Discovery of a Potent and Orally Available Acyl-CoA: Cholesterol Acyltransferase Inhibitor as an Anti-atherosclerotic Agent: (4-Phenylcoumarin)acetanilide Derivatives

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Acyl-CoA: cholesterol acyltransferase (ACAT) is an intracellular enzyme that catalyzes cholesterol esterification. ACAT inhibitors are expected to be potent therapeutic agents for the treatment of atherosclerosis. A series of potent ACAT inhibitors based on an (4-phenylcoumarin)acetanilide scaffold was identified. Evaluation of the structure–activity relationships of a substituent on this scaffold, with an emphasis on improving the pharmacokinetic profile led to the discovery of 2-[7-chloro-4-(3-chlorophenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (23), which exhibited potent ACAT inhibitory activity (IC_{50}=12 nM) and good pharmacokinetic profile in mice. Compound 23 also showed regressive effects on atherosclerotic plaques in apolipoprotein (apo)E knock out (KO) mice at a dose of 0.3 mg/kg *per os* (*p.o.*).

Key words acyl-CoA: cholesterol acyltransferase inhibitor; cholesterol; atherosclerosis; (4-phenylcoumarin)acetanilide

Atherosclerosis is a risk factor for stroke and coronary heart diseases. Current medical management of atherosclerosis is based on the control of the risk factors and predisposed conditions through lipid-lowering therapy involving diet or drugs.[1] However, additional therapies to act directly on atherosclerotic lesions would provide a powerful treatment method.[2]

Atherosclerotic lesions are typically composed of large lipid cores with underlying thin fibrous caps, so-called vulnerable plaques.[3] The progression of atherosclerotic lesions is associated with the accumulation of esterified cholesterol in macrophage-derived foam cells.[3]

Acyl-CoA: cholesterol acyltransferase (ACAT) is an intracellular enzyme that catalyzes cholesterol esterification and is highly expressed in macrophage-derived foam cells.[4—7] Therefore, inhibiting ACAT may directly reduce atherosclerotic lesions. Approaches targeting arterial plaques by using ACAT inhibitors have been investigated for 2 decades.[8] Experimental evidence suggested that ACAT inhibitors reduced and stabilized atherosclerotic plaques in animals.[9—13]

Two ACAT inhibitors, avasimibe and pactimibe, failed to reduce surrogate markers for coronary artery disease in recent clinical trials (Fig. 1).[14—17] Several explanations were proposed, including insufficient ACAT inhibitory activity (IC_{50}: avasimibe, 1980 nM; pactimibe, 216 nM).[18] Thus, our initial effort focused on identifying ACAT inhibitors with both potent activity and a good pharmacokinetic (PK) profile.

A screening of library compounds identified a (4-phenylcoumarin)acetanilide derivative **1** as a novel potent ACAT inhibitor (IC_{50}=5 nM) (Fig. 1). However, the PK profile of this compound was poor, and the anti-atherosclerotic efficacy was not evaluated. For optimization, we divided (4-phenylcoumarin)acetanilide scaffold (**2**) into 3 aromatic moieties, an anilide, a coumarin, and a pendant phenyl (Fig. 2). Herein, we describe the structure–activity relationship (SAR) study of substituents on these moieties with an emphasis on improving the PK profile. Effects of an optimized compound on atherosclerotic plaques in mice are also explained.

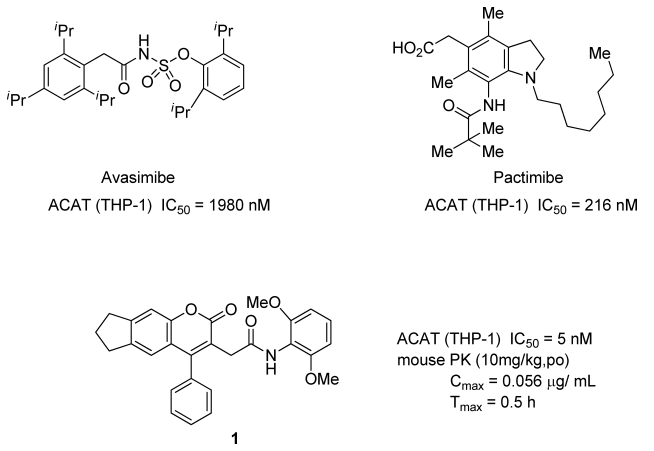

Fig. 1. Structures of ACAT Inhibitors, Avasimibe, Pactimibe and Lead Compound **1**

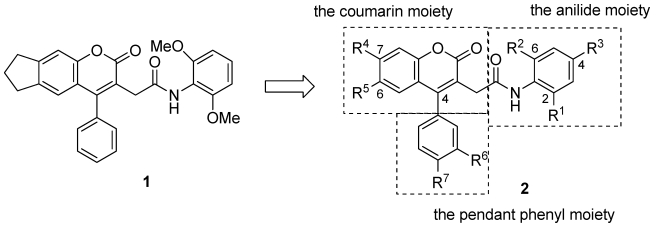

Fig. 2. Optimization Plan of the Substituent on the (4-Phenylcoumarin)-acetanilide Scaffold

Chemistry The general synthetic approach for the preparation of (4-phenylcoumarin)acetanilide derivatives is shown in Chart 1. The starting 2-hydroxybenzophenones **3**, which can be easily prepared from the substituted anisoles using known methods,[19] were converted into the corresponding coumarinacetates **4** by esterification with ethyl succinyl chloride and successive cyclization mediated by 1,8-diazabicyclo[5.4.0]undec-7-ene. After the hydrolysis of the ester

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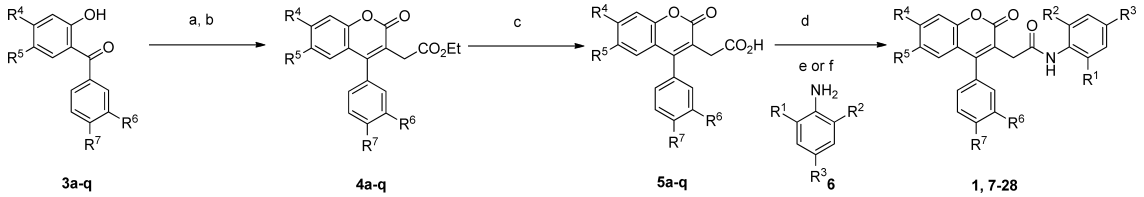


