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Novel 4,1-Benzoxazepine Derivatives with Potent Squalene Synthase Inhibitory Activities

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Abstract—A series of (3,5-*trans*)-2-oxo-5-phenyl-1,2,3,5-tetrahydro-4,1-benzoxazepine derivatives were synthesized and evaluated for squalene synthase inhibitory and cholesterol biosynthesis inhibitory activities. Through modification of substituents of the lead compounds **1a** and **1b**, it was found that 4,1-benzoxazepine-3-acetic acid derivatives with isobutyl and neopentyl groups at the 1-position, the chloro atom at the 7-position, and the chloro and methoxy groups at the 2′-position on the 5-phenyl ring, had potent squalene synthase inhibitory activity. Among such compounds, the 5-(2,3-dimethoxyphenyl) derivative **2t** exhibited potent inhibition of cholesterol biosynthesis in HepG2 cells. As a result of optical resolution study of **2t**, the absolute stereochemistry required for inhibitory activity was determined to be 3*R*,5*S*. In vivo study showed that the sodium salt of (3*R*,5*S*)-7-chloro-5-(2,3-dimethoxyphenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetic acid **20** effectively reduced plasma cholesterol in marmosets.

Introduction

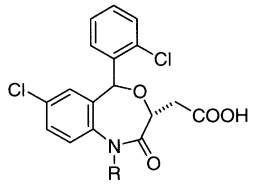
Squalene synthase [EC 2.5.1.21] catalyzes the formation of squalene from farnesyl diphosphate in cholesterol biosynthesis. This enzymatic step occurs after the pathway branches to other isoprene derivatives such as dolicol, ubiquinones and isopentenyl *t*-RNA. Since squalene synthase inhibitors do not interfere with the biosynthesis of these isoprene derivatives, inhibition of this step arrests only cholesterol biosynthesis and might be useful for the treatment of hyperlipidemia.

We previously reported[1] that a novel class of squalene synthase inhibitors, 4,1-benzoxazepine-3-acetic acid derivatives **1a**,**b** (Fig. 1), exhibited potent inhibition of rat enzyme ($IC_{50} = 0.061$–$0.072\ \mu M$) and HepG2 enzyme ($IC_{50} = 0.024$–$0.034\ \mu M$).

In this paper, we describe our synthetic studies of various 4,1-benzoxazepine derivatives,[2] which led to the discovery of a potent squalene synthase inhibitor, the sodium salt of (3*R*,5*S*)-7-chloro-5-(2,3-dimethoxyphenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetic acid **20**, the oral administration of which lowered plasma cholesterol in marmosets.

Chemistry

The syntheses of the 4,1-benzoxazepine-3-acetic acid derivatives **2** listed in Tables 1 and 3 are shown in Scheme 1.[1] Reduction of 2-aminobenzophenones **4** with sodium borohydride ($NaBH_4$) yielded aminoalcohols **5**, which were treated with aldehydes or ketones and $NaBH_4$ or sodium cyano borohydride ($NaBH_3CN$) to afford the alkylated compounds **6**. The compounds **6** were also synthesized by acylation of **5** with acid chloride, followed by reduction of the amides **11** with



1a R=CH_2Bu^t

1b R=Bu^i

Figure 1. Structures of 4,1-benzoxazepine-3-acetic acid derivatives.

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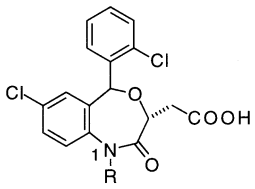
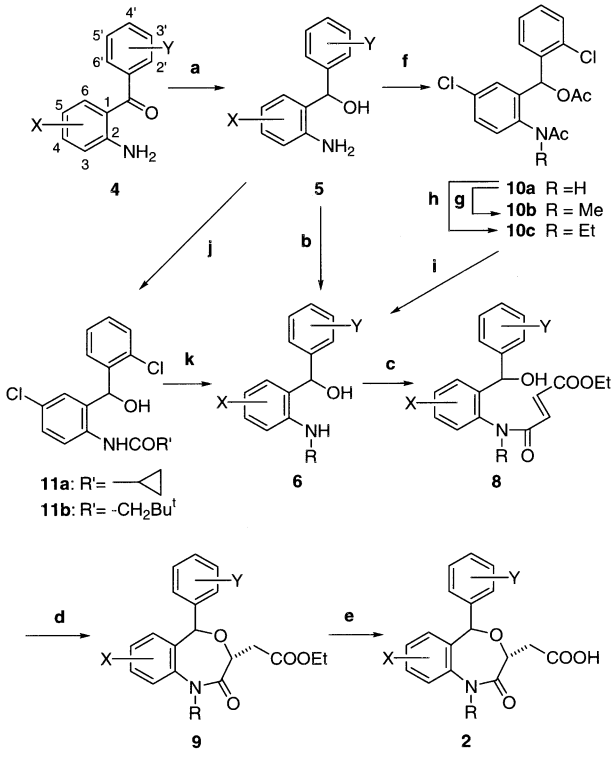
Table 1. Physicochemical and biological properties of 1-substituted (3,5-*trans*)-7-chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetic acid derivatives **1a**,**b** and **2a**–**l**



