67 ± 0.29	7.37 ± 6.61	2.80 ± 0.50

Mean ± SD (n = 3).

4. Experimental

4.1. General procedures

Chemicals were obtained from commercial sources and used without purification. Intermediates **7**, *N*-(1-acetyl-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (**18**) and **12** were synthesized according to the methods previously reported.[15,19] 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.1001 W; 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, Foster City, CA, USA).

4.2. Procedure for preparation of 1a

Compound **1a** was prepared from compound **7** via compound **18** as follows:

4.2.1. *N*-(4,6-Dimethyl-5-nitroindolin-7-yl)-2,2-dimethylpropanamide (19)

To a solution of **18** (100 g, 0.347 mol) in AcOH (100 mL) were added concd H_2SO_4 (36.9 mL, 0.692 mol) and concd HNO_3 (21.8 mL, 0.519 mol) below 20 °C. The reaction mixture was stirred at 20 °C for 1 h, and then poured into water (3.0 L). The formed precipitate was collected by filtration to give *N*-(1-acetyl-4,6-dimethyl-5-nitroindolin-7-yl)-2,2-dimethylpropanamide **(8a)**. To a suspension of **8a** in MeOH (1.2 L) was added 3.5 M NaOH aqueous solution (250 mL, 0.88 mol), followed by stirring at 50 °C for 2.5 h, and then cooled to room temperature. The precipitate was collected by filtration to give **19** (95.0 g, 94% yield) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.23 (9H, s), 1.94 (3H, s), 2.08 (3H, s), 2.97 (2H, t, J = 8.6 Hz), 3.54 (2H, t, J = 8.6 Hz), 5.45 (1H, s), 8.72 (1H, s).

4.2.2. 2,2-Dimethyl-*N*-(1,4,6-trimethyl-5-nitroindolin-7-yl)propanamide (9a)

To a solution of **19** (2.00 g, 3.43 mmol) in DMF (20 mL) was added a 60% NaH (270 mg, 6.75 mmol) at 0 °C under N_2 atmosphere, and the reaction mixture was stirred at the same temperature for 30 min. Methyl iodide (1.10 g, 7.75 mmol) was added to the solution and the reaction mixture was stirred at room temperature for 10 h. After water was added, the mixture was extracted twice with AcOEt. The extracts were combined, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$–MeOH) to give **9a** (1.60 g, 76% yield) as a pale brown solid. ^{1}H NMR ($CDCl_3$) δ: 1.33 (9H, s), 2.01 (3H, s), 2.11 (3H, s), 2.90 (3H, s), 2.99 (2H, t, J = 8.5 Hz), 3.46 (2H, t, J = 8.5 Hz), 6.81 (1H, br s).

4.2.3. *N*-(5-Methanesulfonylamino-1,4,6-trimethylindolin-7-yl)-2,2-dimethylpropanamide (1a)

A solution of **9a** (1.20 g, 3.93 mmol) in MeOH–toluene (4:1, 100 mL) was hydrogenated at 0.3 MPa in the presence of 5% Pd–C (200 mg) at room temperature for 20 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (20 mL), washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give aniline derivative. To a solution of the obtained product in $CHCl_3$ (30 mL) were added Et_3N (1.64 mL, 11.8 mmol) and methanesulfonyl chloride (MsCl) (0.61 mL, 7.9 mmol) at 0 °C, followed by stirring at room temperature for 1 h. The solution was washed with 5% citric acid solution, water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$–MeOH) to give crude **1a**, which was recrystallized from AcOEt to give **1a** (700 mg, 50% yield) as a white crystalline product. Mp 213–217 °C. IR (Nujol): 3197, 1662, 1506 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 1.20 (9H, s), 1.99 (3H, s), 2.11 (3H, s), 2.77 (2H, br t), 2.88 (3H, s), 3.19 (3H, s), 3.37 (2H, br t), 8.52 (1H, br s), 8.67 (1H, br s). MS m/z: 354 [M+H]$^+$. Anal. Calcd for $C_{17}H_{27}N_3O_3S{\cdot}0.1CHCl_3{\cdot}0.5H_2O$: C, 54.85; H, 7.56; N, 11.22. Found: C, 54.87; H, 7.49; N, 10.95.

4.3. Procedure for preparation of 1b–g, i, k, p, q

Compounds **1b–g**, **i**, **k**, **p**, **q** were prepared according to the procedure for **1a.**

4.3.1. *N*-(1-Ethyl-5-methanesulfonylamino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (1b)

Mp 200–207 °C (dec.). IR (Nujol): 3197, 1664, 1506 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 0.94 (3H, t, J = 7.0 Hz), 1.21 (9H, s), 1.99 (3H, s), 2.11 (3H, s), 2.77 (2H, br t), 2.88 (3H, s), 3.19 (2H, br t), 3.37 (2H, br t), 8.52 (1H, br s), 8.67 (1H, br s). MS m/z: 368 [M+H]$^+$. Anal. Calcd for $C_{18}H_{29}N_3O_3S{\cdot}0.15CHCl_3{\cdot}0.5H_2O$: C, 55.27; H, 7.70; N, 10.65. Found: C, 55.26; H, 7.45; N, 10.66.

4.3.2. *N*-(5-Methanesulfonylamino-4,6-dimethyl-1-propylindolin-7-yl)-2,2-dimethylpropanamide (1c)

Mp 225–232 °C (dec.). IR (Nujol): 3205, 1662, 1506 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.90 (3H, t, J = 7.2 Hz), 1.30–1.80 (2H, m), 1.34 (9H, s), 2.07 (3H, s), 2.10 (3H, s), 2.81 (2H, t, J = 8.4 Hz), 2.95 (3H, s), 3.14 (2H, t, J = 7.2 Hz), 3.44 (2H, t, J = 8.4 Hz), 6.23 (1H, br s), 6.88 (1H, br s). MS m/z: 382 [M+H]$^+$. Anal. Calcd for $C_{19}H_{31}N_3O_3S{\cdot}0.2H_2O$: C, 59.25; H, 8.22; N, 10.91. Found: C, 59.03; H, 8.16; N, 11.05.

4.3.3. *N*-(1-Butyl-5-methanesulfonylamino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (1d)

Mp 204–208 °C. IR (Nujol): 3203, 1666 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.90 (3H, br t), 1.10–1.80 (4H, m), 1.33 (9H, s), 2.10 (3H, s), 2.15 (3H, s), 2.83 (2H, t, J = 8.4 Hz), 2.97 (3H, s), 3.18 (2H, t, J = 8.0 Hz), 3.45 (2H, t, J = 8.4 Hz), 6.04 (1H, br s), 6.83 (1H, br s). MS m/z: 397 [M+H]$^+$. Anal. Calcd for $C_{20}H_{33}N_3O_3S{\cdot}0.2H_2O$: C, 60.18; H, 8.43; N, 10.53. Found: C, 60.28; H, 8.35; N, 10.54.

