Novel Acyl-CoA: Cholesterol Acyltransferase Inhibitor: Indoline-Based Sulfamide Derivatives with Low Lipophilicity and Protein Binding Ratio

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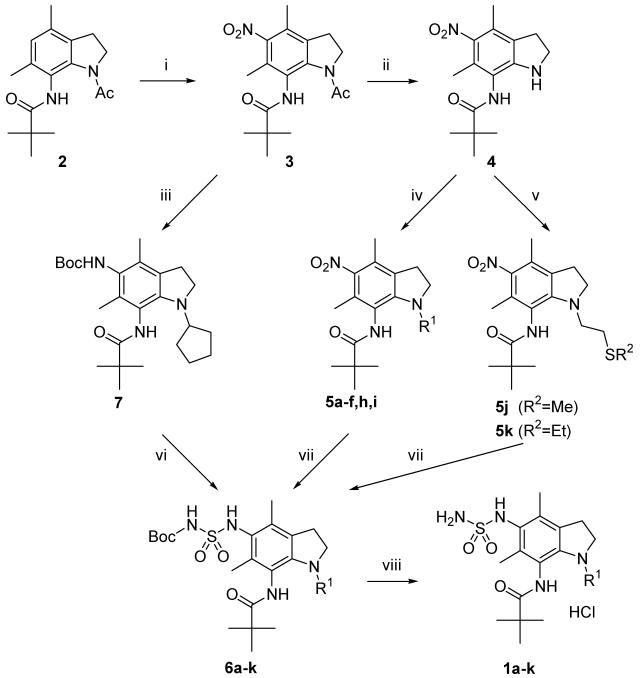
To find a novel acyl-CoA: cholesterol acyltransferase inhibitor, a series of sulfamide derivatives were synthesized and evaluated. Compound 1d, in which carboxymethyl moiety at the 5-position of Pactimibe was replaced by a sulfamoylamino group, showed 150-fold more potent anti-foam cell formation activity (IC_{50}: 0.02 μM), 1.6-fold higher log $D_{7.0}$ (4.63), and a slightly lower protein binding ratio (93.2%) than Pactimibe. Compound 1i, in which the octyl chain at the 1-position in 1d was replaced by an ethoxyethyl, showed markedly low log $D_{7.0}$ (1.73) and maintained 3-fold higher anti-foam cell formation activity (IC_{50}: 1.0 μM), than Pactimibe. The plasma protein binding ratio (PBR) of 1i was much lower than that of Pactimibe (62.5% *vs.* 98.1%), and its partition ratio to the rabbit atherosclerotic aorta after oral administration was higher than that of Pactimibe. Compound 1i at 10 μM markedly inhibited cholesterol esterification in atherosclerotic rabbit aortas even when incubated with serum, while Pactimibe had little effect probably due to its high PBR. In conclusion, compound 1i is expected to more efficiently inhibit the progression of atherosclerosis than Pactimibe.

Key words acyl-CoA: cholesterol acyltransferase; sulfamide derivative; protein binding ratio; lipophilicity; foam cell formation; Pactimibe

Numerous acyl-CoA: cholesterol acyltransferase (ACAT) inhibitors have been reported as potential hypolipidemic drugs, which inhibit intestinal and hepatic esterification of cholesterol,[1—5] and anti-atherosclerotic drugs, which inhibit macrophage esterification of cholesterol in atherosclerotic plaques[6]; however, most of the reported inhibitors have been highly lipophilic and demonstrated to have low bioavailability. We previously synthesized a new indoline-based ACAT inhibitor, Pactimibe, with a carboxymethyl group at the 5-position, which was moderately lipophilic, highly water soluble, showed good oral absorption, and exerted excellent experimental anti-atherosclerotic effects.[7] Pactimibe decreased the atherosclerotic areas in apo-E knockout mice and stabilized aortic plaques in Watanabe heritable hyperlipidemic rabbits (WHHL rabbits)[8,9]; however, a clinical study using an intravascular-ultrasonography catheter (ACTIVATE study) failed to show plaque retardation at a dose of 100 mg in patients with coronary artery disease (CAD).[10] It cannot be excluded that Pactimibe may have not fully inhibited plaque ACAT activity, since Pactimibe probably has a high plasma protein binding ratio (PBR), suggesting low partition to atherosclerotic plaques. A new less lipophilic ACAT inhibitor that has more potent biological effects and lower PBR would more efficiently inhibit plaque ACAT activity and would be a candidate for a new anti-atherosclerotic drug. In the case of Pactimibe, the introduction of a carboxymethyl group to its indoline ring resulted in an increase in its water solubility and bioavailability whilst maintaining its biological activity. In the present study, we replaced a carboxymethyl moiety of Pactimibe with a sulfamoylamino moiety, which markedly enhanced its anti-foam cell formation activity *via* ACAT inhibition and slightly increased its lipophilicity, as well as reducing its PBR. A series of sulfamide derivatives were synthesized and a compound with an ethoxyethyl group at the 1-position was found to show much lower lipophilicity and PBR, and more potent anti-foam cell formation (AFCF) activities than Pactimibe.

Chemistry Indoline-based sulfamide derivatives with no substituents at the 2-position of the indoline ring (**1a**—**k**) were synthesized from *N*-(1-acetyl-4,6-dimethylindolin-7-



Reagents, conditions and yields: (i) fum. HNO_3, conc. H_2SO_4, AcOH, 10 °C; (ii) NaOH solution, MeOH, reflux, 2 steps 94%; (iii) (a) H_2, Pd–C, rt, 93%; (b) Boc_2O, Et_3N, 90 °C, 37%; (c) NaOH solution, EtOH, reflux, 68%; (d) cyclopentyl bromide, *i*-Pr_2NEt, KI, hydroquinone, DMF, 90 °C, 21%; (iv) alkyl halide, NaH, DMF, 0 °C–rt or alkoxyethyl halide, *i*-Pr_2NEt, DMF, 110 °C, 32—80%; (v) (a) 2-bromoethanol, *i*-Pr_2NEt, DMF, 120 °C, 67%; (b) MsCl, Et_3N, $CHCl_3$, 0 °C; (c) AcSK, DMF, 70 °C; (d) NaOH solution, MeOH, 0 °C, 3 steps 85%; (e) MeI (for **5j**) or EtI (for **5k**), *i*-Pr_2NEt, DMF, rt, 91 or 86%, respectively; (vi) (a) HCl in *i*-PrOH, HCO_2H, 0 °C; (b) $BocNHSO_2Cl$ (prepared from *tert*-BuOH and chlorosulfonyl isocyanate), Et_3N, CH_2Cl_2, −10 °C, 2 steps 69%; (vii) (a) H_2, Pd–C, MeOH, 35 °C; (b) $BocNHSO_2Cl$, Et_3N, CH_2Cl_2, −10°C, 2 steps 36—77%; (viii) HCl in *i*-PrOH, HCO_2H, 0 °C, 52—94%.

Chart 1. Synthesis of Indoline-Based Sulfamide Derivatives with No Substitution at the 2-Position

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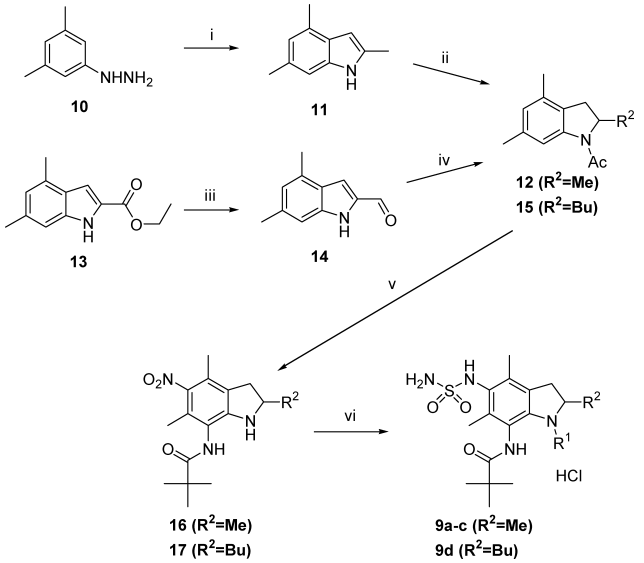
yl)-2,2-dimethylpropanamide (**2**) as outlined in Chart 1. Compound **2** was synthesized from 1-acetyl-4,6-dimethylindoline according to the method previously reported[11] and then nitrated to give compound **3** with a nitro group at the 5-position. To prepare **1a**—**f**, **h**—**k**, compound **3** was hydrolyzed to give compound **4** with no substituents at the 1-position, and then various chains, *e.g.* straight alkyl, branched alkyl, and ether chains, were introduced to **4** at the 1-position using *i*-Pr_2NEt or NaH as a base to give **5a**—**f**, **h**, **i**. Separately, compound **4** was alkylated with 2-bromoethanol at the 1-position, followed by mesylation, and then the mesylate compound was converted to thioether derivatives **5j**, **k**. The nitro moiety of compounds **5a**—**f**, **h**—**k** was hydrogenated, followed by the reaction with Boc-$NHSO_2Cl$, which was previously generated by the reaction of chlorosulfonyl isocyanate with *tert*-BuOH, to give **6a**—**f**, **h**—**k** with Boc-protected sulfamoylamino moiety at the 5-position. To prepare compound **6g** with a cyclopentyl moiety at the 1-position, the nitro moiety of **3** was converted to Boc-protected amino moiety. The acetyl moiety was hydrolyzed, and then a cyclopentyl moiety was introduced to give compound **7**. The Boc group of **7** was cleaved by treating with HCl, followed by conversion to **6g** according to the method described above. Finally, the Boc group of **6a**—**k** was deprotected with HCl to obtain compounds **1a**—**k** as a hydrochloride salt.

Indoline-based sulfamide derivatives with a methyl or butyl moiety at the 2-position (**9a**—**d**) were prepared as outlined in Chart 2. 3,5-Dimethylphenylhydrazine (**10**) was converted to 2,4,6-trimethylindole (**11**) by Fisher indole synthesis. Compound **11** was reduced by $NaBH_3CN$ and acetylated at the 1-position to give compound **12**. Separately, the ester function of ethyl 4,6-dimethylindole-2-carboxylate (**13**)[12] was reduced with $LiAlH_4$, followed by successive oxidation with activated MnO_2 to afford an aldehyde derivative **14**. Then, compound **14** was converted to **15** with a butyl moiety at the 2-position by the Wittig reaction and successive hydrogenation, followed by reduction of the indole ring. Compounds **12** and **15** were converted to 1,2-disubstituted indolines **9a**—**d** *via* deacetylated compounds **16** and **17** according to the method described in Chart 1.