Compd	R	Yield[a] (%)	Mp (°C)	Formula[b]	IC_{50} (μM)[c] (rat enzyme)
2a	CH_2Ph	63	241–242	$C_{24}H_{25}C_{l2}NO_4$	8.3
2b	Me	45	211–213	$C_{18}H_{15}Cl_2NO_4$	6.4
2c	Et	75	215–216	$C_{19}H_{17}Cl_2NO_4$	0.53
2d	Pr	59	189–190	$C_{20}H_{19}Cl_2NO_4$	0.14
2e	Bu	79	195–196	$C_{21}H_{21}Cl_2NO_4$	2.2
2f	Pr	61	208–209	$C_{20}H_{19}Cl_2NO_4$	0.71
2g	CH_2CHEt_2	76	188–189	$C_{23}H_{25}Cl_2NO_4$	0.79
2h	CH_2–cyclopropyl	80	230–232	$C_{21}H_{19}Cl_2NO_4$	0.21
2i	CH_2–cyclohexyl	63	241–242	$C_{24}H_{25}Cl_2NO_4$	6.9
2j	$CHEt_2$	95	220–221	$C_{22}H_{23}Cl_2NO_4$	0.92
2k	$CH_2CH_2CHMe_2$	48	166–167	$C_{22}H_{23}Cl_2NO_4$	2.3
2l	$CH_2CH_2CMe_3$	63	176–177	$C_{23}H_{25}Cl_2NO_4$	>10 (25.6)[d]
1a[1]	CH_2CMe_3				0.072
1b[1]	Bu[i]				0.061

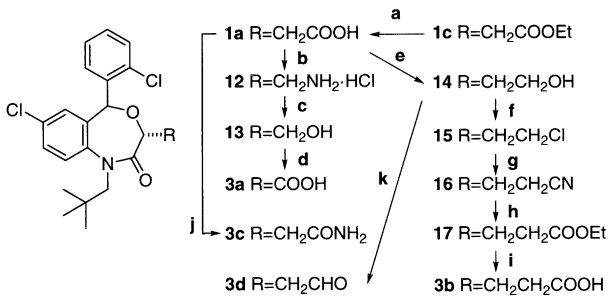
[a]Yield of final step.
[b]Analysis for C, H, N were correct within ±0.4%.
[c]IC_{50} values were determined by a single experiment run in duplicate.
[d]%Inhibition at 10 μM.



Scheme 1. Reagents and conditions: (a) $NaBH_4$; (b) aldehyde, ketone, $NaBH_4$ or $NaBH_3CN$, AcOH; (c) fumalic acid chloride monoethyl ester **7**, $NaHCO_3$, CH_2Cl_2; (d) K_2CO_3, EtOH; (e) K_2CO_3, MeOH–H_2O; (f) Ac_2O, DMAP, pyridine; (g) MeI, NaH, DMF; (h) EtI, NaH, DMF; (i) NaOH, EtOH; (j) R'COCl; (k) $LiAlH_4$.

lithium aluminum hydride ($LiAlH_4$). The *N*-methyl and *N*-ethyl analogues **6b**,**c** were prepared by alkylation of the diacetylated compound **10a** obtained from **5a**, followed by alkaline hydrolysis of the alkylated compounds **10b**,**c**. After condensation of **6** and fumaric acid chloride monoethyl ester **7**, intramolecular Michael addition of the obtained amides **8** afforded 4,1-benzoxazepine-3-acetates **9**. In this reaction, thermodynamically stable 3,5-*trans*-isomers were obtained, except with **2m**, which was obtained as a mixture of *trans*/*cis* isomers (1:1). Hydrolysis of the esters **9** gave the carboxylic acids **2**.