3–5	R^4	R^5	R^6	R^7
a	$-(CH_2)_3-$		H	H
b	$-(CH_2)_4-$		H	H
c	Me	Me	H	H
d	F	F	H	H
e	Cl	Cl	H	H
f	H	Cl	H	H
g	Me	Cl	H	H
h	Cl	F	H	H
i	Cl	Me	H	H
j	Cl	OMe	H	H
k	Cl	Me	Me	H
l	Cl	Me	Cl	H
m	Cl	Me	OCF_3	H
n	Cl	Me	H	Cl
o	Cl	Me	H	F
p	Cl	Me	Cl	F
q	Cl	Me	Me	Me

Compds	R^1	R^2	R^3	R^4	R^5	R^6	R^7
1	OMe	OMe	H	$-(CH_2)_3-$		H	H
7	OMe	H	H	$-(CH_2)_3-$		H	H
8	Me	H	H	$-(CH_2)_3-$		H	H
9	CF_3	H	H	$-(CH_2)_3-$		H	H
10	CF_3	F	H	$-(CH_2)_3-$		H	H
11	CF_3	H	F	$-(CH_2)_3-$		H	H
12	CF_3	H	Cl	$-(CH_2)_3-$		H	H
13	CF_3	H	Cl	$-(CH_2)_4-$		H	H
14	CF_3	H	Cl	Me	Me	H	H
15	CF_3	H	Cl	F	F	H	H
16	CF_3	H	Cl	Cl	Cl	H	H
17	CF_3	H	Cl	H	Cl	H	H
18	CF_3	H	Cl	Me	Cl	H	H
19	CF_3	H	Cl	Cl	F	H	H
20	CF_3	H	Cl	Cl	Me	H	H
21	CF_3	H	Cl	Cl	OMe	H	H
22	CF_3	H	Cl	Cl	Me	Me	H
23	CF_3	H	Cl	Cl	Me	Cl	H
24	CF_3	H	Cl	Cl	Me	OCF_3	H
25	CF_3	H	Cl	Cl	Me	H	Cl
26	CF_3	H	Cl	Cl	Me	H	F
27	CF_3	H	Cl	Cl	Me	Cl	F
28	CF_3	H	Cl	Cl	Me	Me	Me

Reagents and conditions: (a) $EtO_2CCH_2CH_2COCl$, Et_3N, THF, 0 °C, 1 h; (b) DBU, toluene, reflux, 2.5 h; (c) c HCl, AcOH, reflux, 1 h; (d) $(COCl)_2$, DMF (cat.), THF, rt, 0.5 h; (e) **6**, Et_3N, THF, rt, 1 h; (f) **6**, NaH, THF, rt, overnight.

Chart 1. Synthesis of (4-Phenylcoumarin)acetanilides Derivatives

group, resulting acid **5** was converted to the acid chloride and coupled with aniline **6** to produce (4-phenylcoumarin)acetanilides **1** and **7**—**28**.

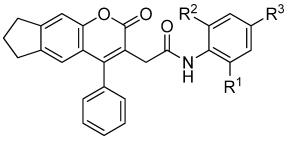
Results and Discussion

The synthesized compounds were examined for ACAT inhibitory activity, which was measured using human macrophage homogenates derived from THP-1 cell lines.

First, the SAR of R^1, R^2, and R^3 groups on the anilide moiety was explored (Table 1). The methoxy groups and the *para* position were thought to be metabolically vulnerable sites of the anilide moiety. Removal of one of the methoxy groups (compound **7**) and replacement by an ethyl group (compound **8**) led to reduced ACAT inhibitory activity. 2-Trifluoromethyl analogue **9** showed more potent ACAT inhibitory activity than compounds **7** and **8**. Further introduction of a fluorine group improved ACAT inhibitory activity (compounds **10**, **11**). These results suggested that the trifluoromethyl group acts as not only a bulky substituent but also an electron-withdrawing substituent to decrease electron density of the anilide moiety, leading to enhanced ACAT inhibitory activity. On the other hand, 4-chloro analogue **12** showed a weak ACAT inhibitory activity (IC_{50}=96 nM). This showed that the introduction of a substituent at the 4-position on the anilide moiety, except small molecules such as a fluorine, does not result in potent ACAT inhibition.

We also evaluated oral absorption of these compounds in mice and plasma concentrations 2 h (C_{2h}) after oral administration (10 mg/kg) to C57/BL/6 mice (Table 1). Compounds **9**, **10**, and **11** showed slightly improved oral availability relative to compound **1**, potentially due to blockage of the metabolic site by the trifluoromethyl group and fluorine atom. Surprisingly, compound **12** exhibited significantly high C_{2h} values. The electron withdrawing property of the chlorine atom is thought to contribute to the resistive effect on oxida-

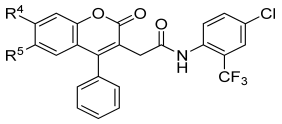
Table 1. Effects of Substituent on the Anilide Moiety of the (4-Phenylcoumarine)acetanilide on ACAT Inhibitory Activity[a] and PK Profile[b]



Compound	R^1	R^2	R^3	ACAT[a] IC_{50} (nM)	C_{2h}[b] (μg/ml)
1	OMe	OMe	H	5	0.007
7	OMe	H	H	100	ND
8	Et	H	H	86	ND
9	CF_3	H	H	36	0.032
10	CF_3	F	H	6	0.082
11	CF_3	H	F	19	0.159
12	CF_3	H	Cl	96	3.109

a) ACAT inhibitory activity was determined by using homogenized THP-1 cells. *b*) Compounds were orally administrated in C57/BL/6 mice (n=3) at a dose of 10 mg/kg (suspension in 0.5% methylcellulose). The values of drug concentration in plasma at 2h after oral administration (C_{2h}) were measured. ND: not determined.