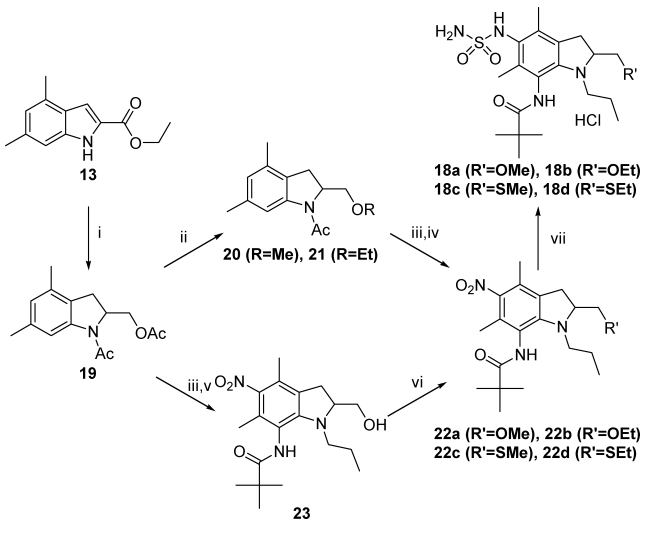
Indoline-based sulfamide derivatives with ether or thioether moiety at the 2-position (**18a**—**d**) were synthesized as outlined in Chart 3. An ester compound **13** was reduced with $LiAlH_4$ and $NaBH_3CN$, and then acetylated to give compound **19**. Compound **19** was selectively deacetylated at the 2-position, and then alkylated with MeI and EtI to give **20** and **21**, respectively. Compounds **20** and **21** were converted to **18a**, **b** *via* 5-nitroindoline derivatives **22a**, **b** as described in Chart 1. Separately, compound **19** was converted to **23** with a hydroxymethyl moiety at the 2-position as described in Chart 1, and then the hydroxymethyl moiety was converted to the thioether moiety to give **22c**, **d**, followed by conversion to compounds **18c**, **d**.

An indoline-based sulfamide derivative with a methoxyethyl moiety at the 3-position (**24**) was synthesized as outlined in Chart 4. 4,6-Dimethylindole (**25**) was reacted with oxalyl chloride, and successively esterified to give a product with ethyl oxalate at the 3-position, which was reduced with $LiAlH_4$ and $NaBH_3CN$, and protected with Boc group to obtain a compound with a hydroxyethyl moiety at the 3-position. Then, the compound was methylated at the hydroxyl group, followed by exchanging Boc for an acetyl



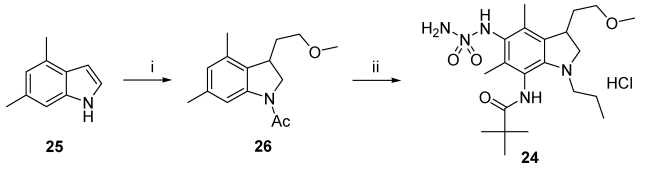
Reagents, conditions and yields: (i) acetone, PPA, 140 °C, 80%; (ii) (a) $NaBH_3CN$, AcOH, 0 °C; (b) Ac_2O, benzene, rt, 2 steps 85%; (iii) (a) $LiAlH_4$, Et_2O, rt; (b) MnO_2, CH_2Cl_2, rt, 2 steps 62%; (iv) (a) allyltriphenylphosphonium bromide, *n*-BuLi, THF, 10 °C; (b) H_2, Pd–C, MeOH, 35 °C; (c) $NaBH_3CN$, AcOH, 10 °C; (d) Ac_2O, $CHCl_3$, Et_3N, rt, 4 steps 42%; (v) (a) Br_2, AcOH, rt, 94—100%; (b) fum. HNO_3, conc. H_2SO_4, AcOH, −10 °C, 70—100%; (c) H_2, Pd–C, MeOH, 40 °C; (d) pivaloyl chloride, Et_3N, CH_2Cl_2, 0 °C, 2 steps 72—100%; (e) fum. HNO_3, conc. H_2SO_4, AcOH, 0 °C, 72—84%; (f) NaOH solution, EtOH, 60 °C, 97—98%; (vi) (a) alkyl halide, *i*-Pr_2NEt, DMF, 110 °C, or MeI, NaH, DMF, rt, 52—89%; (b) H_2, Pd–C, MeOH, 35 °C; (c) Boc$NHSO_2Cl$, Et_3N, CH_2Cl_2, −10 °C, 2 steps 61—89%; (d) HCl in *i*-PrOH, HCO_2H, 0 °C, 62—67%.

Chart 2. Synthesis of Indoline-Based Sulfamide Derivatives with Methyl or Butyl Moiety at the 2-Position



Reagents, conditions and yields: (i) (a) $LiAlH_4$, Et_2O, rt; (b) $NaBH_3CN$, AcOH, 10 °C; (c) Ac_2O, Et_3N, $CHCl_3$, rt, 3 steps 77%; (ii) (a) LiOH solution, MeOH, 0 °C, 96%; (b) MeI (for **20**) or EtI (for **21**), NaH, DMF, 45 or 41%, respectively; (iii) (a) Br_2, $CHCl_3$, 0 °C, 93—99%; (b) fum. HNO_3, conc. H_2SO_4, AcOH, 10—15 °C, 95—99%; (c) H_2, Pd–C, MeOH, 35 °C; (d) pivaloyl chloride, Et_3N, CH_2Cl_2, 0 °C, 2 steps 83—95%; (e) fum. HNO_3, conc. H_2SO_4, AcOH, 10 °C, 92%; (iv) (a) LiOH solution, MeOH, 0 °C, 91%; (b) PrI, *i*-Pr_2NEt, DMF, 110 °C, 61%; (v) (a) NaOH solution, EtOH, reflux, 88%; (b) PrI, *i*-Pr_2NEt, DMF, 100 °C, 91%; (vi) (a) MsCl, Et_3N, $CHCl_3$, 0 °C, 41%; (b) AcSK, DMF, 70 °C, 80%; (c) NaOH solution, MeOH, 0 °C, 92%; (d) MeI (for **22c**) or EtI (for **22d**), *i*-Pr_2NEt, DMF, rt, 93%; (vii) (a) H_2, Pd–C, MeOH, rt, 82%; (b) Boc$NHSO_2Cl$, Et_3N, CH_2Cl_2, −10 °C, 72—78%; (c) HCl in *i*-PrOH, HCO_2H, 0 °C, 67—75%.

Chart 3. Synthesis of Indoline-Based Sulfamide Derivatives with Ether or Thioether Moiety at the 2-Position



Reagents, conditions and yields: (i) (a) oxalyl chloride, Et_2O, rt; (b) EtOH, rt, 2 steps 54%; (c) $LiAlH_4$, THF, reflux, 100%. (d) $NaBH_3CN$, AcOH, 10 °C, 98%; (e) Boc_2O, THF, rt, 89%; (f) MeI, NaH, DMF, rt; (g) HCl in *i*-PrOH, HCO_2H, 0 °C, 2 steps 97%; (h) Ac_2O, Et_3N, $CHCl_3$, rt, 100%; (ii) (a) Br_2, $CHCl_3$, rt; (b) fum. HNO_3, conc. H_2SO_4, AcOH, 10 °C, 2 steps 66%; (c) H_2, Pd–C, MeOH, 35 °C; (d) pivaloyl chloride, Et_3N, CH_2Cl_2, 0 °C, 2 steps 100%; (e) fum. HNO_3, conc. H_2SO_4, AcOH, 10 °C, 100%; (f) NaOH solution, MeOH, reflux, 74%; (g) PrI, NaH, DMF, 0 °C, 86%; (h) H_2, Pd–C, MeOH, 35 °C; (i) $BocNHSO_2Cl$, Et_3N, CH_2Cl_2, −10 °C, 2 steps 92%; (j) HCl in *i*-PrOH, HCO_2H, 0 °C, 72%.

Chart 4. Synthesis of Indoline-Based Sulfamide Derivative **24**

group to give a 3-methoxyethyl indoline derivative **26**, which was then converted to **24** in a similar manner to that described in Chart 1. Compound **27** (Pactimibe) with a carboxymethyl moiety, and compounds **28** (KY-455) and **29** with no substituents at the 5-position were synthesized according to the methods previously reported.[7,11]

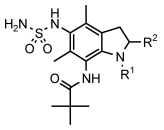
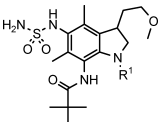
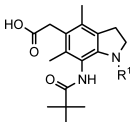
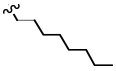
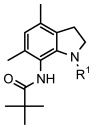
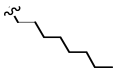
Results and Discussion