4.3.4. *N*-(5-Methanesulfonylamino-4,6-dimethyl-1-pentylindolin-7-yl)-2,2-dimethylpropanamide (1e)

Mp 207–208 °C. IR (Nujol): 3203, 1664 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.90 (3H, br t), 1.10–1.70 (6H, m), 1.33 (9H, s), 2.11 (3H, s), 2.15 (3H, s), 2.97 (2H, t, J = 8.4 Hz), 3.08 (3H, s), 3.18 (2H, t, J = 8.0 Hz), 3.45 (2H, t, J = 8.4 Hz), 6.09 (1H, br s), 6.84 (1H, br s). MS m/z: 410 [M+H]$^+$. Anal. Calcd for $C_{21}H_{35}N_3O_3S$: C, 61.58; H, 8.61; N, 10.01. Found: C, 61.31; H, 8.74; N, 10.01.

4.3.5. *N*-(1-Hexyl-5-methanesulfonylamino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (1f)

Mp 144–150 °C. IR (Nujol): 3360, 1665 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 5.0 Hz), 1.16–1.69 (8H, m), 1.33 (9H, s), 2.10 (3H, s), 2.14 (3H, s), 2.73–3.55 (6H, m), 2.97 (3H, s), 6.09 (1H, br s), 6.83 (1H, br s). MS m/z: 425 $[M+H]^+$. Anal. Calcd for $C_{22}H_{37}N_3O_3S{\cdot}0.3H_2O$: C, 61.59; H, 8.83; N, 9.79. Found: C, 61.51; H, 8.81; N, 9.77.

4.3.6. *N*-(5-Methanesulfonylamino-4,6-dimethyl-1-octylindolin-7-yl)-2,2-dimethylpropanamide (1g)

Mp 154–157 °C. IR (Nujol): 2924, 1462 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 5.0 Hz), 1.18–1.58 (12H, m), 1.34 (9H, s), 2.10 (3H, s), 2.15 (3H, s), 2.72–3.55 (6H, m), 2.97 (3H, s), 6.10 (1H, br s), 6.88 (1H, br s). MS m/z: 453 $[M+H]^+$. Anal. Calcd for $C_{24}H_{41}N_3O_3S$: C, 63.52; H, 9.02; N, 9.30. Found: C, 63.82; H, 9.15; N, 9.30.

4.3.7. *N*-[5-Methanesulfonylamino-4,6-dimethyl-1-(2-methylpropyl)indolin-7-yl]-2,2-dimethylpropanamide (1i)

Mp 228–238 °C (dec.). IR (Nujol): 3269, 1658, 1596 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.93 (6H, d, J = 6.4 Hz), 1.20–1.60 (1H, m), 1.34 (9H, s), 2.06 (3H, s), 2.09 (3H, s), 2.82 (2H, t, J = 8.4 Hz), 2.96 (3H, s), 3.01 (2H, d, J = 6.4 Hz), 3.41 (2H, t, J = 8.4 Hz), 6.27 (1H, br s), 6.81 (1H, br s). MS m/z: 397 $[M+H]^+$. Anal. Calcd for $C_{20}H_{33}N_3O_3S{\cdot}0.1H_2O$: C, 60.45; H, 8.42; N, 10.57. Found: C, 60.30; H, 8.32; N, 10.49.

4.3.8. *N*-[5-Methanesulfonylamino-4,6-dimethyl-1-(3-methylbutyl)indolin-7-yl]-2,2-dimethylpropanamide (1k)

Mp 197–199 °C. IR (Nujol): 3205, 1666 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.92 (6H, d, J = 6.0 Hz), 1.20–1.60 (3H, m), 1.34 (9H, s), 2.09 (3H, s), 2.14 (3H, s), 2.81 (2H, t, J = 8.3 Hz), 2.96 (3H, s), 3.19 (2H, br t), 3.43 (2H, t, J = 8.3 Hz), 6.15 (1H, br s), 6.86 (1H, br s). MS m/z: 410 $[M+H]^+$. Anal. Calcd for $C_{21}H_{35}N_3O_3S$: C, 61.58; H, 8.61; N, 10.26. Found: C, 61.58; H, 8.66; N, 10.19.

4.3.9. *N*-[5-Methanesulfonylamino-1-(2-methoxyethyl)-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (1p)

Mp 176–180 °C. IR (Nujol): 3362, 3200, 1662 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.31 (9H, s), 2.09 (3H, s), 2.17 (3H, s), 2.88 (2H, t, J = 8.8 Hz), 2.98 (3H, s), 3.36 (3H, s), 3.45–3.65 (6H, m), 5.80–6.00 (1H, br), 7.74 (1H, br s). MS m/z: 398 $[M+H]^+$. Anal. Calcd for $C_{19}H_{31}N_3O_4S$: C, 57.40; H, 7.86; N, 10.57. Found: C, 57.23; H, 7.71; N, 10.46.

4.3.10. *N*-[1-(2-Ethoxyethyl)-5-methanesulfonylamino-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (1q)

Mp 157–159 °C. IR (Nujol): 3350, 3200, 1663 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.16 (3H, t, J = 7.0 Hz), 1.32 (9H, s), 2.10 (3H, s), 2.19 (3H, s), 2.88 (2H, t, J = 8.8 Hz), 2.98 (3H, s), 3.45–3.65 (6H, m), 3.49 (2H, q, J = 7.0 Hz), 5.70–5.90 (1H, br), 7.87 (1H, br s). MS m/z: 412 $[M+H]^+$. Anal. Calcd for $C_{20}H_{33}N_3O_4S{\cdot}0.1H_2O$: C, 58.11; H, 8.10; N, 10.17. Found: C, 58.02; H, 7.84; N, 10.12.

4.4. Procedure for preparation of 1h

Compound **1h** was prepared from **8a** as follows:

4.4.1. *N*-(1-Acetyl-5-amino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (20)

A solution of **8a** (20.0 g, 60.0 mmol) in MeOH (300 mL) was hydrogenated at 0.3 MPa in the presence of 5% Pd–C (2.0 mg) at room temperature for 20 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The solid residue was rinsed with Et_2O to give **20** (17.0 g, 93% yield) as a pale brown solid. Mp 257–258 °C. ^1H NMR ($CDCl_3$) δ: 1.28 (9H, s), 1.98 (3H, s), 2.06 (3H, s), 2.28 (3H, s), 2.75–3.25 (2H, m), 3.55–3.62 (2H, br), 3.90–4.20 (2H, m), 9.30–9.40 (1H, br).

4.4.2. *N*-(1-Acetyl-5-methanesulfonylamino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (21)

To a solution of **20** (17.0 g, 56.0 mmol) in CH_2Cl_2 (200 mL) were added pyridine (9.06 mL, 112 mmol) and MsCl (8.67 mL, 112 mmol) at 0 °C, followed by stirring at room temperature for 2 h. The solution was washed with 10% citric acid solution, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The solid residue was rinsed with i-Pr_2O to give **21** (21.3 g, quant.) as a brown solid. Mp 230–231 °C. ^1H NMR ($CDCl_3$) δ: 1.28 (9H, s), 2.21 (3H, s), 2.28 (3H, s), 2.32 (3H, s), 2.80–2.96 (2H, m), 3.02 (3H, s), 3.08–3.26 (1H, m), 4.00–4.30 (2H, m), 6.17–6.22 (1H, br), 9.18–9.23 (1H, br).