Table 2. Effects of Substituent on the Coumarin Moiety of the (4-Phenylcoumarine)acetanilide on ACAT Inhibitory Activity[a)]



Compound	R^4	R^5	ACAT[a)] IC_{50} (nM)
12	$-(CH_2)_3-$		96
13	$-(CH_2)_4-$		>100
14	Me	Me	>100
15	F	F	>100
16	Cl	Cl	>100
17	H	Cl	>100
18	Me	Cl	100
19	Cl	F	90
20	Cl	Me	44
21	Cl	OMe	>100

a) ACAT inhibitory activity was determined by using homogenized THP-1 cells.

tive metabolism on the anilide moiety. After modification of the anilide moiety, we found that the 2-trifluoro-4-chloroanilide analogue has an attractive PK profile and moderate ACAT inhibitory activity.

Next, we focused on the modification of R^4 and R^5 groups on the coumarin ring based on compound **12** (Table 2) to improve the potency of the ACAT inhibition. In most cases, the potencies were reduced; however, compound **20** showed improved potency (IC_{50}=44 nM). These results suggested that a chloro group and a methyl group are the most effective combination of R^4 and R^5 groups, respectively, on the coumarin ring for potent ACAT inhibition.

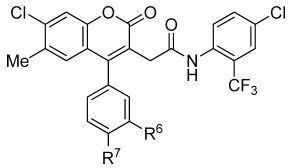
Introduction of some substituents on the pendant phenyl moiety was examined for further potency improvement (Table 3). Most compounds showed more potent inhibitory activity than compound **20**. However, the potency of ACAT inhibition of compound **25** was weak. Introduction of a hydrophobic substituent at the *meta*-position (R^6) led to increased ACAT inhibitory activity (compounds **22**—**24**). This hydrophobic substituent (R^6) might interact with ACAT proteins directly and/or create the proper torsional angle between the coumarin moiety and the pendant phenyl moiety. On the other hand, only a small group such as a fluorine group or a methyl group was tolerable for potent ACAT inhibition as an R^7 group (compounds **26**—**28**).

PK profile of compounds **22**—**24**, **27** and **28**, which exhibited potent ACAT inhibitory activity, were also examined in mice (Table 3). Among them, compound **23** showed the best bioavailability.

As a result of the optimization of the substituent on the (4-phenylcoumarin)acetanilide moiety, we chose compound **23**, which exhibited potent ACAT inhibitory activity and an improved PK profile (at a dose of 10 mg/kg, *per os* (*p.o.*), C_{max}=6.83 μg/ml, AUC=61.4 μg·h/ml)[20)] in comparison with lead compound **1**.

Finally, compound **23** was examined anti-atherosclerotic effects in apolipoprotein E knock out mice (apoE KO mice).[21,22)] After daily oral administration of compound **23** at doses of 0.3, 1, 3, and 10 mg/kg for 4 weeks, regressive effects were estimated by reduction of the lesion area (Fig. 3A) and the content of aortic cholesteryl ester (CE) (Fig. 3B).

Table 3. Effects of Substituent on the Pendant Phenyl Moiety of the (4-Phenylcoumarine)acetanilide on ACAT Inhibitory Activity[a)] and PK Profile[b)]



Compound	R^6	R^7	ACAT[a)] IC_{50} (nM)	C_{max} (ng/ml)	$AUC_{0-8\,h}$ (ng·h/ml)	F (%)
20	H	H	44	ND	ND	ND
22	Me	H	4	503.5	2839.6	15.9
23	Cl	H	12	1734.6	8771.1	34.5
24	OCF_3	H	14	80.7	355.7	5.7
25	H	Cl	>100	ND	ND	ND
26	H	F	33	ND	ND	ND
27	Cl	F	9	477.6	2587.7	7.4
28	Me	Me	6	118.7	633.1	2.2

a) ACAT inhibitory activity was determined by using homogenized THP-1 cells. *b*) Compounds were administrated as a cassette dosing to nonfasted mice at 0.1 mg/kg, i.v. and 1 mg/kg, *p.o.* ND: not determined.

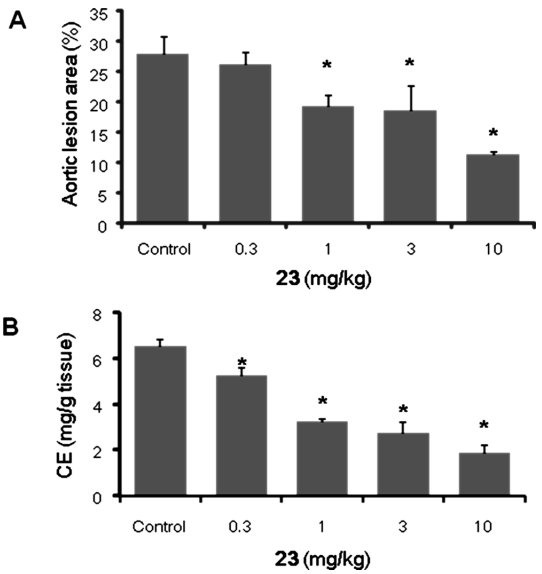


Fig. 3. Anti-atherosclerotic Effects of Compound **23** in apoE KO Mice

Six-week-old, male apoE KO mice were fed atherogenic diet for 7 weeks and nomal chow for one week. Compound **23** was then administered orally once daily for 4 week at a dose of 0.3, 1, 3 and 10 mg/kg (solution in Gelucire).[21)] (n=3—10). Regressive effect on (A) the atherosclerotic lesion and (B) the aortic cholesteryl ester (CE) contents was determined. Data represent mean±S.E. $*p<0.025$ *vs.* control by Williams test.

Compound **23** exhibited regressive effects in a dose-dependent manner, particularly on aortic CE contents at a dose of 0.3 mg/kg.