We have reported the synthesis and biological activities of Pactimibe, an indoline-based ACAT inhibitor with a carboxymethyl group at the 5-position.[7] Pactimibe exerted inhibitory effects on macrophage ACAT activities and was efficiently absorbed orally, suggesting that it would have potent anti-atherosclerotic effects; however, clinical studies failed to show a plaque-reducing effect in CAD patients.[10] It remains to be determined whether Pactimibe is unable to retard atherosclerosis due to insufficient ACAT inhibition and/or whether ACAT inhibition itself is ineffective at reducing human atherosclerotic plaques. Pactimibe showed high water solubility and bioavailability whilst maintaining high ACAT inhibitory activity, while its PBR was high because it is a relatively large lipophilic molecule with an ionizable moiety. The high PBR may have resulted in low partition into atherosclerotic plaques in some patients. Thus, we attempted to introduce a sulfamoylamino group instead of the carboxyl moiety at the 5-position, which is unionized around neutral pH, to find a new bioavailable inhibitor with low lipophilicity and PBR. Lipophilicity ($\log D_{7.0}$), rabbit PBR and inhibitory activity against foam cell formation of the sulfamide derivatives and related compounds were determined (Table 1). Compound **1d** with a sulfamoylamino group at the 5-position and an octyl chain at the 1-position showed lower $\log D_{7.0}$ (4.63) than **29** (5.10), which had no substituent at the 5-position,[11] and higher $\log D_{7.0}$ than Pactimibe (2.85), which had a carboxymethyl moiety, while compound **1d** showed lower PBR than **29** and Pactimibe (93.2, 99.2, 98.1%, respectively). On the other hand, the AFCF activity of **1d** (IC_{50}: 0.02 μM) was about 6-fold and 150-fold higher than those of **29** (IC_{50}: 0.11 μM) and Pactimibe (IC_{50}: 3.0 μM), respectively, indicating that introduction of a sulfamoylamino group is useful for increasing activity. To find a new well-balanced compound for lipophilicity, PBR and AFCF activity, various alkyl chains were introduced at the 1-, 2- and 3-positions of the indoline ring. Shortening the alkyl chain at the 1-position from octyl (**1d**) to pentyl (**1c**), butyl (**1b**) and propyl (**1a**) decreased $\log D_{7.0}$, PBR, and AFCF activity. Compound **1a** showed much lower $\log D_{7.0}$ and PBR (2.03, 72.5%, respectively) than Pactimibe, while it still had about 3-fold more potent AFCF activity (IC_{50}: 0.95 μM) than Pactimibe. Compound **1c** with a pentyl chain showed much lower $\log D_{7.0}$ (2.87) and PBR (80.1%), and 30-fold stronger activity (IC_{50}: 0.094 μM) than the corresponding compound **28** (KY-455) with no substituents at the 5-position.[11,13] All sulfamide derivatives prepared, except for **24**, showed lower PBR and stronger AFCF activities than Pactimibe. Interestingly, **1b** and **9a**, and **1c** and **9d** showed similar AFCF activities, respectively, suggesting that a relatively small lipophilic chain at the 1- or 2-position is enough to interact with ACAT protein in sulfamide derivatives. Overall, AFCF activity and PBR increased in dependence on lipophilicity ($\log D_{7.0}$) in the sulfamide derivatives synthesized: there were significant co-relationships between log(AFCF IC_{50}) and $\log D_{7.0}$ (r^2=0.777), between PBR and $\log D_{7.0}$ (r^2=0.763), and between log(AFCF IC_{50}) and PBR (r^2=0.831), respectively. Sulfamide derivatives were demonstrated to maintain potent AFCF activities at much lower $\log D_{7.0}$ and PBR than Pactimibe and KY-455 derivatives (**28**, **29**). Low lipophilic sulfamide derivatives may have interacted with an ACAT protein in a manner different from those of high lipophilic inhibitors. Furthermore, in macrophages, the sulfamide derivatives may have efficiently reached the enzyme without being trapped by culture medium proteins, cell membrane, intracellular lipid and/or proteins. In particular, compounds **1h**, **1i** and **9b** with an alkoxy chain showed $\log D_{7.0}$ lower than 2.0 and PBR around 60%. Among these, **1i** with the most potent AFCF activity was selected for further evaluation.

Compound **1i** potently inhibited hepatic ACAT activity (IC_{50}: 0.21 μM), suggesting that it would exert hypolipidemic effects by inhibiting hepatic very low density lipoprotein (VLDL) secretion. Compound **1i** showed good oral absorption in rats, rabbits, and dogs (Table 2). The maximal plasma concentration of **1i** was higher than its IC_{50} values for AFCF and hepatic ACAT inhibitory activities. Unlike Pactimibe, compound **1i** showed very low PBR; thus, the plasma concentration of the unbound form available for partition to the target organ was considered to be much higher than that of Pactimibe. Indeed, the partition ratio of Pactimibe from plasma to the atherosclerotic aorta was significantly lower than that of **1i**: 0.10±0.03 *vs.* 0.85±0.10 (n=3, p<0.01, Student's *t*-test). Furthermore, the anti-foam cell formation activity of **1i** at 10 μM was not affected by 5% albumin (% inhibition; 65.7±9.9 *vs.* 60.5±5.4, n=3), while that of Pactimibe was markedly reduced (% inhibition; 74.5±11.5 *vs.* 33.1±6.2, n=3). Compound **1i** at 10 μM markedly inhibited the cholesterol esterification in isolated atherosclerotic aortas of Kurosawa and Kusanagi hypercholesterolemic (KHC) rabbits incubated with serum (% inhibition; 70.8±3.6), while Pactimibe had little effect, probably due to its high PBR.

In conclusion, an indoline structure with a sulfamoylamino moiety is a useful scaffold to design a potent ACAT inhibitor with low lipophilicity and PBR. Compound **1i** showed lower lipophilicity and PBR, and maintained higher inhibitory activity against cholesterol esterification in macrophages and atherosclerotic aorta than Pactimibe, even in the presence of albumin or serum, probably due to its lower PBR. In addition, it also inhibited hepatic ACAT activity. Compound **1i** would more potently and consistently prevent atherosclerotic

Table 1. Chemical Structures, Molecular Weights, log $D_{7.0}$, Inhibitory Activity against Foam Cell Formation (AFCF), and Plasma Protein Binding Ratio (PBR) of Indoline-Based Sulfamide Derivatives

Structure	Compound	R^1	R^2	M.W. (free)	log $D_{7.0}$	AFCF[a] (IC_{50}, μM)	PBR[b] (%)
	**1a**		–H	382.52	2.03	0.95	72.5
	1b		–H	396.55	2.45	0.44	70.5
	1c		–H	410.58	2.87	0.094	80.1
	1d		–H	452.65	4.63	0.02	93.2
	1e		–H	396.55	2.52	0.30	72.3
	1f		–H	410.58	2.93	0.17	85.6
	1g		–H	408.56	2.42	0.62	69.8
	1h		–H	398.52	1.40	2.7	58.6
	1i		–H	412.55	1.73	1.0	62.5
	1j		–H	414.59	1.88	0.96	68.2
	1k		–H	428.61	2.29	0.33	70.3
	9a		$-CH_3$	396.55	2.45	0.54	72.5
	9b		$-CH_3$	412.55	1.84	1.8	58.2
	9c		$-CH_3$	426.57	2.25	0.63	66.3
	18a			426.57	2.22	0.44	71.9
	18b			440.60	2.61	0.15	73.9
	18c			442.64	2.71	0.21	73.6
	18d			456.67	3.14	0.036	80.3
	9d			396.55	3.02	0.056	82.3
	**24**			440.60	2.34	4.0	63.2
	**27** (Pactimibe)			416.60	2.85	3.0	98.1
	**28** (KY-455)			316.48	3.67	3.0	99.6
	29			358.56	5.10	0.11	99.2

a) Inhibitory activity against foam cell formation in THP-1 cells, $n=2$. *b*) Mean of duplicate assay.

Table 2. Maximal Plasma Concentrations of Compound **1i** and Pactimibe after Oral Administration at 10 mg/kg in Male Rats, Rabbits, and Dogs

	Rat	Rabbit	Dog
		C_{max} (μg/ml)	
1i	0.72±0.07	1.3±0.48	4.9
27 (Pactimibe)	0.15±0.02	3.0±1.50	7.3

Mean±S.E. (n=2—3).

plaque formation and promote its regression than Pactimibe.

Experimental

General Procedures Chemicals were obtained from commercial sources and used without purification. Reactions were monitored by TLC on Merck precoated silica gel 60 F_{254} (0.25 mm) plates. Column chromatography was performed on silica gel (Daisogel No. 1001W; Daiso, Osaka, Japan). Melting points were measured on a melting point apparatus (MP-500P; Yanaco, Kyoto, Japan) and are uncorrected. IR spectra were obtained with an infrared spectrometer (FT-IR 8200PC; Shimadzu, Kyoto, Japan). ^{1}H-NMR spectra were recorded on a nuclear magnetic resonance spectrometer at 400 MHz (JNM-AL400; JEOL, Tokyo, Japan) or 90 MHz (R-1900; Hitachi, Tokyo) using tetramethylsilane as an internal standard. MS spectra were obtained on a QTRAP LC/MS/MS system (API2000, Applied Biosystems; Foster, CA, U.S.A.).

Procedure for the Synthesis of 1i Compound **1i** was synthesized from **2** *via* **3**, **4**, **5i**, and **6i** as follows:

N-(1-Acetyl-4,6-dimethyl-5-nitroindolin-7-yl)-2,2-dimethylpropanamide (**3**): To a solution of **2** (1.99 g, 6.90 mmol) in AcOH (20 ml) in an ice bath was added fuming HNO_3 (0.41 ml, 10 mmol). The reaction mixture was stirred at room temperature overnight and at 50 °C for 4 h. After the mixture was poured into water, the precipitate formed was collected by filtration. A solution of the collected material in $CHCl_3$ (300 ml) was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and then evaporated under reduced pressure. The residue was purified by column chromatography (AcOEt : *n*-hexane=1 : 1) to give **3** as a crystalline solid (2.2 g, 96% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (9H, s), 2.11 (3H, s), 2.15 (3H, s), 2.32 (3H, s), 3.04 (2H, t, J=8.0 Hz), 4.16 (2H, t, J=8.0 Hz), 9.07 (1H, s).

N-(4,6-Dimethyl-5-nitroindolin-7-yl)-2,2-dimethylpropanamide (**4**): To a solution of **3** (800 mg, 2.4 mmol) in MeOH (8 ml) was added NaOH aqueous solution (480 mg, 12.0 mmol/3 ml), and the mixture was stirred at 80 °C for 15 min. After evaporation under reduced pressure, the residue was dissolved in $CHCl_3$ (50 ml), and the solution was washed with water and brine, dried over Na_2SO_4, and then evaporated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$: MeOH=50 : 1) to give **4** as a crystalline solid (680 mg, 97% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.23 (9H, s), 2.14 (3H, s), 2.16 (3H, s), 3.01 (2H, t, J=8.5 Hz), 3.67 (2H, t, J=8.5 Hz), 4.26 (1H, s), 7.03 (1H, s).

N-[1-(2-Ethoxyethyl)-4,6-dimethyl-5-nitroindolin-7-yl]-2,2-dimethylpropanamide (**5i**): To a solution of **4** (50 g, 0.17 mol) in *N*,*N*-dimethylformamide (DMF) (500 ml) were added *i*-Pr_2NEt (58.5 ml, 0.34 mol) and 2-bromoethyl ethyl ether (58 ml, 0.51 mol), and the mixture was stirred at 110 °C for 18 h under an N_2 atmosphere. After cooling, the reaction mixture was diluted with AcOEt (2 l), and washed with water, 5% citric acid solution, and brine. The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure to give a crystalline product. The products were recrystallized from MeOH (60 ml) to give **5i** (45.0 g, 72% yield). IR (Nujol) cm^{-1}: 1668. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, t, J=7.0 Hz), 1.21 (9H, s), 1.88 (3H, s), 2.04 (3H, s), 2.89 (2H, t, J=8.9 Hz), 3.40 (2H, q, J=7.0 Hz), 3.40—3.53 (4H, m), 3.62 (2H, t, J=8.9 Hz), 8.82 (1H, s).