4.4.3. *N*-(5-Methanesulfonylamino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (10)

To a solution of **21** (22.0 g, 57.7 mmol) in EtOH (160 mL) was added 9.0 M NaOH aqueous solution (32 mL, 0.29 mol), was refluxed for 3 h. After water (80 mL) was added, the reaction mixture was evaporated under reduced pressure and neutralized by citric acid. The precipitate was collected by filtration to give **10** (17.8 g, 91% yield) as a pale yellow solid. Mp 225–233 °C (dec) ^1H NMR ($CDCl_3$) δ: 1.35 (9H, s), 2.22 (6H, s), 2.95–3.02 (5H, m), 3.63 (2H, t, J = 8.5 Hz), 4.30–4.48 (1H, br), 5.92–5.98 (1H, br), 7.03–7.10 (1H, br).

4.4.4. *N*-(1-Isopropyl-5-methanesulfonylamino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (1h)

To a solution of **10** (2.00 g, 5.90 mmol) in DMF (20 mL) was added 2-iodopropane (2.00 g, 12.0 mmol) and Et_3N (1.19 g, 11.8 mmol) at room temperature under N_2 atmosphere. The reaction mixture was stirred at 80 °C for 15 h. After AcOEt (200 mL) was added, the mixture was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (n-hexane–AcOEt) to give crude **1h** (800 mg), which was recrystallized from AcOEt to give **1h** (650 mg, 29% yield) as a white crystalline product. Mp 232–234 °C (dec.). IR (Nujol): 3176, 1656 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.03 (6H, d, J = 6.6 Hz), 1.21 (9H, s), 2.00 (3H, s), 2.12 (3H, s), 2.76 (2H, br t), 2.89 (3H, s), 3.37 (2H, br t), 4.00–4.20 (1H, m), 8.53 (1H, br s), 8.70 (1H, br s). MS m/z: 382 $[M+H]^+$. Anal. Calcd for $C_{19}H_{31}N_3O_3S{\cdot}0.1H_2O$: C, 59.53; H, 8.20; N, 10.96. Found: C, 59.42; H, 8.16; N, 10.89.

4.5. Procedure for preparation of 1l–o

Compounds **1l–o** were prepared according to the procedure for **1h.**

4.5.1. *N*-[5-Methanesulfonylamino-4,6-dimethyl-1-(3-methyl-2-butenyl)indolin-7-yl]-2,2-dimethylpropanamide (1l)

Mp 218–221 °C (dec.). IR (Nujol): 3130, 1641, 1600 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.19 (9H, s), 1.61 (3H, s), 1.65 (3H, s), 2.00 (3H, s), 2.12 (3H, s), 2.74 (2H, br t), 2.89 (3H, s), 3.34 (2H, br t), 3.78 (2H, d, J = 6.3 Hz), 5.00–5.30 (1H, m), 8.53 (1H, br s), 8.69 (1H, br s). MS m/z: 408 $[M+H]^+$. Anal. Calcd for $C_{21}H_{33}N_3O_3S$: C, 61.88; H, 8.16; N, 10.31. Found: C, 61.92; H, 8.00; N, 10.25.

4.5.2. *N*-[1-(Cyclopropylmethyl)-5-methanesulfonylamino-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (1m)

Mp 245–250 °C (dec.). IR (Nujol): 3258, 1655 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 0.10–1.10 (5H, m), 1.21 (9H, s), 1.99 (3H, s), 2.10 (3H, s), 2.78 (2H, t, J = 8.1 Hz), 2.78 (3H, s), 3.07 (2H, t, J = 8.1 Hz), 3.48 (2H, t, J = 6.5 Hz), 8.52 (1H, br s), 8.68 (1H, br s). MS m/z:

394 $[M+H]^+$. Anal. Calcd for $C_{20}H_{31}N_3O_3S$: C, 61.04; H, 7.94; N, 10.68. Found: C, 61.16; H, 7.72; N, 10.69.

4.5.3. *N*-[1-(Cyclobutylmethyl)-5-methanesulfonylamino-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (1n)

Mp 208–210 °C. IR (Nujol): 3205, 1662 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.21 (9H, s), 1.50–2.10 (7H, m), 1.99 (3H, s), 2.11 (3H, s), 2.75 (2H, br t), 2.88 (3H, s), 3.10–3.60 (4H, m), 8.52 (1H, br s), 8.63 (1H, br s). MS *m*/*z*: 408 $[M+H]^+$. Anal. Calcd for $C_{21}H_{33}N_3O_3S$: C, 61.88; H, 8.16; N, 10.31. Found: C, 61.60; H, 7.98; N, 10.30.

4.5.4. *N*-[1-(Cyclopentyl)-5-methanesulfonylamino-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (1o)

Mp 254–256 °C (dec.). IR (Nujol): 3219, 1647 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 1.21 (9H, s), 1.30–1.80 (8H, m), 1.98 (3H, s), 2.10 (3H, s), 2.70 (2H, br t), 2.77 (3H, s), 3.37 (2H, br t), 4.20–4.60 (1H, m), 8.51 (1H, br s), 8.68 (1H, br s). MS *m*/*z*: 408 $[M+H]^+$. Anal. Calcd for $C_{21}H_{33}N_3O_3S{\cdot}0.6H_2O$: C, 60.29; H, 8.24; N, 10.04. Found: C, 60.23; H, 8.00; N, 10.14.

4.6. Procedure for preparation 1j

Compound **1j** was prepared from compound **12** as follows:

4.6.1. 1-(5-Bromo-4,6-dimethyl-7-nitroindolin-1-yl)-2,2-dimethylpropan-1-one (22)

A solution of **12** (10.0 g, 36.9 mmol), Et_3N (11.3 mL, 81.1 mmol) and pivaloyl chloride (9.08 mL, 73.7 mmol) in $CHCl_3$ (100 mL) was refluxed for 40 h. The solution was washed with 10% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography, (toluene–AcOEt) to give **22** (13.0 g, 99% yield) as a pale brown solid. ^1H NMR ($CDCl_3$) δ: 1.34 (9H, s), 2.37 (3H, s), 2.48 (3H, s), 3.09 (2H, t, *J* = 7.8 Hz), 4.24 (2H, t, *J* = 7.8 Hz).

4.6.2. 1-(7-Amino-4,6-dimethylindolin-1-yl)-2,2-dimethylpropan-1-one (13)

A solution of **22** (13.0 g, 36.6 mmol) in MeOH (50 mL) was hydrogenated at 0.3 MPa in the presence of 10% Pd–C (2.0 g) at room temperature for 20 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL), washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give **13** (7.50 g, 83% yield) as a brown solid. ^1H NMR ($CDCl_3$) δ: 1.40 (9H, s), 2.14 (3H, s), 2.17 (3H, s), 2.89 (2H, t, *J* = 7.2 Hz), 3.70–4.60 (2H, br), 4.19 (2H, t, *J* = 7.2 Hz), 6.72 (1H, s).