Conclusion

We developed (4-phenylcoumarin)acetanilide derivatives as a novel potent and oral available ACAT inhibitors. Evaluation of the structure–activity relationships of various substituents on the (4-phenylcoumarin)acetanilide scaffold with an emphasis on improving the PK profile led to the discovery of 2-[7-chloro-4-(3-chlorophenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]-*N*-[4-chloro-2-(trifluoromethyl)phenyl]-acetamide (**23**), which exhibits a potent ACAT inhibitory activity and good PK profile in mice. Compound **23** also show-

ed the regressive effects on atherosclerotic plaques in apoE KO mice at a dose of 0.3 mg/kg *p.o.*

Experimental

Melting points (mp) were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Gemini 200 (200 MHz) or Varian Mercury 300 (300 MHz) spectrometer. Chemical shifts are reported as δ values (ppm) downfield from internal tetramethylsilane of the indicated organic solution. Peak multiplicities are expressed as follow: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; br s, broad singlet; m, multiplet. Coupling constants (J values) are given in hertz (Hz). Elemental analyses were carried out by Takeda Analytical Research Laboratories Ltd. Reaction progress was determined by thin layer chromatography analysis on silica gel 60 F_{254} plate (Merck). Chromatographic purification was carried out on silica gel columns 60 (0.063—0.200 mm, Merck). Commercial reagents and solvents were used without additional purification. Abbreviations are used as follows: $CDCl_3$, deuterated chloroform; DMSO-d_6, dimethyl sulfoxide-d_6; EtOAc, ethyl acetate; AcOH, acetic acid; DMF, *N*,*N*-dimethylformamide; MeOH, methanol; THF, tetrahydrofuran; EtOH, ethanol; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene.

2-Hydroxybenzophenones **3a**—**q** were prepared from anisoles and benzoyl chlorides by the Friedel–Crafts acylation according to known methods.[19] These products were subjected to the next recation without further purification.

2-(2-Oxo-4-phenyl-2,6,7,8-tetrahydrocyclopenta[*g*]chromen-3-yl)acetic Acid (5a) To a solution of **3a** (1.00 g, 4.20 mmol) in THF (20 ml) was added dropwise triethylamine (0.98 ml, 7.03 mmol) and ethyl succinyl chloride (0.55 ml, 3.89 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and diluted with water. The reaction mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in toluene (10 ml) and DBU (0.25 ml, 1.66 mmol) was added. The mixture was refluxed for 2.5 h with a Dean–Stark apparatus to remove water azeotropically. After cooling, the reaction mixture was diluted with water, extracted with EtOAc, washed with 1 M HCl and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in c HCl aq. (10 ml) and AcOH (20 ml) and the mixture was refluxed for 1 h. The reaction mixture was evaporated and the residue was extracted with EtOAc, washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was crystallized from EtOAc to give **5a** (0.95 g, 70%) as colorless crystals: mp 216—218 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.09 (2H, m), 2.81 (2H, d, J=7.0 Hz), 2.99 (2H, d, J=7.0 Hz), 3.41 (2H, s), 6.82 (1H, s), 7.20—7.30 (3H, m), 7.50—7.60 (3H, m); *Anal.* Calcd for $C_{20}H_{16}O_4$: C, 74.99; H, 5.03. Found: C, 74.75; H, 5.13.

Compounds **5b**—**q** were prepared in a manner similar to that described for **5a**.

2-(2-Oxo-4-phenyl-6,7,8,9-tetrahydro-2*H*-benzo[*g*]chromen-3-yl)acetic Acid (5b) Colorless crystals (52%): mp 196—197 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.64—1.96 (4H, m), 2.50—2.76 (2H, m), 2.80—3.00 (2H, m), 3.40 (2H, s), 6.69 (1H, s), 7.09 (1H, s), 7.20—7.40 (2H, m), 7.40—7.70 (3H, m); *Anal.* Calcd for $C_{21}H_{18}O_4$: C, 75.43; H, 5.43. Found: C, 75.18; H, 5.43.

(6,7-Dimethyl-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetic Acid (5c) Colorless crystals (43%): mp 228—229 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.16 (3H, s), 2.34 (3H, s), 3.41 (2H, s), 6.75 (1H, s), 7.18 (1H, s), 7.20—7.36 (2H, m), 7.40—7.66 (3H, m); *Anal.* Calcd for $C_{19}H_{16}O_4$: C, 74.01; H, 5.23. Found: C, 73.96; H, 5.39.

(6,7-Difluoro-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetic Acid (5d) Colorless crystals (64%): mp 164—166 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.43 (2H, s), 4.86 (2H, dd, J=8.0 Hz, 10.0 Hz), 7.15—7.35 (3H, m), 7.50—7.65 (3H, m); *Anal.* Calcd for $C_{17}H_{10}F_2O_4\cdot 0.5H_2O$: C, 62.77; H, 3.41. Found: C, 62.71; H, 3.33.

(6,7-Dichloro-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetic Acid (5e) Colorless crystals (62%): mp 222—223 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.43 (2H, s), 7.10 (1H, s), 7.20—7.36 (2H, m), 7.50—7.64 (4H, m); *Anal.* Calcd for $C_{17}H_{10}Cl_2O_4\cdot 0.3H_2O$: C, 57.59; H, 3.01. Found: C, 57.44; H, 2.99.

(6-Chloro-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetic Acid (5f) Colorless crystals (59%): mp 174—177 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.44 (2H, s), 7.01 (1H, d, J=2.4 Hz), 7.20—7.60 (7H, m); *Anal.* Calcd for $C_{17}H_{10}ClFO_4$: C, 64.88; H, 3.52. Found: C, 65.13; H, 3.54.

(6-Chloro-7-methyl-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetic Acid (5g) Colorless crystals (50%): mp 216—217 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.45 (3H, s), 3.43 (2H, s), 7.00 (1H, s), 7.20—7.30 (3H, m), 7.50—7.62 (3H, m); *Anal.* Calcd for $C_{18}H_{13}ClO_4$: C, 65.76; H, 3.99. Found: C, 65.62; H, 4.12.

(7-Chloro-6-fluoro-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetic Acid (5h) Colorless crystals (92%): mp 209—210 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.44 (2H, s), 6.82 (1H, d, J=9.2 Hz), 7.21—7.32 (2H, m), 7.48 (1H, d, J=5.8 Hz), 7.52—7.62 (3H, m); *Anal.* Calcd for $C_{17}H_{10}ClFO_4$: C, 61.37; H, 3.03. Found: C, 61.35; H, 2.88.

(7-Chloro-6-methyl-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetic Acid (5i) Colorless crystals (64%): mp 222—224 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.28 (3H, s), 3.41 (2H, s), 6.86 (1H, s), 7.20—7.30 (2H, m), 7.42 (1H, s), 7.50—7.65 (3H, m); *Anal.* Calcd for $C_{18}H_{13}ClO_4$: C, 65.76; H, 3.99. Found: C, 65.72; H, 3.90.