N-[5-(*tert*-Butoxycarbonylamino)sulfonylamino-1-(2-ethoxyethyl)-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (**6i**): A suspension of **5i** (45.0 g, 0.12 mol) in MeOH (700 ml) was hydrogenated at 0.3 MPa in the presence of 10% Pd–C (9.0 g) at 40 °C for 20 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was rinsed with *i*-Pr_2O (150 ml), and then the precipitate was collected by filtration. *N*-[5-Amino-1-(2-ethoxyethyl)-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (**30**) was obtained as a crystalline solid (35.0 g). To a stirred solution of *tert*-BuOH (15.0 ml, 0.157 mol) in CH_2Cl_2 (350 ml) was added chlorosulfonyl isocyanate (13.7 ml, 0.157 mol) at −10 °C, and then the mixture was stirred at the same temperature for 30 min. To the solution were added **30** (35.0 g, 0.105 mol) and Et_3N (22.0 ml, 0.158 mol), and the mixture was stirred for 1 h at the same temperature. After addition of CH_2Cl_2, the solution was washed with 5% citric acid solution, saturated $NaHCO_3$ solution, water, and brine. The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (AcOEt : $CHCl_3$=1 : 1) to give **6i** as a crystalline solid (47.5 g, 75% yield). ^{1}H-NMR (DMSO-d_6) δ: 1.08 (3H, t, J=7.0 Hz), 1.20 (9H, s), 1.43 (9H, s), 1.95 (3H, s), 2.07 (3H, s), 2.77 (2H, t, J=8.7 Hz), 3.30—3.50 (8H, m), 8.66 (1H, s), 9.12 (1H, s), 10.70—10.85 (1H, br).

N-[1-(2-Ethoxyethyl)-4,6-dimethyl-5-sulfamoylaminoindolin-7-yl]-2,2-dimethylpropanamide Hydrochloride (1i): To a solution of **6i** (47.5 g, 0.93 mol) in HCO_2H (190 ml) in an ice bath was added 8.7 M HCl in *i*-PrOH (32 ml, 0.28 mol), and the mixture was stirred at the same temperature for 30 min. After the solution had been poured into Et_2O (5.9 l) with stirring, the precipitate formed was collected by filtration. To a suspension of the precipitate in MeOH (180 ml) was added Et_2O (3.9 l) with stirring, and the precipitate was collected by filtration to obtain compound **1i** as a crystalline solid (33.5 g, 80% yield). mp 144—148 °C. IR (Nujol) cm^{-1}: 1657. ^{1}H-NMR (DMSO-d_6) δ: 1.13 (3H, t, J=7.0 Hz), 1.26 (9H, s), 2.11 (3H, s), 2.27 (3H, s), 3.05—3.15 (2H, m), 3.30—3.40 (2H, m), 3.45 (2H, q, J=7.0 Hz), 3.65—3.75 (2H, m), 3.75—3.85 (2H, m), 8.46 (1H, s), 9.16 (1H, s). MS *m*/*z*: 413 [M+H]$^+$. *Anal.* Calcd for $C_{19}H_{32}N_4O_4S \cdot HCl \cdot H_2O$: C, 48.86; H, 7.55; N, 12.00; Cl, 7.59. Found: C, 48.87; H, 7.43; N, 11.95; Cl, 7.47.

Procedure for the Synthesis of 1a Compound **1a** was synthesized from **4** *via* **5a** according to the procedure for **1i**. The reaction condition of the preparation for **5a** was changed as follows:

N-(4,6-Dimethyl-5-nitro-1-propylindolin-7-yl)-2,2-dimethylpropanamide (**5a**): To a stirred solution of **4** (500 mg, 1.72 mmol) in DMF (5 ml) in an ice bath was added a 60% suspension of NaH in mineral oil (103 mg, 2.6 mmol) portionwise under an N_2 atmosphere. After stirring at the same temperature for 15 min, propyl iodide (0.34 ml, 3.5 mmol) was added to the mixture in an ice bath, and was further stirred for 18 h at room temperature. After addition of water (50 ml), the reaction mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$: MeOH=50 : 1) to give **5a** as a crystalline solid (0.42 g, 80% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, t, J=7.1 Hz), 2.10—2.70 (2H, m), 1.33 (9H, s), 2.02 (3H, s), 2.11 (3H, s), 2.89 (2H, t, J=8.7 Hz), 3.10—3.40 (2H, m), 3.55 (2H, t, J=8.7 Hz), 6.77 (1H, s).

N-(4,6-Dimethyl-1-propyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**1a**): A crystalline solid. mp 175—178 °C. IR (Nujol) cm^{-1}: 1674. ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, t, J=7.3 Hz), 1.30 (9H, s), 1.60—2.00 (2H, m), 2.10 (3H, s), 2.30 (3H, s), 2.90—3.40 (4H, m), 3.65—4.00 (2H, m), 5.00—8.00 (3H, br), 8.53 (1H, s), 9.32 (1H, s). MS *m*/*z*: 383 [M+H]$^+$. *Anal.* Calcd for $C_{18}H_{30}N_4O_3S \cdot HCl \cdot 0.5H_2O$: C, 50.51; H, 7.54; N, 13.09; Cl, 8.28. Found: C, 50.34; H, 7.40; N, 13.01; Cl, 8.24.

Procedure for the Synthesis of 1b—f Compounds **1b**—**f** were synthesized according to the procedure for **1a**.

N-(1-Butyl-4,6-dimethyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**1b**): A crystalline solid. mp 178—181 °C. IR (Nujol) cm^{-1}: 1674. ^{1}H-NMR (DMSO-d_6) δ: 0.70—1.10 (3H, m), 1.30 (9H, s), 1.40—1.90 (4H, m), 2.14 (3H, s), 2.30 (3H, s), 2.90—3.40 (4H, m), 3.60—3.90 (2H, m), 4.50—8.00 (3H, br), 8.55 (1H, s), 9.33 (1H, s). MS *m*/*z*: 397 [M+H]$^+$. *Anal*. Calcd for $C_{19}H_{32}N_4O_3S \cdot HCl \cdot 0.5H_2O$: C, 51.37; H, 7.65; N, 12.56; Cl, 7.84. Found: C, 51.63; H, 7.75; N, 12.68; Cl, 8.02.

N-(4,6-Dimethyl-1-pentyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**1c**): A crystalline solid. mp 170—173 °C. IR (Nujol) cm^{-1}: 1672. ^{1}H-NMR (DMSO-d_6) δ: 0.85 (3H, t, J=5.7 Hz), 1.30 (9H, s), 1.50—2.00 (6H, m), 2.14 (3H, s), 2.30 (3H, s), 2.90—3.40 (4H, m), 3.65—4.00 (2H, m), 5.00—8.00 (3H, br), 8.53 (1H, s), 9.32 (1H, s). MS *m*/*z*: 411 [M+H]$^+$. *Anal.* Calcd for $C_{20}H_{34}N_4O_3S \cdot HCl \cdot 0.5H_2O$: C, 52.68; H, 7.96; N, 12.29; Cl, 7.77. Found: C, 52.41; H, 7.84; N, 12.37; Cl, 7.57.

N-(4,6-Dimethyl-1-octyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**1d**): A crystalline solid. mp 170—173 °C. IR (Nujol) cm^{-1}: 1676. ^{1}H-NMR (DMSO-d_6) δ: 0.84 (3H, t, J=6.0 Hz), 1.00—1.95 (21H, m), 2.13 (3H, s), 2.30 (3H, s), 2.95—3.50 (4H, m), 3.81 (2H, t, J=6.5 Hz), 5.50—9.00 (3H, br), 8.55 (1H, s), 9.37 (1H, s). MS *m*/*z*: 453 [M+H]$^+$. *Anal.* Calcd for $C_{23}H_{40}N_4O_3S \cdot HCl \cdot 0.5H_2O$: C, 55.46; H, 8.50; N, 11.25; Cl, 7.12. Found: C, 55.63; H, 8.26; N, 11.39; Cl, 7.01.

N-[4,6-Dimethyl-1-(2-methylpropyl)-5-sulfamoylaminoindolin-7-yl]-2,2-dimethylpropanamide Hydrochloride (**1e**): A crystalline solid. mp 182—185 °C. IR (Nujol) cm^{-1}: 1668. ^{1}H-NMR (DMSO-d_6) δ: 0.98 (6H, d, J=6.4 Hz), 1.29 (9H, s), 1.90—2.30 (1H, m), 2.12 (3H, s), 2.29 (3H, s), 2.80—3.30 (3H, m), 3.60—3.90 (2H, m), 5.00—7.50 (3H, m), 8.48 (1H, s), 9.27 (1H, s). MS *m*/*z*: 397 [M+H]$^+$. *Anal.* Calcd for $C_{19}H_{32}N_4O_3S \cdot$

$HCl \cdot H_2O$: C, 50.60; H, 7.82; N, 12.42; Cl, 7.86. Found: C, 50.44; H, 7.49; N, 12.51; Cl, 7.86.

N-[4,6-Dimethyl-1-(3-methylbutyl)-5-sulfamoylaminoindolin-7-yl]-2,2-dimethylpropanamide Hydrochloride (**1f**): A crystalline solid. mp 181—183 °C. IR (Nujol) cm^{-1}: 1672. ^{1}H-NMR (DMSO-d_6) δ: 0.85 (6H, d, J=5.0 Hz), 1.30 (9H, s), 1.30—1.80 (3H, m), 2.14 (3H, s), 2.30 (3H, s), 2.90—3.40 (4H, m), 3.60—4.00 (2H, m), 5.50—7.50 (2H, br), 8.54 (1H, s), 9.30 (1H, s). MS *m/z*: 411 $[M+H]^+$. *Anal.* Calcd for $C_{20}H_{34}N_4O_3S \cdot HCl \cdot H_2O$: C, 51.65; H, 8.02; N, 12.05; Cl, 7.62. Found: C, 51.83; H, 7.98; N, 12.02; Cl, 7.64.