Next, we focused on the synthesis of 4,1-benzoxazepine derivatives having various substituents at the 3-position of lead compound **1a** (Scheme 2). The ethyl ester **1c** has been reported in the previous paper.[1] Modification of the tether length between the carboxyl moiety and the



Scheme 2. Reagents and conditions: (a) NaOH, EtOH; (b) (1) $(PhO)_2P(O)N_3$; (2) benzene, reflux; (3) concd HCl; (c) $NaNO_2$, AcOH–H_2O; (d) Jones' oxidation; (e) (1) ClCOOEt, *N*-methylmorpholine; (2) $NaBH_4$; (f) $SOCl_2$, pyridine; (g) NaCN; (h) HCl, EtOH; (i) K_2CO_3, MeOH–H_2O; (j) NH_4Cl, DEPC, Et_3N, DMF; (k) Swern's oxidation.

benzoxazepine ring was carried out as follows. Curtius rearrangement of the 3-acetic acid derivatives **1a** using diphenylphosphorylazide (DPPA), and subsequent acid hydrolysis of the resulting isocyanate gave the 3-aminomethyl analogue **12**.[3] The diazotation of **12** with sodium nitrite ($NaNO_2$) gave the 3-hydroxymethyl analogue **13** which was converted to the 3-carboxylic acid derivative **3a** by Jones' oxidation. The treatment of the 3-acetic acid derivative **1a** with ethyl chloroformate, followed by reduction with $NaBH_4$ gave the 3-(2-hydroxyethyl) analogue **14**.[4] Compound **14** was converted to the 3-(2-chloroethyl) analogue **15** which was reacted with sodium cyanide to provide the 3-propionitrile analogue **16**. Acid hydrolysis of **16** afforded the ethyl ester **17**, and subsequent alkaline hydrolysis gave the 3-propionic acid **3b**. The amide derivative **3c** were obtained by condensation of **1a** with ammonium chloride using diethyl cyanophosphonate (DEPC). Compound **14** was converted to the aldehyde **3d** by Swern's oxidation.[5]

To determine which enantiomer is an active inhibitor, we performed optical resolution of the compound **2t**, which exhibited the most potent inhibiting activity. We initially investigated the separation of the diastereomeric amide which could be prepared by condensation of racemate **2t** with various chiral amines and α-aminoacid esters. After several trials, we found that the diastereomeric amides of the acid **2t** condensed with methyl L-leucinate could easily be separated by silica gel column chromatography to give the less polar amide **18a** and the polar amide **18b** in diastereomerically pure forms. Then the amides **18a** and **18b** were hydrolyzed to yield enantiomerically pure acid **19a** and acid **19b**, respectively (Scheme 3). Determination of the absolute stereochemistry was performed by X-ray diffraction analysis of the compound **19a**. As depicted in Figure 2, the absolute configuration of **19a** (**18a**) was determined to be (3*R*,5*S*). Therefore, **19b** (**18b**) was (3*S*,5*R*).

Biological Results and Discussion

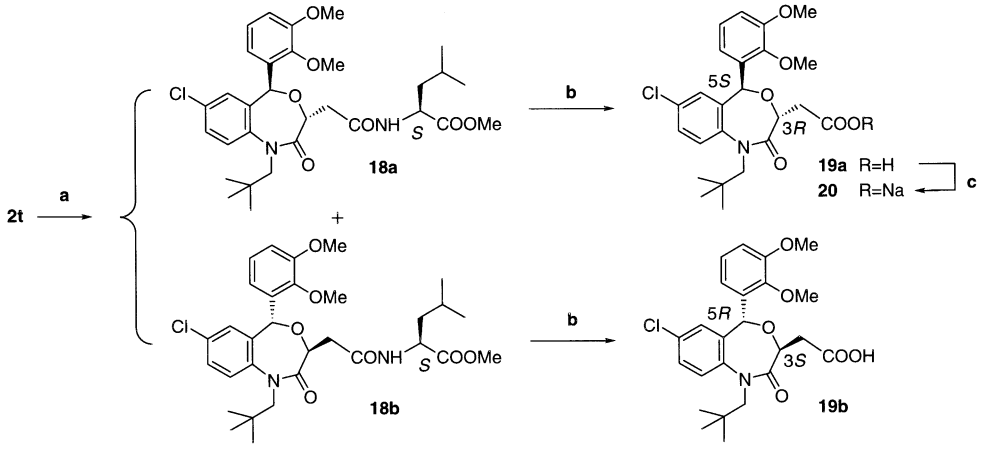
The compounds synthesized were evaluated for inhibition of squalene synthase prepared from rat liver and human hepatoma (HepG2) cells. Inhibitory activities were measured according to the method of Cohen et al. with slight modification.[6] The selected compounds were evaluated for inhibition of cholesterol biosynthesis in HepG2 cells according to the method of Kuroda et al.[7]