(7-Chloro-6-methoxy-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetic Acid (5j) Colorless crystals (61%): mp 254—257 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.43 (2H, s), 3.66 (3H, s), 6.47 (1H, s), 7.24—7.33 (2H, m), 7.47 (1H, s), 7.50—7.61 (3H, m); *Anal.* Calcd for $C_{18}H_{13}ClO_5\cdot 0.1H_2O$: C, 62.39; H, 3.84. Found: C, 62.30; H, 3.91.

[7-Chloro-6-methyl-4-(3-methylphenyl)-2-oxo-2*H*-chromen-3-yl]acetic Acid (5k) Colorless crystals (80%): mp 250—253 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.28 (3H, s), 2.44 (3H, s), 3.41 (2H, s), 6.87 (1H, s), 7.05 (2H, m), 7.40 (3H, m); *Anal.* Calcd for $C_{19}H_{15}ClO_4$: C, 66.58; H, 4.41. Found: C, 66.41; H, 4.24.

[7-Chloro-4-(3-chlorophenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]acetic Acid (5l) Colorless crystals (58%): mp 262—264 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.30 (1H, s), 3.35 (1H, d, J=17.0 Hz), 3.46 (1H, d, J=16.8 Hz), 6.81 (1H, s), 7.14—7.20 (1H, m), 7.25—7.29 (1H, m), 7.43 (1H, s), 7.50—7.55 (1H, m); *Anal.* Calcd for $C_{18}H_{12}Cl_2O_4$: C, 59.53; H, 3.33. Found: C, 59.42; H, 3.18.

{7-Chloro-6-methyl-2-oxo-4-[3-(trifluoromethoxy)phenyl]-2*H*-chromen-3-yl}acetic Acid (5m) Colorless crystals (71%): mp 187—191 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.29 (3H, s), 3.36 (1H, d, J=17.2 Hz), 3.46 (1H, d, J=17.2 Hz), 6.81 (1H, s), 7.21 (2H, m), 7.42 (2H, m), 7.64 (1H, t, J=7.6 Hz); *Anal.* Calcd for $C_{19}H_{12}ClF_3O_5$: C, 55.29; H, 2.93. Found: C, 55.37; H, 2.89.

[7-Chloro-4-(4-chlorophenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]acetic Acid (5n) Colorless crystals (87%): mp 238—241 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.29 (3H, s), 3.40 (2H, s), 6.82 (1H, s), 7.22 (2H, d, J=8.4 Hz), 7.41 (1H, s), 7.56 (2H, d, J=8.4 Hz); *Anal.* Calcd for $C_{18}H_{12}Cl_2O_4$: C, 59.53; H, 3.33. Found: C, 59.47; H, 3.07.

[7-Chloro-4-(4-fluorophenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]acetic Acid (5o) Colorless crystals (80%): mp 253—257 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.29 (3H, s), 3.33 (2H, s), 6.83 (1H, s), 7.20—7.40 (5H, m); *Anal.* Calcd for $C_{18}H_{12}ClFO_4$: C, 62.35; H, 3.49. Found: C, 62.13; H, 3.29.

[7-Chloro-4-(3-chloro-4-fluorophenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]acetic acid (5p) Colorless crystals (88%): mp >258 °C (decomp.); ^{1}H-NMR ($CDCl_3$) δ: 2.31 (3H, s), 3.32 (2H, s), 6.82 (1H, s), 7.22 (1H, m), 7.37 (3H, m); *Anal.* Calcd for $C_{18}H_{11}Cl_2FO_4$: C, 56.72; H, 2.91. Found: C, 56.76; H, 2.83.

[7-Chloro4-(3,4-dimethylphenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]acetic Acid (5q) Colorless crystals (86%): mp 225—227 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.28 (3H, s), 2.34 (3H, s), 2.37 (3H, s), 3.43 (2H, s), 6.91 (1H, s), 6.94—7.01 (2H, m), 7.29 (1H, d, J=7.0 Hz), 7.40 (1H, s); *Anal.* Calcd for $C_{20}H_{17}ClO_4$: C, 67.32; H, 4.80. Found: C, 67.33; H, 4.83.

***N*-(2,6-Dimethoxyphenyl)-2-(2-oxo-4-phenyl-2,6,7,8-tetrahydrocyclopenta[*g*]chromen-3-yl)acetamide (1)** To a solution of **5a** (150 mg, 0.47 mmol) in THF (10 ml) and DMF (one drop) was added oxalyl chloride (60 μl, 0.69 mmol) at room temperature. After stirring for 0.5 h, the mixture was concentrated *in vacuo*. The residue was dissolved with THF (10 ml) and a solution of 2,6-dimethoxyaniline (79.0 mg, 0.52 mmol) and triethylamine (100 μl, 0.72 mmol) in THF (5 ml) was added. After stirring for 1 h at room temperature, the mixture was diluted with water and extracted with EtOAc. The organic layer was separated, washed with 1 M HCl aq., sat. $NaHCO_3$ aq. and water successively, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was crystallized from EtOAc–THF to give **1** (146 mg, 64%) as colorless crystals: mp 213—215 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.09 (2H, m), 2.81 (2H, t, J=7.0 Hz), 2.99 (2H, t, J=7.0 Hz), 3.46 (2H, br s) 3.78 (6H, s), 6.54 (2H, d, J=8.0 Hz), 6.85 (1H, s), 7.14 (1H, t, J=8.0 Hz), 7.26 (1H, s), 7.43 (2H, m), 7.50 (1H, m); *Anal.* Calcd for $C_{28}H_{25}NO_5\cdot 0.2H_2O$: C, 73.25; H, 5.58; N, 3.05. Found: C, 73.04; H, 5.79; N, 3.14.

Compounds **7** and **8** were prepared in a manner similar to that described for **1**.