Procedure for the Synthesis of 1h Compound **1h** was synthesized from **4** in a similar manner to the procedure for **1i**.

N-[1-(2-Methoxyethyl)-4,6-dimethyl-5-sulfamoylaminoindolin-7-yl]-2,2-dimethylpropanamide Hydrochloride (**1h**): A crystalline solid. mp 160—163 °C. IR (Nujol) cm^{-1}: 1680. ^{1}H-NMR (DMSO-d_6) δ: 1.25 (9H, s), 2.10 (3H, s), 2.24 (3H, s), 3.00—3.10 (2H, m), 3.27 (3H, s), 3.35 (2H, t, J=5.2 Hz), 3.59—3.80 (4H, m), 6.40—7.10 (2H, br), 8.39 (1H, s), 9.02 (1H, s). MS *m/z*: 399 $[M+H]^+$. *Anal.* Calcd for $C_{18}H_{30}N_4O_4S \cdot HCl \cdot 0.8H_2O$: C, 48.11; H, 7.31; N, 12.47; Cl, 7.89. Found: C, 47.91; H, 6.97; N, 12.43; Cl, 7.97.

Procedure for the Synthesis of 1g Compound **1g** was synthesized from **3** *via* **7** and **6g**. Compound **6g** was converted to **1g** according to the procedure for **1i**. Compound **6g** was prepared *via* **31**, **33**, and **7** as follows:

N-(1-Acetyl-5-*tert*-butoxycarbonylamino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (**31**): A solution of **3** (20.0 g, 60.0 mmol) in MeOH (300 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 solid residue was rinsed with Et_2O to give *N*-(1-acetyl-5-amino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (**32**) as a solid (17.0 g, 93% yield). A mixture of **32** (5.0 g, 16 mmol), Boc_2O (10.8 g, 49.5 mmol), and Et_3N (6.9 ml, 49 mmol) was stirred at 90 °C for 17 h. After addition of water (50 ml), the reaction mixture was extracted with $CHCl_3$. The organic layer was washed with 10% 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 ($CHCl_3$: *n*-hexane=4 : 1) to give **31** as an oil (2.44 g, 37% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (9H, s), 1.47 (9H, s), 2.08 (3H, s), 2.13 (3H, s), 2.29 (3H, s), 2.70—3.30 (2H, m), 4.10 (2H, br-t), 5.85 (1H, s), 9.20 (1H, s).

N-(5-*tert*-Butoxycarbonylamino-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (**33**): To a solution of **31** (2.40 g, 5.95 mmol) in EtOH (24 ml) was added 5.0 M NaOH aqueous solution (6 ml, 30 mmol), and the mixture was refluxed for 1 h. After addition of water (75 ml), the reaction mixture was stirred for 15 min at room temperature. The precipitate formed was collected by filtration to obtain **33** as a crystalline solid (1.47 g, 68% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.32 (9H, s), 1.46 (9H, s), 2.07 (3H, s), 2.09 (3H, s), 2.20—3.30 (1H, br), 2.95 (2H, t, J=8.1 Hz), 3.57 (2H, t, J=8.1 Hz), 5.77 (1H, s), 7.06 (1H, s).

N-(5-*tert*-Butoxycarbonylamino-1-cyclopentyl-4,6-dimethylindolin-7-yl)-2,2-dimethylpropanamide (**7**): To a solution of **33** (3.14 g, 8.69 mmol) in DMF (30 ml) were added *i*-Pr_2NEt (3.0 ml, 17 mmol), bromocyclopentane (1.86 ml, 17.4 mmol), KI (721 mg, 4.34 mmol), and hydroquinone (48 mg, 0.44 mmol), and the mixture was stirred for 15 h at 90 °C. After the mixture was poured into water (100 ml), the precipitate formed was collected by filtration. A solution of the obtained powder in $CHCl_3$ (100 ml) was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (AcOEt : $CHCl_3$=1 : 5) to give a crystalline powder. The powder was rinsed with *i*-Pr_2O to give **7** as a crystalline solid (0.78 g, 21% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.32 (9H, s), 1.30—1.80 (8H, m), 1.49 (9H, s), 1.99 (3H, s), 2.06 (3H, s), 2.84 (2H, t, J=8.3 Hz), 3.41 (2H, t, J=8.3 Hz), 4.20—4.30 (1H, m), 5.75 (1H, s), 6.85 (1H, s).

N-[5-(*tert*-Butoxycarbonylamino)sulfonylamino-1-cyclopentyl-4,6-dimethylindolin-7-yl]-2,2-dimethylpropanamide (**6g**): To a solution of **7** (0.76 g, 1.8 mmol) in HCO_2H (7.6 ml) in an ice bath was added 8.7 M HCl in *i*-PrOH (0.61 ml, 5.3 mmol), and the mixture was stirred at the same temperature for 15 min. After addition of a mixed solvent of *i*-Pr_2O–*n*-hexane, the supernatant was removed by decantation to give an oily product. A solution of the oil in AcOEt (100 ml) was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was treated with chlorosulfonyl isocyanate according to the method of preparation for **6i** to give **6g** as a crystalline solid (618 mg, 69% yield). ^{1}H-NMR (DMSO-d_6) δ: 1.23 (9H, s), 1.30–1.80 (8H, m), 1.43 (9H, s), 1.99 (3H, s), 2.12 (3H, s), 2.60—3.10 (2H, m), 4.00—4.80 (3H, m), 8.88 (1H, s), 9.24 (1H, s), 10.82 (1H, s).

N-(1-Cyclopentyl-4,6-dimethyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**1g**): A crystalline solid. Yield 89%. mp 173—176 °C. IR (Nujol) cm^{-1}: 1665. ^{1}H-NMR (DMSO-d_6) δ: 1.30 (9H, s), 1.40—1.80 (8H, m), 2.13 (3H, s), 2.27 (3H, s), 3.00—3.20 (2H, m), 3.20—3.80 (4H, m), 3.90—4.00 (1H, m), 6.70—7.00 (1H, br), 8.50 (1H, s), 9.23 (1H, s). MS *m/z*: 409 $[M+H]^+$. *Anal.* Calcd for $C_{20}H_{32}N_4O_3S \cdot HCl \cdot 1.2H_2O$: C, 51.48; H, 7.65; N, 12.01; Cl, 7.60. Found: C, 51.45; H, 7.60; N, 11.87; Cl, 7.27.

Procedure for the Synthesis of 1j Compound **1j** was synthesized from **4** *via* **5j**. Compound **5j** was converted to **1j** according to the procedure for **1i**. Compound **5j** was prepared as follows:

N-[1-(2-Hydroxyethyl)-4,6-dimethyl-5-nitroindolin-7-yl]-2,2-dimethylpropanamide (**34**): To a solution of **4** (8.0 g, 28 mmol) in DMF (40 ml) were added *i*-Pr_2NEt (14 ml, 82 mmol) and 2-bromoethanol (5.8 ml, 82 mmol), and the mixture was stirred for 6.5 h at 120 °C under an N_2 atmosphere. After addition of 5% citric acid solution (200 ml), the mixture was extracted with AcOEt. The organic layer was washed with 5% $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crystalline residue was rinsed with $CHCl_3$ (50 ml) to obtain **34** (4.88 g, 53% yield). The filtrate was evaporated under reduced pressure and purified by column chromatography (AcOEt : $CHCl_3$=1 : 3). The crystalline residue was rinsed with AcOEt to obtain **34** (1.33 g, 14% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (9H, s), 1.88 (3H, s), 2.04 (3H, s), 2.89 (2H, t, J=7.1 Hz), 3.30—3.40 (2H, m), 3.45—3.60 (2H, m), 3.65 (2H, t, J=7.1 Hz), 4.79 (1H, t, J=4.9 Hz), 8.85 (1H, s).

N-[1-(2-Mercaptoethyl)-4,6-dimethyl-5-nitroindolin-7-yl]-2,2-dimethylpropanamide (**35**): To a solution of **34** (6.21 g, 18.5 mmol) in $CHCl_3$ (62 ml) in an ice bath were added MsCl (2.8 ml, 36 mmol) and Et_3N (5.1 ml, 36 mmol), and the mixture was stirred for 30 min at the same temperature. After addition of AcOEt, the mixture was washed with 5% citric acid solution, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crystalline residue was rinsed with Et_2O to give a mesylate derivative. To a solution of the obtained solid (7.38 g, 17.8 mmol) in DMF (73 ml) was added potassium thioacetate (3.05 g, 26.7 mmol), and the mixture was stirred for 30 min at 70 °C. After addition of water, the mixture was extracted with AcOEt. The extract was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crystalline residue was rinsed with Et_2O to give a thioacetate derivative as a solid. To a solution of the solid (6.5 g, 17 mmol) in MeOH (130 ml) in an ice bath was added 2.0 M NaOH aqueous solution (9.9 ml, 20 mmol), and the mixture was stirred for 1 h at the same temperature. After addition of 5% citric acid solution, the mixture was evaporated under reduced pressure, and then the residue was extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crystalline residue was rinsed with Et_2O to obtain **35** (5.54 g, 85% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (9H, s), 1.43 (1H, t, J=7.8 Hz), 2.02 (3H, s), 2.11 (3H, s), 2.67 (2H, td, J=7.8, 7.1 Hz), 2.94 (2H, t, J=9.0 Hz), 3.50 (2H, t, J=7.8 Hz), 3.57 (2H, t, J=9.0 Hz), 6.97 (1H, s).

N-[4,6-Dimethyl-1-(2-methylthioethyl)-5-nitroindolin-7-yl]-2,2-dimethylpropanamide (**5j**): To a solution of **35** (1.5 g, 4.3 mmol) in DMF (15 ml) were added *i*-Pr_2NEt (1.45 ml, 8.5 mmol) and methyl iodide (0.53 ml, 8.5 mmol), and the mixture was stirred for 0.5 h at room temperature. After dilution with AcOEt, the mixture was washed with 5% citric acid solution, water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crystalline residue was rinsed with *i*-Pr_2O to obtain **5j** (1.42 g, 91% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (9H, s), 2.03 (3H, s), 2.11 (3H, s), 2.13 (3H, s), 2.66 (2H, t, J=7.3 Hz), 2.93 (2H, t, J=8.8 Hz), 3.52 (2H, t, J=7.3 Hz), 3.61 (2H, t, J=8.8 Hz), 6.98 (1H, s).