4.6.3. *N*-[1-(2,2-Dimethylpropyl)-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (14)

To a suspension of $LiAlH_4$ (2.77 g, 73.0 mmol) in Et_2O (120 mL) was added **13** (6.00 g, 24.4 mmol) in Et_2O (10 mL) solution at 0 °C, followed by stirring at the same temperature for 1 h. The reaction mixture was poured into ice water, and the precipitate was removed by filtration. The filtrate was extracted with AcOEt, washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the obtained product in $CHCl_3$ (100 mL) were added Et_3N (8.10 mL, 58.0 mmol) and pivaloyl chloride (6.05 mL, 49.1 mmol) at 0 °C, followed by stirring at the same temperature for 2 h. The reaction mixture was washed with 10% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (toluene–AcOEt) to give **14** (3.30 g, 42% yield) as a pale yellow solid. ^1H NMR ($CDCl_3$) δ: 0.99 (9H, s), 1.34 (9H, s), 2.03 (3H, s), 2.09 (3H, s), 2.80 (2H, t, *J* = 8.4 Hz), 3.06 (2H, s), 3.49 (2H, t, *J* = 8.4 Hz), 6.35 (1H, s), 6.67 (1H, br s).

4.6.4. *N*-[1-(2,2-Dimethylpropyl)-4,6-dimethyl-5-nitroindolin-7-yl]-2,2-dimethylpropanamide (23)

To a solution of **14** (3.00 g, 9.48 mmol) in toluene (90 mL) was added concd HNO_3 (0.60 mL, 9.47 mmol) at 0 °C, followed by stirring at room temperature for 1 h. The reaction mixture was neutralized with saturated $NaHCO_3$ solution, extracted with AcOEt (50 mL), washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$). The solid residue was rinsed with AcOEt to give **23** (1.25 g, 36% yield) as a pale yellow solid. ^1H NMR ($CDCl_3$) δ: 0.99 (9H, s), 1.34 (9H, s), 2.01 (3H, s), 2.16 (3H, s), 2.87 (2H, t, *J* = 8.3 Hz), 3.14 (2H, s), 3.58 (2H, t, *J* = 8.3 Hz), 6.68 (1H, s).

4.6.5. *N*-[1-(2,2-Dimethylpropyl)-5-methanesulfonylamino-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide hydrochloride (1j)

A solution of **23** (1.20 g, 3.32 mmol) in MeOH (120 mL) was hydrogenated at 0.3 MPa in the presence of 5% Pd–C (200 mg) at room temperature for 15 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL), washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the obtained product in $CHCl_3$ (50 mL) were added Et_3N (1.85 mL, 13.3 mmol) and MsCl (0.51 mL, 6.59 mmol) at 0 °C, followed by stirring at room temperature for 1 h. The solution was washed with 10% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$–MeOH). To a solution of the obtained product in CH_2Cl_2–MeOH (1:1, 4 mL) was added 8.0 M HCl–*i*-PrOH (0.29 mL, 2.32 mmol) at 0 °C, followed by stirring for 10 min at room temperature. The solid residue was rinsed with *i*-PrOH to give **1j** (1.00 g, 67% yield) as a white solid. Mp 151–157 °C. IR (Nujol): 3348, 1668 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.21 (9H, s), 1.48 (9H, s), 2.16 (3H, s), 2.26 (3H, s), 3.03 (5H, br s), 3.10–3.40 (2H, br), 3.80–4.30 (2H, br), 7.22 (1H, br s), 9.55 (1H, br s). MS *m*/*z*: 410 $[M+H]^+$. Anal. Calcd for $C_{21}H_{35}N_3O_3S{\cdot}HCl{\cdot}0.6CH_2Cl_2$: C, 52.20; H, 7.54; N, 8.45. Found: C, 52.23; H, 7.43; N, 8.53.

4.7. Procedure for preparation of 4a

Compound **4a** was prepared from compound **7** as follows:

4.7.1. *N*-(1-Acetyl-4,6-dimethylindolin-7-yl)isobutyramide (24)

To a solution of **7** (5.00 g, 17.5 mmol) and Et_3N (7.33 mL, 52.5 mmol) in $CHCl_3$ (50 mL) was added isobutyryl chloride (2.05 g, 19.2 mmol) at 0 °C, followed by stirring at room temperature for 1 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 (*n*-hexane–AcOEt) to give **24** (4.70 g, 97% yield) as a colorless oil. ^1H NMR ($CDCl_3$) δ: 1.21 (6H, d, *J* = 7.5 Hz), 2.18 (6H, s), 2.29 (3H, s), 2.20–2.80 (1H, m), 2.94 (2H, t, *J* = 7.7 Hz), 4.10 (2H, t, *J* = 7.7 Hz), 6.88 (1H, s), 9.03 (1H, br s).

4.7.2. *N*-(4,6-Dimethyl-5-nitroindolin-7-yl)isobutyramide (25)

To a solution of **24** (3.70 g, 13.5 mmol) in AcOH (30 mL) were added concd HNO_3 (1.20 mL, 28.6 mmol) at 0 °C, followed by stirring at 60 °C for 1 h. The reaction mixture was poured into ice water and the mixture was extracted with $CHCl_3$. The extract was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a suspension of the obtained *N*-(1-acetyl-4,6-dimethyl-5-nitroindolin-7-yl)isobutyramide **(8b)** in MeOH (50 mL) was added 4.5 M NaOH aqueous solution (15 mL, 67.5 mmol), followed by stirring at 50 °C for 10 h, and then cooled to room temperature. The precipitate was col-

lected by filtration, and the product was dissolved in $CHCl_3$. The solution was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give **25** (2.50 g, 66% yield) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ: 1.12 (6H, d, J = 6.8 Hz), 1.96 (3H, s), 2.08 (3H, s), 2.30–2.70 (1H, m), 2.97 (2H, t, J = 8.4 Hz), 3.55 (2H, t, J = 8.4 Hz), 5.49 (1H, br s), 9.02 (1H, br s).

4.7.3. *N*-(1-Butyl-4,6-dimethyl-5-nitroindolin-7-yl)isobutyramide (9b)

To a solution of **25** (1.40 g, 5.05 mmol) in DMF (15 mL) were added *n*-butyl iodide (1.39 g, 7.55 mmol) and Et_3N (0.84 mL, 6.0 mmol), followed by stirring at 70 °C for 12 h under nitrogen atmosphere. After addition of AcOEt (100 mL), the solution was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The solid residue was rinsed with Et_2O to give **9b** (1.10 g, 65% yield) as a brown solid. ^{1}H NMR ($CDCl_3$) δ: 1.00 (3H, br t), 1.34 (6H, d, J = 6.6 Hz), 1.10–1.80 (4H, m), 2.03 (3H, s), 2.10 (3H, s), 2.88 (2H, t, J = 8.5 Hz), 3.24 (2H, t, J = 7.1 Hz), 3.54 (2H, t, J = 8.5 Hz), 6.63 (1H, br s).