Squalene synthase inhibitory activity (in vitro)

Modification at the 1-position had a great influence on potency of inhibition of rat derived squalene synthase (Table 1). The 1-benzyl compound **2a** showed only weak activity. In the series of *N*-alkyl derivatives (**2b**,**c**,**d**,**e**), *n*-propyl compound **2d** was the most potent inhibitor of squalene synthase, with an IC_{50} value of 0.14 μM, indicating that the optimal carbon chain number is three. The derivatives with β-branched alkyl groups such as neopentyl (**1a**), isobutyl (**1b**) and 2-ethylbutyl (**2g**) exhibited much more potent activities than *n*-alkylated compounds **2d** and **2e**, while the cyclopropylmethyl derivative **2h** did not exhibit improved activity. On the other hand, compounds having α- and γ-branched alkyl substituents (**2f**, **2j** and **2k**, **2l**) did not exhibit improved activity, probably due to the bulkiness of these groups. Unsurprisingly, the cyclohexylmethyl compound **2i** was not a good inhibitor. The most favorable groups at the 1-position were thus found to be neopentyl (**1a**) and isobutyl (**1b**).

Modification of the 3-acetic acid moiety of compound **1a** gave the following results (Table 2). Elongation and shortening of the tether length (**3a**,**b**) resulted in 40- to 50-fold decrease in activity compared with **1a**. The activities of the ethyl ester analogue **1c**[1] and the carboxamide analogue **3c** were significantly weaker than that of the corresponding 3-acetic acid analogues **1a**, but retained IC_{50} values at the 10^{-6}–10^{-7} M level. The 3-acetaldehyde analogue **3d** exhibited only weak activity, with 39% inhibition at 10^{-5} M. The above results indicate that a carboxymethyl group is the best substituent at the 3-position.

Migration of the 7-chloro atom in the fused benzene ring to the 6- and 8-positions yielded compounds **2m** (a mixture of 3,5-*cis* and-*trans* isomers) and **2n**, which had poor activities (Table 3). For the chloro atom, the 7-position was found to be best.[8]



Scheme 3. Reagents and conditions: (a) L-Leu-OMe, DEPC, Et_3N then separation; (b) (1) HCl, MeOH; (2) NaOH; (c) NaOH.

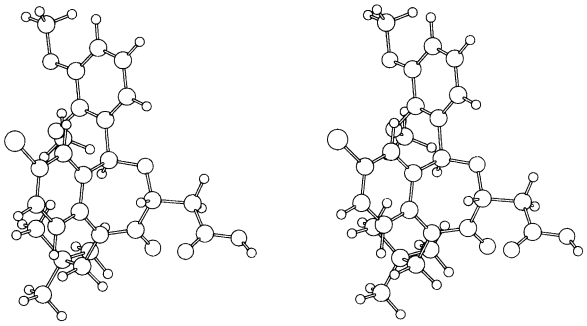


Figure 2. Stereoscopic molecular view of compound **19a.**

Biological data for compounds having various substituents on the 5-phenyl ring are also shown in Table 3. Compound **2o** with no substituent at the 2′-position was weaker than the 2′-chloro analogue **1a**. Replacement of a chloro atom at the 2′-position with a bromo and a methoxy group led to compounds **2p**,**q**, which exhibited potent activity. In order to improve the activity of the 2′-methoxy analogue **2q**, additional substituents such as fluoro, methyl and methoxy groups were introduced. Introduction of a fluoro atom or a methoxy group at the 5′- or 6′-position caused a loss of potency (**2s**,**v**,**w**), while introduction of a methyl group at the 3′-position resulted in a slight decrease of activity (**2y**). On the other hand, introduction of a methoxy group at the 3′- and 4′-positions (**2t**,**u**) and of a fluoro atom at the 4′-position (**2r**) yielded the same level of potency as the 2′-methoxy analogue **2q**. The 2′,4′,6′-trimethoxy analogue **2x** was less active than the 2′,4′-dimethoxy analogue **2u** but more active than the 2′,6′-dimethoxy analogue **2w**. A fluoro atom and a methoxy group are thus favorable as additional substituents at the 3′- and 4′-positions of the 2′-methoxy analogue **2q**.