N-[4,6-Dimethyl-1-(2-methylthioethyl)-5-sulfamoylaminoindolin-7-yl]-2,2-dimethylpropanamide Hydrochloride (**1j**): A crystalline solid. mp 152—156 °C. IR (neat) cm^{-1}: 1674. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (9H, s), 2.07 (3H, s), 2.09 (3H, s), 2.23 (3H, s), 2.65—2.75 (3H, m), 2.99—3.06 (3H, m), 3.30—3.40 (2H, m), 3.40—4.20 (4H, m), 8.35 (1H, s), 9.00 (1H, s). MS *m/z*: 413 $[M-H]^-$. *Anal.* Calcd for $C_{18}H_{30}N_4O_3S_2 \cdot HCl \cdot 0.5H_2O$: C, 46.99; H, 7.01; N, 12.18; Cl, 7.71. Found: C, 46.75; H, 6.68; N, 12.14; Cl, 7.63.

Procedure for the Synthesis of 1k Compound **1k** was synthesized from **4** according to the procedure for **1j**. EtI was used instead of MeI.

N-[1-(2-Ethylthioethyl)-4,6-dimethyl-5-sulfamoylaminoindolin-7-yl]-2,2-dimethylpropanamide Hydrochloride (**1k**): A crystalline solid. mp 143—146 °C. IR (Nujol) cm^{-1}: 1665. ^{1}H-NMR ($CDCl_3$) δ: 1.16 (3H, t, J=7.3 Hz), 1.27 (9H, s), 2.09 (3H, s), 2.24 (3H, s), 2.53 (2H, q, J=7.3 Hz), 2.70—2.80 (2H, m), 3.06 (2H, t, J=7.8 Hz), 3.30—3.40 (2H, m), 3.70 (2H, t,

J=7.8 Hz), 6.30—7.20 (2H, br), 8.39 (1H, s), 9.05 (1H, s). MS m/z: 429 $[M+H]^+$. *Anal.* Calcd for $C_{19}H_{32}N_4O_3S_2 \cdot HCl \cdot H_2O$: C, 47.24; H, 7.30; N, 11.60; Cl, 7.34. Found: C, 47.08; H, 6.84; N, 11.61; Cl, 7.47.

Procedure for the Synthesis of 9a Compound **9a** was synthesized from **10** *via* **11** and **12**. To compound **12** was introduced a pivaloylamino moiety at the 7-position according to the previous report[11] and then the product was converted to **9a** according to the procedure for **1a**. Compound **11** and **12** were prepared as follows:

2,4,6-Trimethylindole (**11**): To a mixture of **10**[14] (533 mg, 3.91 mmol) and acetone (0.29 ml, 3.9 mmol) was added polyphosphoric acid (1.5 g), and the mixture was heated gradually until 140 °C for 30 min. After addition of water (10 ml), the mixture was extracted with Et_2O. The organic layer was washed with water, saturated $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (AcOEt : *n*-hexane=1 : 20) to give **11** as a crystalline solid (500 mg, 80% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.39 (6H, s), 2.45 (3H, s), 6.15 (1H, s), 6.70 (1H, s), 6.87 (1H, s).

1-Acetyl-2,4,6-trimethylindoline (**12**): To a solution of **11** (480 mg, 3.01 mmol) in AcOH (2 ml) in an ice bath was added $NaBH_3CN$ (378 mg, 6.02 mmol), and then the mixture was stirred for 2 h. After the mixture was poured into cold water (8 ml), AcOEt (5 ml) was added, and then the mixture was neutralized with NaOH. The organic layer was separated, washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the residue in benzene (2 ml) was added Ac_2O (0.4 ml, 4 mmol), and then the mixture was stirred for 1 h. After removal of the solvent under reduced pressure, a solution of the residue in $CHCl_3$ (50 ml) was washed with water, saturated $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (AcOEt : *n*-hexane=1 : 1) to give **12** as a crystalline solid (520 mg, 85% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, d, J=6.4 Hz), 2.19 (3H, s), 2.30 (6H, s), 2.40—3.40 (2H, m), 4.47—4.57 (1H, m), 6.83 (1H, s), 7.81 (1H, s).

N-(2,4,6-Trimethyl-1-propyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**9a**): A crystalline solid. mp 156—160 °C. IR (Nujol) cm^{-1}: 1666. ^{1}H-NMR (DMSO-d_6) δ: 0.83 (3H, t, J=7.1 Hz), 1.29 (9H, s), 1.39 (3H, d, J=6.1 Hz), 1.50—1.85 (2H, m), 2.12 (3H, s), 2.26 (3H, s), 2.68—2.80 (1H, m), 2.95—3.05 (1H, m), 3.20—3.30 (1H, m), 3.35—4.00 (2H, m), 4.15—4.40 (1H, m), 6.50—7.50 (2H, br), 8.49 (1H, s), 9.30—9.70 (1H, br). MS m/z: 397 $[M+H]^+$. *Anal.* Calcd for $C_{19}H_{32}N_4O_4S \cdot HCl \cdot H_2O \cdot 0.1Et_2O$: C, 49.11; H, 7.65; N, 11.81; Cl, 7.47. Found: C, 48.95; H, 7.35; N, 11.93; Cl, 7.24.

Procedure for the Synthesis of 9b and 9c Compounds **9b** and **9c** were synthesized from **12** according to the procedure for **9a**.

N-[1-(2-Methoxyethyl)-2,4,6-trimethyl-5-sulfamoylaminoindolin-7-yl]-2,2-dimethylpropanamide Hydrochloride (**9b**): A crystalline solid. mp 164—167 °C. IR (Nujol) cm^{-1}: 1653. ^{1}H-NMR (DMSO-d_6) δ: 1.25 (9H, s), 1.34 (3H, d, J=6.1 Hz), 2.10 (3H, s), 2.22 (3H, s), 2.45—2.70 (1H, m), 3.15—3.45 (1H, m), 3.26 (3H, s), 3.40—3.70 (4H, m), 3.90 (1H, s), 4.00—4.30 (1H, br), 6.20—7.20 (2H, br), 8.37 (1H, s), 9.22 (1H, s). MS m/z: 411 $[M-H]^-$. *Anal.* Calcd for $C_{19}H_{32}N_4O_4S \cdot HCl \cdot H_2O$: C, 50.42; H, 7.44; N, 12.38; Cl, 7.83. Found: C, 50.45; H, 7.35; N, 12.29; Cl, 7.60.

N-[1-(2-Ethoxyethyl)-2,4,6-trimethyl-5-sulfamoylaminoindolin-7-yl]-2,2-dimethylpropanamide Hydrochloride (**9c**): A crystalline solid. mp 165—168 °C. IR (Nujol) cm^{-1}: 1654. ^{1}H-NMR (DMSO-d_6) δ: 1.12 (3H, t, J=7.1 Hz), 1.26 (9H, s), 1.34 (3H, d, J=5.9 Hz), 2.10 (3H, s), 2.22 (3H, s), 2.50—2.70 (1H, m), 3.15—3.55 (7H, m), 4.00—4.40 (2H, br), 6.40—7.20 (2H, br), 8.37 (1H, s), 9.15—9.40 (1H, br). MS m/z: 427 $[M+H]^+$. *Anal.* Calcd for $C_{20}H_{34}N_4O_4S \cdot HCl \cdot 0.5H_2O$: C, 50.89; H, 7.69; N, 11.87; Cl, 7.51. Found: C, 50.66; H, 7.41; N, 11.84; Cl, 7.48.

Procedure for the Synthesis of 9d Compound **9d** was synthesized from **13** *via* **14** and **15**. Compound **15** was converted to **9d** according to the procedure for **9a**. Compound **15** was prepared as follows:

4,6-Dimethylindole-2-carbaldehyde (**14**): To a suspension of $LiAlH_4$ (4.11 g, 108 mmol) in Et_2O (320 ml) in an ice bath was added **13**[12] (15.7 g, 72.2 mmol) portionwise, and then the mixture was stirred at the same temperature for 1 h. After addition of water and AcOEt (300 ml), the mixture was filtrated. The two layers of the filtrate were separated, and the organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the residue in CH_2Cl_2 (540 ml) was added activated MnO_2 (43.9 g, 505 mmol). After stirring at room temperature for 15 h, activated MnO_2 (10.0 g, 115 mmol) was added, and stirred for 6 h. The mixture was filtrated, and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (AcOEt : *n*-hexane=1 : 1) to give **14** as a crystalline solid (7.78 g, 62% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.43 (3H, s), 2.54 (3H, s), 6.81 (1H, s), 7.06 (1H, s), 7.25 (1H, s), 9.15 (1H, s), 9.78 (1H, s).

1-Acetyl-2-butyl-4,6-dimethylindoline (**15**): To a suspension of allyltriphenylphosphonium bromide (25.0 g, 65.3 mmol) in tetrahydrofuran (THF) (200 ml) was added 1.6 M *n*-BuLi in hexane (65 ml, 65 mmol) dropwise below 10 °C. The reaction mixture was stirred at the same temperature for 20 min. To the mixture was added **14** (5.66 g, 32.7 mmol) in THF (50 ml) at the same temperature. After stirring for 20 min, water was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. A solution of the residue in MeOH (400 ml) was hydrogenated at 0.4 MPa in the presence of 10% Pd–C (2.70 g) at 35 °C for 16 h. After removal of the catalyst by filtration, the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (AcOEt : *n*-hexane=1 : 3). To a solution of the product in AcOH (38 ml) was added $NaBH_3CN$ (2.64 g, 37.8 mmol) portionwise at 10 °C, and then the mixture was stirred for 30 min at the same temperature. After addition of NaOH aqueous solution (22 g/72 ml) at below 20 °C, the mixture was extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the residue in $CHCl_3$ (39 ml) were added Ac_2O (2.7 ml, 29 mmol), and Et_3N (4.0 ml, 29 mmol). After stirring at room temperature for 30 min, the reaction mixture was washed with 10% 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 (AcOEt : *n*-hexane=1 : 2) to give **15** (3.48 g, 42% yield). ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.00 (3H, m), 1.20—1.80 (6H, m), 2.20 (3H, s), 2.26 (3H, s), 2.31 (3H, s), 2.50—2.74 (1H, m), 2.95—3.20 (1H, m), 4.20—4.40 (0.7H, m), 4.70—4.85 (0.3H, m), 6.68 (1H, s), 6.74 (0.3H, s), 7.82 (0.7H, s).