4.7.4. *N*-(1-Butyl-5-methanesulfonylamino-4,6-dimethylindolin-7-yl)isobutyramide (4a)

A solution of **9b** (1.90 g, 5.68 mmol) in MeOH (150 mL) was hydrogenated at 0.3 MPa in the presence of 5% Pd–C (200 mg) at 40 °C for 10 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL), washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give aniline derivative. To a solution of the obtained product in $CHCl_3$ (40 mL) were added Et_3N (2.40 mL, 17.2 mmol) and MsCl (0.88 mL, 11.4 mmol) at 0 °C, followed by stirring at room temperature for 1 h. The solution was washed with 10% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$–MeOH). The solid residue was rinsed with AcOEt to give **4a** (630 mg, 29% yield) as a white crystalline product. Mp 235–240 °C (dec.). IR (Nujol): 3263, 1656 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 0.80–1.60 (7H, m), 1.10 (6H, d, J = 6.6 Hz), 2.00 (3H, s), 2.11 (3H, s), 2.20–2.70 (1H, m), 2.77 (2H, br t), 2.89 (3H, s), 3.00–3.60 (4H, m), 8.51 (1H, br s), 8.93 (1H, br s). MS m/z: 382 $[M+H]^+$. Anal. Calcd for $C_{19}H_{31}N_3O_3S$: C, 59.81; H, 8.19; N, 11.01. Found: C, 59.68; H, 8.04; N, 11.04.

4.8. Procedure for preparation of 4b

Compound **4b** was prepared according to the procedure for **4a**.

4.8.1. *N*-(1-Butyl-5-methanesulfonylamino-4,6-dimethylindolin-7-yl)-2,2-dimethylbutyramide (4b)

Mp 170–174 °C. IR (Nujol): 3201, 1660 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.80–1.20 (6H, m), 1.20–1.90 (6H, m), 1.28 (6H, s), 2.10 (3H, s), 2.13 (3H, s), 2.82 (2H, t, J = 8.4 Hz), 2.96 (3H, s), 3.18 (2H, t, J = 7.1 Hz), 3.44 (2H, t, J = 8.4 Hz), 6.16 (1H, br s), 6.85 (1H, br s). MS m/z: 410 $[M+H]^+$. Anal. Calcd for $C_{21}H_{35}N_3O_3S$: C, 61.58; H, 8.61; N, 10.26. Found: C, 61.40; H, 8.65; N, 10.26.

4.9. Procedure for preparation of 5a

Compound **5a** was prepared from *N*-(1-butyl-4,6-dimethyl-5-nitroindolin-7-yl)-2,2-dimethylpropanamide **(9c)** which was synthesized according to the same method of **9a**.

4.9.1. *N*-(5-Amino-1-butyl-4,6-dimethylindol-7-yl)-2,2-dimethylpropanamide (11a)

A solution of **9c** (2.00 g, 5.76 mmol) in MeOH–toluene (4:1, 80 mL) was hydrogenated at 0.3 MPa in the presence of 5% Pd–C (500 mg) at room temperature for 15 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL), washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. A solution of the obtained product and 5% Pd–C (1.0 g) in toluene (100 mL) was refluxed for 20 h. After removal of the catalyst by filtration, the filtrate was evaporated. The residue was purified by column chromatography (toluene–AcOEt) to give **11a** (850 mg, 46% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ: 0.82 (3H, br t), 1.10–1.80 (4H, m), 1.32 (9H, s), 2.06 (3H, s), 2.32 (3H, s), 3.37 (2H, br s), 4.09 (2H, t, J = 6.7 Hz), 6.31 (1H, d, J = 3.1 Hz), 6.87 (1H, d, J = 3.1 Hz), 7.10 (1H, br s).

4.9.2. *N*-(1-Butyl-5-methanesulfonylamino-4,6-dimethylindol-7-yl)-2,2-dimethylpropanamide (5a)

To a solution of **11a** (850 mg, 2.69 mmol) in $CHCl_3$ (20 mL) were added Et_3N (1.50 mL, 10.8 mmol) and MsCl (0.42 mL, 5.4 mmol) at 0 °C, followed by stirring at room temperature for 30 min. The solution was washed with 10% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (toluene–AcOEt) to give crude **5a**, which was recrystallized from AcOEt to give **5a** (350 mg, 33% yield) as a white crystalline product. Mp 217–223 °C. IR (Nujol): 3186, 1652 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.86 (3H, br t), 1.00–1.80 (4H, m), 1.40 (9H, s), 2.13 (3H, s), 2.23 (3H, s), 2.91 (3H, s), 3.80–4.20 (2H, m), 6.32 (1H, d, J = 3.1 Hz), 6.94 (1H, d, J = 3.1 Hz), 6.65 (1H, br s), 7.25 (1H, br s). MS m/z: 394 $[M+H]^+$. Anal. Calcd for $C_{20}H_{31}N_3O_3S$: C, 61.04; H, 7.94; N, 10.68. Found: C, 60.77; H, 7.82; N, 10.60.

4.10. Procedure for preparation of 5b

Compound **5b** was prepared according to the procedure for **5a**.

4.10.1. *N*-(1-Isobutyl-5-methanesulfonylamino-4,6-dimethylindol-7-yl)-2,2-dimethylpropanamide (5b)

Mp 240–242 °C. IR (Nujol): 3192, 1651 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.74 (6H, br d), 1.29 (9H, s), 1.80–2.20 (1H, m), 2.19 (3H, s), 2.48 (3H, s), 2.97 (3H, s), 3.92 (2H, d, J = 7.5 Hz), 6.43 (1H, d, J = 2.9 Hz), 7.14 (1H, d, J = 2.9 Hz), 8.77 (1H, br s), 9.07 (1H, br s). MS m/z: 394 $[M+H]^+$. Anal. Calcd for $C_{20}H_{31}N_3O_3S$: C, 61.04; H, 7.94; N, 10.68. Found: C, 60.77; H, 7.80; N, 10.63.

4.11. Procedure for preparation of 6

Compound **6** was prepared from compound **15** as follows:

4.11.1. 1-Acetyl-6-bromo-5,7-dimethyl-1,2,3,4-tetrahydroquinoline (26)

To a solution of **15** (10.0 g, 49.2 mmol) in AcOH (100 mL) was added Br_2 (2.54 mL, 49.3 mmol), followed by stirring at room temperature for 30 min. The reaction mixture was poured into water and the formed precipitate was collected by filtration. The obtained product was dissolved in $CHCl_3$ (100 mL), washed with 5% $NaHSO_3$ solution, water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (toluene–AcOEt). The solid residue was recrystallized from MeOH to give **26** (7.30 g, 52% yield) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.80–2.20 (2H, m), 2.19 (3H, s), 2.39 (6H, s), 2.70 (2H, t, J = 6.7 Hz), 3.74 (2H, t, J = 6.7 Hz), 7.01 (1H, s).