***N*-(2-Methoxyphenyl)-2-(2-oxo-4-phenyl-2,6,7,8-tetrahydrocyclopenta[*g*]chromen-3-yl)acetamide (7)** Colorless crystals (40%): mp 219—222 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J=8.0 Hz), 2.09 (2H, m), 2.66 (2H, q, J=8.0 Hz), 2.82 (2H, t, J=7.0 Hz), 3.00 (2H, t, J=7.0 Hz), 3.46 (2H, s), 6.86 (1H, s), 7.1—7.2 (4H, m), 7.26 (1H, s), 7.39 (2H, m), 7.56 (3H, m), 8.43 (1H, br s); *Anal.* Calcd for $C_{25}H_{18}Cl_2N_2O_3\cdot 0.3H_2O$: C, 74.64; H, 5.57; N, 3.22. Found: C, 74.55; H, 5.66; N, 3.08.

***N*-(2-Ethylphenyl)-2-(2-oxo-4-phenyl-2,6,7,8-tetrahydrocyclopenta[*g*]chromen-3-yl)acetamide (8)** Colorless crystals (46%): mp 234—237 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.11 (2H, m), 2.83 (2H, t, J=7.0 Hz), 3.02 (2H, t, J=7.0 Hz), 3.54 (2H, s), 6.89 (1H, s), 7.30 (1H, s), 7.35 (2H, m), 7.56 (3H, m), 8.49 (2H, s), 8.74 (1H, br s); *Anal.* Calcd for $C_{28}H_{25}NO_3 \cdot 0.3H_2O$: C, 78.41; H, 6.02; N, 3.27. Found: C, 78.11; H, 6.04; N, 3.28.

***N*-[4-Chloro-2-(trifluoromethyl)phenyl]-2-(2-oxo-4-phenyl-2,6,7,8-tetrahydrocyclopenta[*g*]chromen-3-yl)acetamide (12)** To a solution of **5a** (150 mg, 0.47 mmol) in THF (10 ml) and DMF (one drop) was added oxalyl chloride (60 μl, 0.69 mmol). The mixture was stirred at room temperature for 0.5 h. After concentration of the solvent, the residue was dissolved with THF (10 ml). To the solution was added 4-chloro-2-(trifluoromethyl)aniline (**6a**) (80 mg, 0.41 mmol) and the mixture was stirred at room temperature overnight. The mixture was diluted with water and extracted with EtOAc. The organic layer was separated, washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was crystallized from EtOAc–THF to give **12** (125 mg, 55%) as colorless crystals: mp 198—200 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.00—2.20 (2H, m), 2.82 (2H, d, J=8.0 Hz), 3.00 (2H, d, J=8.0 Hz), 3.47 (2H, s), 6.86 (1H, s), 7.20—7.35 (3H, m), 7.40—7.60 (5H, m), 8.08 (1H, d, J=9.0 Hz), 8.43 (1H, br s); *Anal.* Calcd for $C_{27}H_{19}ClF_3NO_3$: C, 65.13; H, 3.85; N, 2.81. Found: C, 64.88; H, 3.98; N: 2.73.

The compounds **9**—**11** and **13**—**29** were prepared in a manner similar to that described for **12**.

2-(2-Oxo-4-phenyl-2,6,7,8-tetrahydrocyclopenta[*g*]chromen-3-yl)-*N*-[2-(trifluoromethyl)phenyl)]acetamide (9) Colorless crystals (79%): mp 191—193 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.02—2.17 (2H, m), 2.82 (2H, t, J=7.5 Hz), 3.00 (2H, t, J=7.5 Hz), 3.48 (2H, s), 6.86 (1H, s), 7.16—7.39 (4H, m), 7.46—7.62 (5H, m), 8.09 (1H, d, J=8.4 Hz), 8.35 (1H, br s); *Anal.* Calcd for $C_{27}H_{20}F_3NO_3 \cdot 0.1H_2O$: C, 69.70; H, 4.38; N, 3.01. Found: C, 69.64; H, 4.36; N, 2.87.

***N*-[2-Fluoro-6-(trifluoromethyl)phenyl]-2-(2-oxo-4-phenyl-2,6,7,8-tetrahydrocyclopenta[*g*]chromen-3-yl)acetamide (10)** Colorless crystals (19%): mp 194—196 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.00—2.20 (2H, s), 2.83 (2H, t, J=7.0 Hz), 3.01 (2H, t, J=7.0 Hz), 3.50 (2H, s), 6.89 (1H, s), 7.25—7.60 (9H, m), 8.32 (1H, br s); *Anal.* Calcd for $C_{27}H_{19}F_4NO_3$: C, 67.36; H: 3.98; N, 2.91. Found: C, 67.58; H, 3.82; N, 2.59.

***N*-[4-Fluoro-2-(trifluoromethyl)phenyl]-2-(2-oxo-4-phenyl-2,6,7,8-tetrahydrocyclopenta[*g*]chromen-3-yl)acetamide (11)** Colorless crystals (57%): mp 186—187 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.00—2.20 (2H, m), 2.82 (2H, d, J=8.0 Hz), 3.00 (2H, d, J=8.0 Hz), 3.46 (2H, s), 6.86 (1H, s), 7.10—7.40 (5H, m), 7.50—7.60 (3H, m), 8.00 (1H, dd, J=9.0, 5.0 Hz), 8.36 (1H, br s); *Anal.* Calcd for $C_{27}H_{19}F_4NO_3$: C, 67.36; H, 3.98; N, 2.91. Found: C, 67.35; H, 3.74; N, 2.91.

***N*-[4-Chloro-2-(trifluoromethyl)phenyl]-2-(2-oxo-4-phenyl-6,7,8,9-tetrahydro-2*H*-benzo[*g*]chromen-3-yl)acetamide (13)** Colorless crystals (85%): mp 232—234 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.78 (4H, m), 2.65 (2H, m), 2.87 (2H, m), 3.46 (2H, s), 6.73 (1H, s), 7.12 (1H, s), 7.32 (2H, m), 7.40-7.60 (5H, m), 8.08 (1H, d, J=8.8 Hz), 8.42 (1H, br s); *Anal.* Calcd for $C_{28}H_{21}ClF_3NO_3$: C, 65.69; H, 4.13; N, 2.74. Found: C, 65.67; H, 4.27; N, 2.68.