N-(2-Butyl-1,4,6-trimethyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**9d**): A crystalline solid. mp 180—183 °C. IR (Nujol) cm^{-1}: 1672. ^{1}H-NMR (DMSO-d_6) δ: 0.85—0.95 (3H, m), 1.26 (9H, s), 1.30—1.40 (4H, m), 1.50—1.65 (1H, m), 1.85—2.00 (1H, m), 2.10 (3H, s), 2.25 (3H, s), 2.70—2.90 (1H, m), 2.83 (3H, s), 3.27 (1H, dd, J=15.6, 7.1 Hz), 3.40—4.00 (1H, m), 6.40—7.20 (2H, br), 8.44 (1H, s), 9.24 (1H, s). MS m/z: 409 $[M-H]^-$. *Anal.* Calcd for $C_{20}H_{34}N_4O_3S \cdot HCl \cdot 0.7H_2O$: C, 52.26; H, 7.98; N, 12.19; Cl, 7.71. Found: C, 52.02; H, 7.60; N, 12.07; Cl, 7.59.

Procedure for the Synthesis of 18a Compound **18a** was synthesized from **13** *via* **19**, **20**, and **22a**. Compound **20** was converted to **22a** according to the procedure for **1a**. Compound **20** was prepared as follows:

2-Acetoxymethyl-1-acetyl-4,6-dimethylindoline (**19**): To a suspension of $LiAlH_4$ (2.10 g, 55.3 mmol) in Et_2O (160 ml) in an ice bath was added **13** (8.0 g, 37 mmol) portionwise, and then the mixture was stirred at the same temperature for 1 h. After addition of water and AcOEt (300 ml), the mixture was filtrated. The two layers of the filtrate were separated, and the organic layer was dried over Na_2SO_4, and evaporated under reduced pressure to give 2-hydroxymethyl-4,6-dimethylindole (**36**) as a crystalline solid. To a solution of **36** in AcOH (60 ml) was added $NaBH_3CN$ (5.14 g, 73.6 mmol) portionwise at 10 °C, and then the mixture was stirred for 30 min at the same temperature. After addition of NaOH aqueous solution (40 g/140 ml) below 20 °C, the mixture was extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. To a solution of the residue in $CHCl_3$ (70 ml) were added Ac_2O (10.4 ml, 110 mmol), and Et_3N (15.4 ml, 110 mmol). After stirring at room temperature for 16 h, the reaction mixture was washed with 10% citric acid solution, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crystalline residue was rinsed with *i*-Pr_2O to obtain **19** (7.43 g, 77% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.00 (3H, s), 2.19 (3H, s), 2.31 (3H, s), 2.36 (3H, s), 2.70 (1H, d, J=16.0 Hz), 3.15 (1H, dd, J=16.0, 8.6 Hz), 3.80—4.30 (2H, m), 4.50—5.20 (1H, m), 6.69 (1H, s), 7.40—8.00 (1H, br).

1-Acetyl-2-hydroxymethyl-4,6-dimethylindoline (**37**): To a solution of **19** (17.4 g, 66.6 mmol) in MeOH (175 ml) in an ice bath was added 1.0 M LiOH aqueous solution (80 ml, 80 mmol), and then the mixture was stirred at the same temperature for 0.5 h. After addition of 5% citric acid solution (100 ml), the solvent was removed under reduced pressure. The precipitate formed was collected by filtration to obtain **37** as a crystalline solid (14.0 g, 96% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.50—2.20 (1H, br), 2.19 (3H, s), 2.31 (3H, s), 2.40 (3H, s), 2.30—2.80 (1H, m), 3.00—3.40 (1H, m), 3.65 (2H, d, J=6.4 Hz), 4.50—5.20 (1H, m), 6.20—8.00 (2H, m).

1-Acetyl-2-methoxymethyl-4,6-dimethylindoline (**20**): To a solution of **37** (8.34 g, 38.0 mmol) in DMF (83 ml) in an ice bath was added 60% suspension of NaH in mineral oil (1.37 g, 34 mmol). After stirring for 10 min at the same temperature, MeI (11.8 ml, 190 mmol) was added, and stirred for 1.5 h

at 80 °C. After addition of 5% citric acid solution (500 ml), the mixture was extracted with AcOEt. The organic layer 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 (AcOEt : *n*-hexane=2 : 1) to give **20** as a crystalline solid (3.95 g, 45% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.20 (3H, s), 2.31 (6H, s), 2.70—2.90 (1H, m), 3.00—3.20 (1H, m), 3.25—3.65 (2H, m), 3.34 (3H, s), 4.54—4.98 (1H, m), 6.69 (1H, s), 6.60—6.90 (0.5H, br), 7.70—7.90 (0.5H, br).

N-(2-Methoxymethyl-4,6-dimethyl-1-propyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**18a**): A crystalline solid. mp 170—173 °C. IR (Nujol) cm^{-1}: 1649. ^{1}H-NMR (DMSO-d_6) δ: 0.80 (3H, t, J=7.3 Hz), 1.26 (9H, s), 1.40—1.70 (2H, m), 2.09 (3H, s), 2.20 (3H, s), 2.60—2.80 (1H, m), 2.95—3.05 (1H, m), 3.20—3.35 (5H, m), 3.40—4.50 (6H, m), 8.20—8.50 (1H, br), 9.00—9.40 (1H, br). MS *m/z*: 427 $[M+H]^+$. *Anal.* Calcd for $C_{20}H_{34}N_4O_4S \cdot HCl \cdot 0.5H_2O$: C, 50.89; H, 7.69; N, 11.87; Cl, 7.51. Found: C, 50.63; H, 7.49; N, 11.62; Cl, 7.52.

Procedure for the Synthesis of 18b Compound **18b** was synthesized from **19** according to the procedure for **18a**. EtI was used instead of MeI.

N-(2-Ethoxymethyl-4,6-dimethyl-1-propyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**18b**): A crystalline solid. mp 177—180 °C. IR (Nujol) cm^{-1}: 1651. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, t, J=7.3 Hz), 1.14 (3H, t, J=7.1 Hz), 1.26 (9H, s), 1.49—1.66 (2H, m), 2.12 (3H, s), 2.21 (3H, s), 2.63—2.79 (1H, m), 2.95—3.06 (1H, m), 3.19—3.36 (2H, m), 3.46—3.54 (4H, m), 3.60—4.35 (1H, m), 6.50—6.98 (2H, br), 8.20—8.50 (1H, br), 9.02—9.50 (1H, br). MS *m/z*: 441 $[M+H]^+$. *Anal.* Calcd for $C_{21}H_{37}N_4O_4S \cdot HCl \cdot 0.2H_2O$: C, 52.37; H, 8.04; N, 11.63; Cl, 7.36. Found: C, 52.23; H, 7.80; N, 11.66; Cl, 7.59.

Procedure for the Synthesis of 18c and 18d Compound **18c** and **18d** were synthesized from **19** *via* **23**. Compound **23** was prepared according to the procedure for **9a**. The hydroxymethyl moiety of **23** was converted to thioether moiety in a similar manner to that described in the procedure for **1j** and **1k**.

N-(2-Hydroxymethyl-4,6-dimethyl-5-nitro-1-propylindolin-7-yl)-2,2-dimethylpropanamide (**23**): A crystalline solid. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, t, J=7.3 Hz), 1.35 (9H, s), 1.40—1.55 (2H, m), 2.07 (3H, s), 2.12 (3H, s), 2.76 (1H, d, J=16.1, 4.4 Hz), 2.85—3.00 (2H, m), 3.10—3.30 (2H, m), 3.40—3.50 (1H, m), 3.70—3.90 (2H, m), 6.94 (1H, s).

N-(4,6-Dimethyl-2-methylthiomethyl-1-propyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**18c**): A crystalline solid. mp 158—161 °C. IR (Nujol) cm^{-1}: 1657. ^{1}H-NMR (DMSO-d_6) δ: 0.81 (3H, t, J=7.3 Hz), 1.26 (9H, s), 1.45—1.70 (2H, m), 2.01 (3H, s), 2.08 (3H, s), 2.14 (3H, s), 2.57 (1H, dd, J=13.5, 8.7 Hz), 2.75—3.05 (2H, m), 3.20—3.35 (2H, m), 3.90—4.20 (2H, m), 5.80—7.40 (2H, br), 8.20—8.40 (1H, br), 9.00—9.20 (1H, br). MS *m/z*: 443 $[M+H]^+$. *Anal.* Calcd for $C_{20}H_{34}N_4O_3S_2 \cdot HCl \cdot 0.6H_2O$: C, 49.03; H, 7.45; N, 11.44; Cl, 7.24. Found: C, 48.78; H, 7.12; N, 11.42; Cl, 7.56.

N-(2-Ethylthiomethyl-4,6-dimethyl-1-propyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**18d**): A crystalline solid. mp 155—159 °C. IR (Nujol) cm^{-1}: 1651. ^{1}H-NMR (DMSO-d_6) δ: 0.83 (3H, t, J=7.2 Hz), 1.20 (3H, t, J=7.3 Hz), 1.25 (9H, s), 1.40—1.70 (2H, m), 2.08 (3H, s), 2.21 (3H, s), 2.50—3.10 (6H, m), 3.20—4.40 (4H, m), 6.50—7.50 (2H, br), 8.30 (1H, s), 9.08 (1H, s). MS *m/z*: 457 $[M+H]^+$. *Anal.* Calcd for $C_{21}H_{36}N_4O_3S_2 \cdot HCl \cdot 0.4H_2O$: C, 50.41; H, 7.61; N, 11.20; Cl, 7.09. Found: C, 50.37; H, 7.54; N, 11.19; Cl, 7.21.