In order to clarify the absolute stereochemistry required for inhibitory activity, optical resolution of the compound **2t** was performed. The (3*R*,5*S*)-enantiomer **19a** exhibited remarkably potent inhibition of both rat and human HepG2 enzymes, while the (3*S*,5*R*)-enantiomer **19b** exhibited modest inhibition (Table 4). Thus the stereochemistry at C(3) and C(5) on the benzoxazepine skeleton is of crucial importance in obtaining potent inhibitory activity.

The IC_{50} values of the sodium salt **20** for human enzyme and rat enzyme were 7.7 and 16 nM, respectively.[9] Lineweaver–Burk analysis of HepG2 enzyme inhibition revealed that compound **20** was a competitive inhibitor with respect to farnesyl pyrophosphate. The K_i value of compound **20** was 1.1 nM and was independent of enzyme concentration.[10]

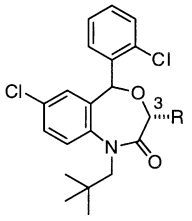
HepG2 cell assay

Since cholesterol is synthesized intracellularly, good cell membrane permeability is necessary for squalene synthase inhibitors. Selected 1-neopentyl compounds (**1a** and **2q**,**t**,**u**) which exhibited potent inhibitory activity at enzyme level (IC_{50} = 18–24 nM) were assayed for their ability to inhibit cholesterol synthesis in HepG2 cells (Table 5). The 2′-chloro compound **1a** exhibited only moderate inhibitory activity, with an IC_{50} value of 3.2 μM, despite potent activity (IC_{50} = 24 nM) at the enzyme level. The two-order difference in potency between the enzyme and cell assay might be due to cell permeability. Replacement of the chloro atom in compound **1a** with a methoxy group to yield compound **2q** resulted in approximately 3-fold increase in activity. Furthermore, introduction of an additional methoxy group into the 3′- or 4′-position gave rise to the much more potent inhibitors **2t**,**u**, with IC_{50} values of 0.12 and 0.40 μM, respectively. Compound **2t** was thus found to be the most potent inhibitor at cell and enzyme levels.

In vivo activity

In vivo activities in rats and marmosets were studied using the sodium salt **20**, because of its good oral bio-

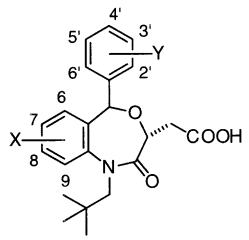
Table 2. Physicochemical and biological properties of (3,5-*trans*)-7-chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine derivatives **1c** and **3a**–**d**



Compd	R	Yield[a] (%)	Mp (°C)	Formula[b]	IC_{50} (μM)[c] (rat enzyme)
3a	COOH	48	166–167	$C_{21}H_{21}Cl_2NO_4$	3.7
3b	CH_2CH_2COOH	94	225–227	$C_{23}H_{25}Cl_2NO_4$	3.1
3c	CH_2CONH_2	65	291–292	$C_{22}H_{24}Cl_2N_2O_3$	0.20
3d	CH_2CHO	64	173–176	$C_{22}H_{23}Cl_2NO_3$	>10 (39.3)[d]
1c[1]	CH_2COOEt				3.8

[a]Yield of final step.
[b]Analysis for C, H, N were correct within ±0.4%.
[c]IC_{50} values were determined by a single experiment run in duplicate.
[d]%Inhibition at 10 μM.

Table 3. Physicochemical and biological properties of (3,5-*trans*)-1-neopentyl-2-oxo-5-phenyl-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetic acid derivatives **2m**–**y**