***N*-[4-Chloro-2-(trifluoromethyl)phenyl]-2-(6,7-dimethyl-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetamide (14)** Colorless crystals (65%): mp 201—203 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.18 (3H, s), 2.35 (3H, s), 3.47 (2H, s), 6.80 (1H, s), 7.22 (1H, s), 7.34 (2H, m), 7.56 (5H, m), 8.09 (1H, d, J=8.0 Hz), 8.34 (1H, br s); *Anal.* Calcd for $C_{26}H_{19}ClF_3NO_3$: C, 64.27; H, 3.94; N, 2.88. Found: C, 64.46; H, 4.03; N, 2.59.

***N*-[4-Chloro-2-(trifluoromethyl)phenyl]-2-(6,7-difluoro-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetamide (15)** Colorless crystals (55%): mp 172—174 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.48 (2H, s), 6.90 (1H, dd, J=8.0, 11.0 Hz), 7.30—7.40 (3H, m), 7.49 (1H, m), 7.57 (4H, m), 8.08 (1H, d, J=9.0 Hz), 8.14 (1H, br s); *Anal.* Calcd for $C_{24}H_{13}ClF_5NO_3$: C, 58.37; H, 2.65; N, 2.84. Found: C, 58.12; H, 2.52; N, 2.68.

***N*-[4-Chloro-2-(trifluoromethyl)phenyl]-2-(6,7-dichloro-2-oxo-4-phenyl-2*H*-chromen-3-yl)acetamide (16)** Colorless crystals (61%): mp 203—206 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.47 (2H, s), 7.15 (1H, s), 7.34 (2H, m), 7.57 (6H, m), 8.07 (2H, m); *Anal.* Calcd for $C_{24}H_{13}Cl_3F_3NO_3$: C, 54.73; H, 2.49; N, 2.66. Found: C, 55.10; H, 2.51; N, 2.34.

2-(6-Chloro-2-oxo-4-phenyl-2*H*-chromen-3-yl)-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (17) Colorless crystals (46%): mp 178—180 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.49 (2H, s), 7.05 (1H, d, J=2.6 Hz), 7.34 (3H, m), 7.57 (6H, m), 8.09 (1H, d, J=9.2 Hz), 8.20 (1H, br s); *Anal.* Calcd for $C_{24}H_{14}Cl_2F_3NO_3$: C, 56.49; H, 3.16; N, 2.74. Found: C, 56.39; H, 2.93; N, 2.59.

2-(6-Chloro-7-methyl-2-oxo-4-phenyl-2*H*-chromen-3-yl)-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (18) Colorless crystals (93%): mp 214—216 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.47 (3H, s), 3.47 (2H, s), 7.04 (1H, s), 7.32 (3H, m), 7.55 (5H, m), 8.09 (1H, d, J=9.0 Hz), 8.25 (1H, br s); *Anal.* Calcd for $C_{25}H_{16}Cl_2F_3NO_3$: C, 59.31; H, 3.19; N, 2.77. Found: C, 59.15; H, 3.21; N, 2.65.

2-(7-Chloro-6-fluoro-2-oxo-4-phenyl-2*H*-chromen-3-yl)-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (19) Colorless crystals (63%): mp 199—203 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.49 (2H, s), 6.86 (1H, d, J=9.2 Hz), 7.30—7.40 (2H, m), 7.45—7.62 (6H, m), 8.07 (1H, d, J=8.8 Hz), 8.15 (1H, br s); *Anal.* Calcd for $C_{24}H_{13}Cl_2F_4NO_3$: C, 56.49; H, 2.57; N, 2.74. Found: C, 56.35; H, 2.50; N, 2.60.

2-(7-Chloro-6-methyl-2-oxo-4-phenyl-2*H*-chromen-3-yl)-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (20) Colorless crystals (74%): mp 236—239 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.29 (3H, s), 3.46 (2H, s), 6.90 (1H, s), 7.27—7.60 (8H, m), 8.08 (1H, d, J=8.4 Hz), 8.25 (1H, br s); *Anal.* Calcd for $C_{25}H_{16}Cl_2F_3NO_3$: C, 59.31; H, 3.19; N, 2.77. Found: C, 59.10; H, 3.01; N, 2.68.

2-(7-Chloro-6-methoxy-2-oxo-4-phenyl-2*H*-chromen-3-yl)-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (21) Colorless crystals (81%): mp 219—221 °C; ^{1}H-NMR ($CDCl_3$) δ: 3.48 (2H, s), 6.50 (1H, s), 7.33—7.40 (2H, m), 7.43—7.52 (1H, m), 7.49 (1H, s), 7.52—7.61 (4H, m), 8.07 (1H, d, J=8.8 Hz), 8.30 (1H, br s); *Anal.* Calcd for $C_{25}H_{16}Cl_2F_3NO_4$: C, 57.49; H, 3.09; N, 2.68. Found: C, 57.51; H, 3.35; N, 2.67.

2-[7-Chloro-6-methyl-4-(3-methylphenyl)-2-oxo-2*H*-chromen-3-yl]-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (22) Colorless crystals (85%): mp 235—237 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.30 (3H, s), 2.44 (3H, s), 3.46 (2H, s), 6.92 (1H, s), 7.12 (2H, m), 7.30—7.50 (4H, m), 7.57 (1H, m), 8.08 (1H, d, J=8.8 Hz), 8.21 (1H, br s); *Anal.* Calcd for $C_{26}H_{18}Cl_2F_3NO_3$: C, 60.02; H, 3.49; N, 2.69. Found: C, 60.07; H, 3.62; N, 2.67.

2-[7-Chloro-4-(3-chlorophenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (23) Colorless crystals (51%): mp 202—204 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.31 (3H, s), 3.39 (1H, d, J=14.0 Hz), 3.51 (1H, d, J=14.4 Hz), 6.86 (1H, s), 7.23—7.30 (2H, m), 7.33—7.37 (1H, m), 7.45 (1H, s), 7.46—7.57 (2H, m), 7.58 (1H, d, J=2.6 Hz), 8.06 (1H, d, J=8.8 Hz), 8.21 (1H, br s); *Anal.* Calcd for $C_{25}H_{15}Cl_3F_3NO_3$: C, 55.53; H, 2.80; N, 2.59. Found: C, 55.83; H, 2.97; N, 2.42.