Procedure for the Synthesis of 24 Compound **24** was synthesized from **25** *via* **26**. Conversion of **26** to **24** was accomplished according the procedure for **9a**. Compound **26** was obtained as follows:

2-(4,6-Dimethylindol-3-yl)ethanol (**38**): To a solution of 4,6-dimethylindole (**25**) (26.0 g, 179 mmol) in Et_2O (260 ml) in an ice bath was added oxalyl chloride (30.6 ml, 322 mmol). After stirring for 4 h at room temperature, the reaction mixture was evaporated under reduced pressure. A solution of the residue in EtOH (200 ml) was stirred for 1 h at room temperature, and then the solvent was evaporated under reduced pressure. After dilution with AcOEt, the solution was washed with water, brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography to give a crystalline residue. The residue was rinsed with Et_2O to give ethyl (4,6-dimethylindol-3-yl)oxoacetate (**39**) as a crystalline solid (21.2 g, 54% yield). To a suspension of $LiAlH_4$ (9.36 g, 247 mmol) in THF (200 ml) was added the compound **39** (20.2 g, 82.4 mmol) at room temperature. The reaction mixture was refluxed for 1 h, and then water was added. After removal of insoluble material by filtration, the organic layer was separated, dried over Na_2SO_4, and evaporated under reduced pressure to give **38** as a crystalline solid (15.8 g, 100% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.39 (3H, s), 2.63 (3H, s), 3.13 (2H, t, J=7.0 Hz), 3.86 (2H, t, J=7.0 Hz), 6.69 (1H, s), 6.91 (1H, s), 6.92 (1H, s), 7.90 (1H, s).

2-(4,6-Dimethylindolin-3-yl)ethanol (**40**): To a solution of **38** (16.5 g, 87.2 mmol) in AcOH (83 ml) was added $NaBH_3CN$ (10.7 g, 170 mmol) at 10 °C, and the mixture was stirred for 0.5 h at the same temperature. After addition of NaOH aqueous solution (60 g/200 ml) dropwise below 40 °C, the mixture was extracted with AcOEt. The organic layer was washed with water, saturated $NaHCO_3$ solution, brine, dried over Na_2SO_4, and evaporated under reduced pressure to give **40** (16.3 g, 98% yield) as a crystalline solid. ^{1}H-NMR ($CDCl_3$) δ: 1.73—1.83 (1H, m), 1.85—1.94 (1H, m), 2.22 (3H, s), 2.23 (3H, s), 3.30—3.60 (6H, m), 6.33 (1H, s), 6.37 (1H, s).

3-(2-Methoxyethyl)-4,6-dimethylindoline (**41**): To a solution of **40** (16.3 g, 85.3 mmol) in THF (160 ml) was added Boc_2O (23.3 g, 107 mmol), and the reaction mixture was stirred for 2 h at room temperature. After evaporation under reduced pressure, the residue was purified by column chromatography (AcOEt : *n*-hexane=1 : 3) to give *tert*-butyl 3-(2-hydroxyethyl)-4,6-dimethylindoline-1-carboxylate (**42**) as an oil (22.1 g, 89% yield). To a solution of **42** (22.1 g, 75.8 mmol) in DMF (110 ml) in an ice bath were added methyl iodide (9.6 ml, 0.15 mol) and 60% suspension of NaH in mineral oil (3.98 g, 99.5 mmol), and the mixture was stirred for 1 h at the same temperature. After addition of 5% citric acid solution, the mixture was extracted with AcOEt. The organic layer was washed with water, saturated $NaHCO_3$ solution, brine, dried over Na_2SO_4, and evaporated under reduced pressure to give *tert*-butyl 3-(2-methoxyethyl)-4,6-dimethylindoline-1-carboxylate (**43**) as an oil (24.1 g). To a solution of **43** (24.0 g, 78.6 mmol) in HCO_2H (72 ml) in an ice bath was added 8.7 M HCl in *i*-PrOH (29 ml, 0.25 mol). The mixture was stirred for 15 min at the same temperature. After addition of water, the mixture was extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$ solution, brine, dried over Na_2SO_4, and evaporated under reduced pressure to give **41** as an oil (15.6 g, 97% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.73—1.94 (2H, m), 2.22 (3H, s), 2.23 (3H, s), 3.30—3.59 (8H, m), 6.33 (1H, s), 6.37 (1H, s).

1-Acetyl-3-(2-methoxyethyl)-4,6-dimethylindoline (**26**): To a solution of **41** (15.5 g, 75.5 mmol) in $CHCl_3$ (155 ml) in an ice bath were added Ac_2O (10.7 ml, 113 mmol) and Et_3N (15.8 ml, 113 mmol). After stirring for 1 h at room temperature, the mixture was washed with 5% citric acid solution, saturated $NaHCO_3$ solution, brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (AcOEt : *n*-hexane=1 : 2) to give **26** as a crystalline solid (19.7 g, 100% yield). ^{1}H-NMR ($CDCl_3$) δ: 1.66—1.74 (1H, m), 1.82—1.90 (1H, m), 2.22 (3H, s), 2.25 (3H, s), 2.34 (3H, s), 3.32 (3H, s), 3.36—3.44 (3H, m), 3.85—4.06 (2H, m), 6.68 (1H, s), 7.89 (1H, s).

N-[3-(2-Methoxyethyl)-4,6-dimethyl-1-propyl-5-sulfamoylaminoindolin-7-yl)-2,2-dimethylpropanamide Hydrochloride (**24**): A crystalline solid. mp 171—174 °C. IR (Nujol) cm^{-1}: 1672. ^{1}H-NMR (DMSO-d_6) δ: 0.87 (3H, t, J=7.3 Hz), 1.27 (9H, s), 1.57—1.78 (2H, m), 1.88—2.00 (2H, m), 2.12 (3H, s), 2.33 (3H, s), 3.02—3.12 (1H, m), 3.20—3.80 (9H, m), 6.60—7.10 (2H, br), 8.45 (1H, s), 9.16 (1H, s). MS *m/z*: 441 $[M+H]^+$. *Anal.* Calcd for $C_{21}H_{36}N_4O_4S \cdot HCl \cdot 0.2H_2O$: C, 52.48; H, 7.84; N, 11.66; Cl, 7.38. Found: C, 52.37; H, 7.82; N, 11.62; Cl, 7.48.

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.[15] Acetanilide, benzonitrile, 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)–MeOH (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.

Biological Evaluations of Compounds All experiments were conducted according to the guidelines for animal experiments of our institute and the Guidelines for Animal Experimentation approved by the Japanese Association of Laboratory Animal Science and the Japanese Pharmacological Society.

Plasma Protein Binding ratio (PBR): The plasma protein binding ratio was estimated by an ultracentrifugation method.[16] Test compounds were dissolved in rabbit plasma (10 μg/ml), which was isolated from blood taken from male Japanese white rabbits (JW rabbits, 6 months old; Japan SLC Inc., Hamamatsu, Japan). 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 436000×***g*** for 150 min with a small ultracentrifuge (CS100GX; Hitachi Koki, Hitachinaka, Japan). The concentrations of the test compounds in the middle layer (protein-free fraction) were determined using the API 2000 QTRAP LC-MS/MS system. From the total and protein-free fraction concentrations, the protein binding ratio of the test compounds was calculated.

Plasma and Arterial Levels of Compounds after Oral Administration: Compound **1i** and Pactimibe sulfate were suspended in 5% arabic gum and administered orally at 10 mg/kg to male Sprague-Dawley (SD) rats (7 weeks old; Japan SLC Inc.), beagles (47, 60 months; NARC Co., Yamatake, Japan), and male JW rabbits (5—7 months; Japan SLC Inc.). Blood samples were drawn using a heparinized syringe from the jugular or ear vein at 0.25, 0.5, 1, 2, 3, 5, 8, and 24 h after administration. Blood was centrifuged at 3000 rpm for 10 min at room temperature. The concentrations of **1i** and Pactimibe in the plasma were determined using HPLC. In another set of experiments, **1i** (30 mg/kg) and Pactimibe (10 mg/kg) were orally administered, blood was taken, and the atherosclerotic aorta was isolated after euthanasia by deep anesthesia. The concentrations of both compounds in the plasma and aorta were determined using HPLC, and the partition ratio from plasma to the aorta was calculated.

Esterified Cholesterol (EC) Accumulation in THP-1 Cell-Derived Macrophages: The effects of the test compounds on EC accumulation in THP-1 cells were determined during differentiation and foam cell formation. In order to cause the cells to differentiate into macrophages and to form foam cells, THP-1 cells were suspended in RPMI-1640 medium containing fetal bovine serum (FBS, 10%) and phorbol 12-myristate 13-acetate (PMA, 200 nM) with acetyl low density lipoprotein (LDL) (400 μg protein/ml), and 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 d in the presence or absence of the test compounds. Acetyl LDL was prepared from the serum of male KHC rabbits (6—8 months old; Japan Laboratory Animals, Inc., Tokyo, Japan). In another set of experiments, the effects of compound **1i** and Pactimibe on EC accumulation by high-cholesterol serum (HCS) were investigated in the presence of bovine serum albumin (BSA). THP-1 cells were suspended in RPMI-1640 medium containing FBS (10%) and PMA (200 nM), incubated at 4×10^5 cells/well in a humidified atmosphere of 95% air, 5% CO_2 at 37 °C for 3 d, and then they were incubated in RPMI-1640 medium containing HCS (3%) with or without BSA (5%) in the presence or absence of the test compounds for a day. HCS was isolated from male JW rabbits (4—5 months old; Japan SLC Inc.) fed a high cholesterol diet for 1 month. Cellular cholesterol was extracted by *n*-hexane/*i*–PrOH (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.

In Vitro ACAT Activity: Male JW rabbits (2.5 kg, Japan SLC) were anesthetized with sodium pentobarbital (30 mg/kg, intravenously (i.v.)) and exsanguinated from the common carotid artery, and then the liver was isolated. Microsomes were prepared according to the method of Field and Mathur.[17] Briefly, each sample was homogenized in a buffered sucrose solution (250 mM sucrose, 5 mM K_2HPO_4/KH_2PO_4, 1 mM ethylenediaminetetraacetic acid (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.*[18] 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, U.S.A.) was added. The reaction mixture was incubated at 37 °C for 20 min. Lipids were extracted with $CHCl_3$/MeOH (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 2 experiments.

In Vitro Cholesterol Esterification in Atherosclerotic Aorta The aorta was isolated from KHC rabbits, and atherosclerotic areas were minced and then equally weighed out 200 mg samples. These aortic samples were treated with compounds **1i** and Pactimibe and incubated with ^{14}C-oleic acid at 37 °C for 24 h in JW rabbit serum. Then, lipids were extracted with $CHCl_3$/MeOH (2 : 1) and separated by TLC. The EC produced in the samples incubated with vehicle or test compounds was determined.

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