4.11.2. 1-Acetyl-6-bromo-5,7-dimethyl-7-nitro-1,2,3,4-tetrahydroquinoline (27)

To a suspension of **26** (6.50 g, 23.0 mmol) in Ac_2O (15 mL) was added concd HNO_3 (2.19 mL, 49.4 mmol) in Ac_2O (15 mL) at 0 °C,

followed by stirring at room temperature for 1 h. The reaction mixture was quenched with saturated $NaHCO_3$ solution, and the mixture was extracted with AcOEt, washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (*n*-hexane–AcOEt) to give **27** (7.00 g, 93% yield) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.80–2.20 (2H, m), 2.11 (3H, s), 2.36 (3H, s), 2.49 (3H, s), 2.73 (2H, br t), 3.30–4.00 (2H, br).

4.11.3. 6-Bromo-5,7-dimethyl-7-nitro-1,2,3,4-tetrahydroquinoline (16)

A solution of **27** (2.00 g, 6.11 mmol) and 2.0 M NaOH aqueous solution (15 mL, 30 mmol) in EtOH (40 mL) was refluxed for 2 h. The reaction mixture was evaporated under reduced pressure, and the residue was dissolved in $CHCl_3$, washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography (toluene) to give **16** (1.40 g, 80% yield) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.70–2.10 (2H, m), 2.34 (3H, s), 2.41 (3H, s), 2.72 (2H, t, J = 6.3 Hz), 3.10–3.50 (2H, m), 5.87 (1H, br s).

4.11.4. *N*-(5,7-Dimethyl-1,2,3,4-tetrahydroquinolin-8-yl)-2,2-dimethylpropanamide (28)

A solution of **16** (4.30 g, 15.1 mmol) in MeOH (400 mL) was hydrogenated at 0.3 MPa in the presence of 5% Pd–C (400 mg) at 40 °C for 12 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL), washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the obtained product in $CHCl_3$ (100 mL) were added Et_3N (0.45 mL, 3.2 mmol) and pivaloyl chloride (1.11 mL, 13.6 mmol), followed by stirring at room temperature for 1 h. The solution was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure to give **28** (3.10 g, 78% yield) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 1.35 (9H, s), 1.70–2.10 (2H, m), 2.09 (3H, s), 2.12 (3H, s), 2.64 (2H, t, J = 6.3 Hz), 3.25 (2H, t, J = 6.3 Hz), 3.80–4.10 (1H, br s), 6.41 (1H, s), 6.70 (1H, br s).

4.11.5. *N*-(5,7-Dimethyl-1-propyl-1,2,3,4-tetrahydroquinolin-8-yl)-2,2-dimethylpropanamide (17)

To a solution of **28** (3.10 g, 11.9 mmol) in DMF (30 mL) were added *n*-propyl iodide (4.00 g, 23.6 mmol) and Et_3N (3.20 mL, 22.9 mmol), followed by stirring at 80 °C for 15 h under N_2 atmosphere. After the addition of AcOEt (100 mL), 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 (*n*-hexane–AcOEt) to give **17** (2.70 g, 74% yield) as a pale brown solid. ^{1}H NMR ($CDCl_3$) δ: 0.85 (3H, t, J = 7.3 Hz), 1.35 (9H, s), 1.60–2.00 (4H, m), 2.09 (3H, s), 2.13 (3H, s), 2.40–2.70 (4H, m), 2.80–3.20 (2H, m), 6.71 (1H, s), 7.25 (1H, br s).

4.11.6. *N*-(5,7-Dimethyl-6-nitro-1-propyl-1,2,3,4-tetrahydroquinolin-8-yl)-2,2-dimethylpropanamide (29)

To a solution of **17** (1.00 g, 3.31 mmol) in Ac_2O (10 mL) was added fum. HNO_3 (0.59 mL, 14.1 mmol) in Ac_2O (5 mL) at 5 °C, followed by stirring at the same temperature for 45 min. The reaction mixture was neutralized with saturated $NaHCO_3$ solution, and extracted with AcOEt (50 mL). The extract was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (*n*-hexane–AcOEt) to give **29** (500 mg, 43% yield) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ: 0.86 (3H, t, J = 7.2 Hz), 1.35 (9H, s), 1.40–1.90 (4H, m), 1.98 (3H, s), 2.08 (3H, s), 2.50–2.70 (4H, m), 2.90–3.10 (2H, m), 7.26 (1H, br s).

4.11.7. *N*-(6-Methanesulfonylamino-5,7-dimethyl-1-propyl-1,2,3,4-tetrahydroquinolin-8-yl)-2,2-dimethylpropanamide hydrochloride (6)

A solution of **29** (500 mg, 1.44 mmol) in MeOH (50 mL) was hydrogenated at 0.3 MPa in the presence of 5% Pd–C (100 mg) at 40 °C for 10 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL), washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the obtained product in $CHCl_3$ (5 mL) were added Et_3N (0.58 mL, 4.3 mmol) and MsCl (0.22 mL, 2.84 mmol) at 0 °C, followed by stirring at room temperature for 1 h. The solution was washed with 10% citric acid solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (toluene–AcOEt). To a solution of the obtained product in MeOH (5 mL) was added 8.0 M HCl–*i*-PrOH (0.14 mL, 1.13 mmol) at 0 °C, followed by stirring for 10 min at same temperature. The reaction mixture was evaporated under reduced pressure. The solid residue was rinsed with Et_2O to give **6** (230 mg, 36% yield) as a white solid. Mp 163–170 °C. IR (Nujol): 1668, 1456 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.95–1.15 (3H, br), 1.47 (9H, s), 1.60–2.00 (4H, m), 2.16 (6H, s), 2.60–2.90 (2H, m), 3.00 (3H, s), 3.10–3.80 (4H, m), 7.34 (1H, br s), 9.83 (1H, br s), 12.90 (1H, br s). MS *m*/*z*: 396 [M+H]$^+$.

4.12. Procedure for preparation of 2a–g

Compounds **2a**–**g** were synthesized according to the methods described in a previous report.[15]

4.12.1. [7-(2,2-Dimethylpropanamido)-1-hexyl-4,6-dimethylindolin-5-yl]acetic acid (2a)

Mp 93–104 °C. IR (Nujol): 1761, 1651 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.70–1.10 (3H, t, J = 7.1 Hz), 1.10–1.70 (8H, m), 1.33 (9H, s), 2.01 (3H, s), 2.15 (3H, s), 2.70–3.20 (4H, m), 3.41 (2H, t, J = 8.5 Hz), 3.56 (2H, s), 7.60–8.10 (2H, br). MS *m*/*z*: 389 [M+H]$^+$.