2-{7-Chloro-6-methyl-2-oxo-4-[3-(trifluoromethoxy)phenyl]-2*H*-chromen-3-yl}-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (24) Colorless crystals (40%): mp 171—175 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.31 (3H, s), 3.39 (1H, d, J=14.4 Hz), 3.52 (1H, d, J=14.4 Hz), 6.85 (1H, s), 7.20—7.50 (5H, m), 7.63 (2H, m), 8.06 (1H, d, J=9.0 Hz), 8.20 (1H, br s); *Anal.* Calcd for $C_{26}H_{15}Cl_2F_6NO_4$: C, 52.90; H, 2.56; N, 2.37. Found: C, 52.71; H, 2.55; N, 2.35.

2-[7-Chloro-4-(4-chlorophenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (25) Colorless crystals (80%): mp 284—286 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.30 (3H, s), 3.45 (2H, s), 6.87 (1H, s), 7.31 (2H, d, J=8.4 Hz), 7.45 (1H, s), 7.54 (4H, m), 8.05 (1H, d, J=9.2 Hz), 8.29 (1H, br s); *Anal.* Calcd for $C_{25}H_{15}Cl_3F_3NO_3$: C, 55.53; H, 2.80; N, 2.59. Found: C, 55.64; H, 3.00; N, 2.54.

2-[7-Chloro-4-(4-fluorophenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (26) Colorless crystals (73%): mp 272—273 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.30 (3H, s), 3.46 (2H, s), 6.88 (1H, s), 7.20—7.40 (4H, m), 7.45 (1H, s), 7.48 (1H, m), 7.58 (1H, m), 8.05 (1H, d, J=8.8 Hz), 8.30 (1H, br s); *Anal.* Calcd for $C_{25}H_{15}Cl_2F_4NO_3$: C, 57.27; H, 2.88; N, 2.67. Found: C, 57.30; H, 2.97; N, 2.65.

2-[7-Chloro-4-(3-chloro-4-fluorophenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (27) Colorless crystals (83%): mp 240—241 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.32 (3H, s), 3.38 (1H, d, J=14.2 Hz), 3.51 (1H, d, J=14.2 Hz), 6.85 (1H, s), 7.29 (2H, m), 7.45 (1H, s), 7.46 (2H, m), 7.59 (1H, m), 8.08 (1H, d, J=8.8 Hz), 8.25 (1H, br s); *Anal.* Calcd for $C_{25}H_{14}Cl_3F_4NO_3$: C, 53.74; H, 2.53; N, 2.51. Found: C, 53.81; H, 2.73; N, 2.46.

2-[7-Chloro-4-(3,4-dimethylphenyl)-6-methyl-2-oxo-2*H*-chromen-3-yl]-*N*-[4-chloro-2-(trifluoromethyl)phenyl]acetamide (28) Colorless crystals (67%): mp 253—254 °C; ^{1}H-NMR ($CDCl_3$) δ: 2.30 (3H, s), 2.34 (3H, s), 2.37 (3H, s), 3.48 (2H, s), 6.96 (1H, s), 7.00—7.10 (2H, m), 7.30 (1H, d, J=7.0 Hz), 7.43 (1H, s), 7.48 (1H, dd, J=3.0, 8.0 Hz), 7.57 (1H, d, J=3.0 Hz), 8.09 (1H, d, J=8.0 Hz), 8.23 (1H, br s); *Anal.* Calcd for $C_{27}H_{20}Cl_2F_3NO_3$: C, 60.69; H, 3.77; N, 2.62. Found: C, 60.75; H, 3.89; N, 2.53.

Inhibitory Effects of Compounds on ACAT Activity ACAT activities

were determined by incorporation of [^{3}H] oleoyl-CoA into cholesteryl esters using various enzyme sources. These were prepared as homogenates or microsome fractions from human THP-1 macrophages or mouse peritoneal macrophages. Inhibitory effects of compounds on ACAT activity were determined by measuring the radioactivities of [^{3}H] cholesteryl oleate fraction with or without compounds and these IC_{50} values was calculated from means of ACAT inhibition in duplicate measurements.

Pharmakokinetic Study in Mouse Cassette Dosing Compounds **22**—**24**, **27** and **28** were administrated as a cassette dosing to nonfasted mice. Afteroral and intravenous administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile. After centrifugation, the obtained supernatant was diluted and centrifuged again. The compound concentration in the supernatant was measured by LC/MS/MS.

Pharmakokinetic Study in Mice Compound **23** was administrated to nonfasted C57BL6 mice (male, 8 weeks old, $n=3$) orally (10 mg/kg, suspension in 0.5% methylcellulose). At 15 and 30 min and 1, 2, 4, 8 and 24 h after oral administration, blood samples were collected from tail vein. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile. After centrifugation, the obtained supernatant was diluted with 0.01 mol/l $CH_3CO_2NH_4$ and acetonitrile. The compound concentration in the supernatant was measured by HPLC system equipped with a UV detector. The HPLC conditions were as follows: column, L-column ODS (4.6 mm×150 mm); mobile phase, 0.01 mol/l $CH_3CO_2NH_4$/acetonitrile=24/76; flow rate, 1 ml/min; column temperature, 40 °C.

Preliminary Test for Oral Absorption Test compounds were administrated to nonfasted C57BL6 mice (male, 8 weeks old, $n=3$) orally (10 mg/kg, suspension in 0.5% methylcellulose). At 2 h after oral administration, blood samples were collected from tail vein. The concentration of the compounds was measured by similar manner as PK study.

Anti-atherosclerotic Effects in apoE KO Mice Six weeks old male apoE KO mice were fed atherogenic diet containing of 1.25% cholesterol and 0.5% cholate for 8 weeks and then normal chow for 1 week. Mice were divided into 5 groups based on plasma total cholesterol level and administered compound **23** orally (Gelucire solution) at doses of 0.3, 1, 3 and 10 mg/kg once daily for 4 weeks. Aorta was dissected after 4 weeks administration and the atherosclerotic lesion area was estimated as the area of oil red O staining quantified by image analysis software. Aortic cholesteryl ester contents were determined by measurements of free cholesterol and total cholesterol contents. Free and total cholesterol concentrations were determined using commercial kits (Wako Pure Chemical Industries). Cholesteryl ester content was calculated by subtracting free cholesterol contents from total cholesterol contents.

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