4.12.2. [7-(2,2-Dimethylpropanamido)-1-heptyl-4,6-dimethylindolin-5-yl]acetic acid hemisulfate (2b)

Mp 140–149 °C. IR (Nujol): 3460, 3219, 2502, 2343, 1713, 1651, 1514 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.80–0.90 (3H, br), 1.00–1.80 (10H, m), 1.38 (9H, s), 1.95 (3H, s), 2.05 (3H, s), 2.60–3.20 (4H, m), 3.40–4.50 (2H, br), 3.50–3.90 (2H, m), 5.20–6.60 (2H, br), 9.50–9.51 (1H, br). MS *m*/*z*: 404 [M+H]$^+$. Anal. Calcd for $C_{24}H_{38}N_2O_3{\cdot}0.5H_2SO_4{\cdot}H_2O$: C, 61.38; H, 8.80; N, 5.97. Found: C, 61.12; H, 8.54; N, 5.89.

4.12.3. [7-(2,2-Dimethylpropanamido)-4,6-dimethyl-1-nonylindolin-5-yl]acetic acid hemisulfate (2d)

Mp 171–182 °C. IR (Nujol): 3267, 2509, 1738, 1682, 1672, 1504 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.80–0.90 (3H, br), 1.03–1.80 (14H, m), 1.38 (9H, s), 1.95 (3H, s), 2.06 (3H, s), 2.70–3.20 (4H, m), 3.40–4.50 (2H, br), 3.45–3.86 (2H, m), 6.40–8.40 (2H, br), 9.40–9.50 (1H, br). MS *m*/*z*: 432 [M+H]$^+$.

4.12.4. [1-Decyl-7-(2,2-dimethylpropanamido)-4,6-dimethylindolin-5-yl]acetic acid hemisulfate (2e)

Mp 173–180 °C. IR (Nujol): 1712, 1656, 1600 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.70–1.10 (3H, t, J = 7.1 Hz), 1.10–1.70 (16H, m), 1.33 (9H, s), 2.01 (3H, s), 2.15 (3H, s), 2.70–3.20 (4H, m), 3.41 (2H, t, J = 8.5 Hz), 3.56 (2H, s), 7.60–8.10 (2H, br). MS *m*/*z*: 446 [M+H]$^+$. Anal. Calcd for $C_{27}H_{44}N_2O_3{\cdot}0.5H_2SO_4$: C, 65.69; H, 9.19; N, 5.67. Found: C, 65.60; H, 9.00; N, 5.74.

4.12.5. [7-(2,2-Dimethylpropanamido)-4,6-dimethyl-1-undecylindolin-5-yl]acetic acid hemisulfate (2f)

Mp 100–108 °C. IR (Nujol): 3186, 2727, 2529, 1728, 1682 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.80–0.90 (3H, br), 1.06–1.92 (18H, m), 1.41

(9H, s), 2.07 (3H, s), 2.23 (3H, s), 2.90–3.35 (4H, m), 3.60–4.00 (2H, br), 3.60–3.70 (2H, m), 4.60–6.20 (2H, br), 9.20–9.30 (1H, br). MS *m*/*z*: 460 $[M+H]^+$. Anal. Calcd for $C_{28}H_{46}N_2O_3{\cdot}0.5H_2SO_4{\cdot}0.1EtOH$: C, 65.99; H, 9.40; N, 5.42. Found: C, 66.12; H, 9.66; N, 5.55.

4.12.6. [7-(2,2-Dimethylpropanamido)-1-dodecyl-4,6-dimethylindolin-5-yl]acetic acid hemisulfate (2g)

Mp 120–130 °C (decomp.). ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 6.6 Hz), 1.15–1.78 (20H, m), 1.38 (9H, s), 1.90 (3H, s), 2.03 (3H, s), 2.90–3.15 (4H, m), 3.30–3.40 (2H, br), 3.50–4.10 (2H, m), 9.40–9.60 (1H, br). MS *m*/*z*: 474 $[M+H]^+$. Anal. Calcd for $C_{29}H_{48}N_2O_3{\cdot}0.5H_2SO_4{\cdot}0.5H_2O$: C, 65.63; H, 9.50; N, 5.28. Found: C, 65.56; H, 9.36; N, 5.21.

4.13. Partition coefficient at pH 7.0

Log $D_{7.0}$ values (logarithm of octanol–water partition coefficients at pH 7.0) were determined by HPLC methods.[22] Benzene, bromobenzene, biphenyl and hexachlorobenzene, the log $D_{7.0}$ values of which are known, were used as reference substances. Test compounds and reference substances were dissolved in acetonitrile containing 1% dimethylsulfoxide (DMSO) at 10 μg/mL, and then 10 μL of the solution was injected into the HPLC system. The HPLC equipment consisted of a pump (PU-980; JASCO, Tokyo, Japan), a UV detector (UV-970; JASCO), an autoinjector (AS-950; JASCO), and a Cosmosil 5C18-AR-II column (5 μm, 4.6 mm × 150 mm; Nacalai Tesque, Kyoto, Japan). Phosphate buffer (pH 7.0)/methanol (8:2) was used as the eluent. The capacity factors of test substances and reference substances were calculated from their retention time. The log $D_{7.0}$ values of test compounds were calculated using these capacity factors and the reported log $D_{7.0}$ values of reference substances.

4.14. Plasma protein binding ratio

The plasma protein binding ratio was estimated by an ultracentrifugation method.[23] Test compounds were dissolved in rat plasma (10 μg/mL). The concentration of the plasma was determined using the API 2000 QTRAP LC–MS/MS system (Applied Biosystems). The plasma was then separated into three layers by ultracentrifugation at 436,000*g* for 150 min with a small ultracentrifuge (CS100GX; Hitachi Koki, Hitachinaka, Japan). The concentration of test compounds in the middle layer (protein-free fraction) was determined using the API 2000 QTRAP LC–MS/MS system. From total and protein-free fraction concentrations, the protein binding ratio of test compounds was calculated.

4.15. Esterified cholesterol (EC) accumulation in THP-1 cell-derived macrophages

THP-1 cells were grown in RPMI-1640 medium containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. In order to differentiate into macrophages, the cells were suspended in RPMI-1640 medium containing 10% FBS and phorbol 12-myristate 13-acetate (PMA, 200 nM). 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. Then, to form foam cells, they were washed and incubated in RPMI-1640 medium containing 10% FBS, PMA (200 nM), and high-cholesterol serum (5%) at 37 °C for a day in the presence or absence of test compounds. The high-cholesterol serum was isolated from Japanese white rabbits (4–6 months old, Japan SLC, Hamamatsu, Japan) fed a high-cholesterol diet. 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.

4.16. In vitro ACAT activity

Male Japanese White rabbits (3 months old, Japan SLC) were anesthetized with sodium pentobarbital (30 mg/kg, iv) and exsanguinated from the common carotid artery, and then the liver was isolated. Microsomes were prepared according to the method of Field and Mathur.[24] 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 12,000*g* for 15 min at 4 °C. The resulting supernatant was centrifuged at 105,000*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.[25] 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, and then 30 nmol of [1-^{14}C]oleoyl-CoA (PerkinElmer, Waltham, MA, USA) was added. The reaction mixture was incubated at 37 °C for 20 min. Lipids were extracted with chloroform/methanol (2:1) and separated by thin-layer chromatography. The EC produced in microsomes treated with vehicle and test compounds were determined. IC_{50} values were calculated using data at the concentrations of test compounds in each experiment and the mean value was calculated for two experiments.

4.17. Plasma levels of compounds after oral administration in rats

Compound **1b** and Pactimibe were suspended in 3% 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. Blood samples were drawn using a heparinized syringe from the jugular vein at 0.25, 0.5, 1, 2, 3, 5, 8 and 24 h after administration to rats. Blood was centrifuged at 3000 rpm for 10 min at room temperature. Concentrations of **1b** and Pactimibe in the plasma were determined using HPLC.

4.18. Effect on hepatic secretion of cholesterol

Triton WR-1339 was intravenously injected to male ICR mice (6 weeks old, Japan SLC) and then immediately **1b** and Pactimibe (10 mg/kg) were orally administered. Blood samples were collected before and 3 h after administration. Serum total cholesterol levels were measured by an enzymatic method using a commercial assay kit (Wako Pure Chemicals Industries, Ltd, Osaka, Japan) and hepatic secretion of cholesterol was calculated.

References and notes

1. Kathawala, F. G.; Heider, J. G. Acyl CoA: Cholesterol Acyltransferase Inhibitors and Lipid–Lipoprotein Metabolism. In *Antilipidemic Drugs*; Witiak, D. T., Newman, H. A. I., Feller, D. R., Eds.; Elsevier Science Ltd: Amsterdam, 1991; pp 159–195.
2. Sliskovic, D. R.; White, A. D. *Trends Pharmacol. Sci.* **1991**, *12*, 194.
3. Chang, C.; Dong, R.; Miyazaki, A.; Sakashita, N.; Zhang, Y.; Liu, J.; Guo, M.; Li, B. L.; Chang, T. Y. *Acta Biochim. Biophys. Sin.* **2006**, *38*, 151.
4. Matsuda, K. *Med. Res. Rev.* **1994**, *14*, 271.
5. Sliskovic, D. R.; Picard, J. A.; Krause, B. R. *Prog. Med. Chem.* **2002**, *39*, 121.
6. Roth, B. D. *Drug Discovery Today* **1998**, *3*, 19.
7. Ashton, M. J.; Bridge, A. W.; Bush, R. C.; Dron, D. I.; Harris, N. V.; Jones, G. D.; Lythgoe, D. J.; Riddell, D.; Smith, C. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 375.
8. Sliskovic, D. R.; Krause, B. R.; Picard, J. A.; Anderson, M.; Bousley, R. F.; Hamelehle, K. L.; Homan, R.; Julian, T. N.; Rashidbaigi, Z. A.; Stanfield, R. L. *J. Med. Chem.* **1994**, *37*, 560.
9. Bani, M.; Bormetti, R.; Ceccarelli, W.; Fiocchi, R.; Gobetti, M.; Lombroso, M.; Magnetti, S.; Olgiati, V.; Palladino, M.; Villa, M.; Vanotti, E. *Eur. J. Med. Chem.* **1995**, *30*, 39.
10. Smith, C.; Ashton, M. J.; Bush, R. C.; Facchini, V.; Harris, N. V.; Hart, T. W.; Jordan, R.; MacKenzie, R.; Riddell, D. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 47.

11. Lee, H. T.; Sliskovic, D. R.; Picard, J. A.; Roth, B. D.; Wierenga, W.; Hicks, J. L.; Bousley, R. F.; Hamelehle, K. L.; Homan, R.; Speyer, C.; Stanfield, R. L.; Krause, B. R. *J. Med. Chem.* **1996**, *39*, 5031.
12. Harte, R. A.; Yeaman, S. J.; Jackson, B.; Suckling, K. E. *Biochim. Biophys. Acta* **1995**, *1258*, 241.
13. Homan, R.; Hamelehle, K. L. *J. Pharm. Sci.* **2001**, *90*, 1859.
14. Takahashi, K.; Kunishiro, K.; Kasai, M.; Miike, T.; Kurahashi, K.; Shirahase, H. *Arzneim. Forsch* **2008**, *58*, 666.
15. Takahashi, K.; Kasai, M.; Ohta, M.; Shoji, Y.; Kunishiro, K.; Kanda, M.; Kurahashi, K.; Shirahase, H. *J. Med. Chem.* **2008**, *51*, 4823.
16. Terasaka, N.; Miyazaki, A.; Kasanuki, N.; Ito, K.; Ubukata, N.; Koieyama, T.; Kitayama, K.; Tanimoto, T.; Maeda, N.; Inaba, T. *Atherosclerosis* **2007**, *190*, 239.
17. Kitayama, K.; Koga, T.; Maeda, N.; Inaba, T.; Fujioka, T. *Eur. J. Pharmacol.* **2006**, *539*, 81.
18. Nissen, S. E.; Tuzcu, E. M.; Brewer, H. B.; Sipahi, I.; Nicholls, S. J.; Ganz, P.; Schoenhagen, P.; Waters, D. D.; Pepine, C. J.; Crowe, T. D.; Davidson, M. H.; Deanfield, J. E.; Wisniewski, L. M.; Hanyok, J. J.; Kassalow, L. M. *N. Engl. J. Med.* **2006**, *354*, 1253.
19. Kamiya, S.; Shirahase, H.; Yoshimi, A.; Nakamura, S.; Kanda, M.; Matsui, H.; Kasai, M.; Takahashi, K.; Kurahashi, K. *Chem. Pharm. Bull.* **2000**, *48*, 817.
20. Winum, J. Y.; Scozzafava, A.; Montero, J. L.; Supuran, C. T. *Med. Res. Rev.* **2005**, *25*, 186.
21. Hughes, J. D.; Blagg, J.; Price, D. A.; Bailey, S.; DeCrescenzo, G. A.; Devraj, R. V.; Ellsworth, E.; Fobian, Y. M.; Gibbs, M. E.; Gilles, R. W.; Greene, N.; Huang, E.; Krieger-Burke, T.; Loesel, J.; Wager, T.; Whiteley, L.; Zhang, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4872.
22. Matsumoto, K.; Takeyasu, A.; Oizumi, K.; Kobayashi, T. *Yakugaku Zasshi (Text in Japanese)* **1995**, *115*, 213.
23. Nakai, D.; Kumamoto, K.; Sakikawa, C.; Kosaka, T.; Tokui, T. *J. Pharma. Sci.* **2004**, *93*, 847.
24. Field, F. J.; Mathur, S. N. *J. Lipid Res.* **1983**, *24*, 409.
25. Heider, J. G.; Pickens, C. E.; Kelly, L. A. *J. Lipid Res.* **1983**, *24*, 1127.