Compd	X	Y	Yield[a] (%)	Mp (°C)	Formula[b]	IC_{50} (μM)[c]	
						Rat enzyme	HepG2 enzyme
2m [a mixture of *cis* and *trans* (1:1)]	6-Cl	2′–Cl	74	223–224	$C_{22}H_{23}Cl_2NO_4$	>10 (24.5)[d]	—[e]
2n	8-Cl	2′-Cl	63	186–187	$C_{22}H_{23}Cl_2NO_4$	>10 (41.9)[d]	—[e]
2o	7-Cl	H	72	247–248	$C_{22}H_{24}ClNO_4$	0.76	0.55
2p	7-Cl	2′-Br	84	251–253	$C_{22}H_{23}BrClNO_4$	0.027	0.019
2q	7-Cl	2′-OMe	64	167–168	$C_{23}H_{26}ClNO_5$	0.028	0.023
2r	7-Cl	2′-OMe, 4′-F	97	237–238	$C_{23}H_{25}ClFNO_5$	0.044	0.027
2s	7-Cl	2′-OMe, 5′-F	88	223–224	$C_{23}H_{25}ClFNO_5$	2.0	—[e]
2t	7-Cl	2′,3′-diOMe	76	244–247	$C_{24}H_{28}ClNO_6$	0.038	0.018
2u	7-Cl	2′,4′-diOMe	90	260–263	$C_{24}H_{28}ClNO_6$	0.049	0.020
2v	7-Cl	2′,5′-diOMe	77	230–232	$C_{24}H_{28}ClNO_6$	>10 (33.7)[d]	—[e]
2w	7-Cl	2′,6′-diOMe	79	263–270 (dec.)	$C_{24}H_{28}ClNO_6$ 3/10H_2O	1.2	—[e]
2x	7-Cl	2′,4′,6′-triOMe	92	260–270 (dec.)	$C_{25}H_{30}ClNO_7$	0.32	—[e]
2y	7-Cl	2′-OMe, 3′-Me	85	230–231	$C_{24}H_{28}ClNO_5$	0.18	0.093

[a]Yield of final step.
[b]Analysis for C, H, N were correct within ±0.4%.
[c]IC_{50} values were determined by a single experiment run in duplicate.
[d]% Inhibition at 10 μM.
[e]Not tested.

availability (BA). The BAs of sodium salt **20** and free acid **19a** in rats (non-fasted state) were 87 and 16%, respectively.

The inhibition by compound **20** of cholesterogenesis from [^{14}C] acetate at 1 h after oral dosing was examined in Wistar rats. The ED_{50} value was 3.0 mg/kg.

Compound **20** was administered orally to marmosets at a dose of 50 mg/kg/day for 4 days and found to decrease plasma total cholesterol and non-HDL cholesterol levels by 32 and 64%, respectively, compared to control (Fig. 3). Based on these responses in marmosets, a primate whose lipoprotein profile is similar to that of humans, efficacy in humans is expected.

Conclusions

We performed chemical modification of 4,1-benzoxazepine derivatives and evaluated squalene synthase inhibitory activity. Compounds **1a** and **2q**,**t**,**u** exhibited potent inhibition of rat and human squalene synthases. Among these, **2t** was the most effective inhibitor of cholesterol synthesis in HepG2 cells. The absolute stereochemistry required for inhibitory activity was determined to be 3*R*,5*S*. The sodium salt of the (3*R*,5*S*)-7-chloro-5-(2,3-dimethoxyphenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetic acid **20** effectively reduced plasma cholesterol by oral administration in marmosets. This compound is one of the hopeful candidates for a cholesterol-lowering and anti-atherosclerotic agent. Further modification of 4,1-benzoxazepine derivatives will be reported.

Table 4. Inhibition of squalene synthase by compounds **19a** and **19b**

Compd	Stereochemistry	IC_{50} (μM)[a]	
		Rat enzyme	HepG2 enzyme
19a	3*R*,5*S*	0.025	0.013
19b	3*S*,5*R*	7.7	3.9

[a]IC_{50} values were determined by a single experiment run in duplicate.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrectad. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian GEMINI-200 (200 MHz) spectrometer (with tetramethylsilane as an internal standard). Infrared (IR) absorption spectra were recorded on a JASCO IR-810. $[\alpha]_D$ values were determined in the indicated solvents on a JASCO DIP-370 polarimeter.

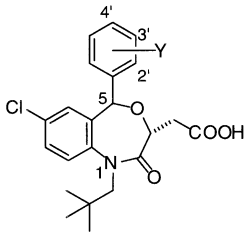
TLC analyses were carried out on Merck Kieselgel 60 F_{254} plates. Elemental analyses were carried out by Takeda Analytical Laboratories, Ltd., and are within ±0.4% of the theoretical values unless otherwise noted. For column chromatography, Merck Kieselgel 60 (70–230 mesh ASTM) was used. Yields were not maximized. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

2-Aminobenzophenone derivatives (4; Table 6).[11]

Method A[12]. A solution of Grignard reagent in THF (15 mL) prepared from 2,3-dimethoxybromobenzene[13] (1.5 g, 6.9 mmol) and Mg (0.17 g, 6.9 mmol) was added dropwise to a solution of 6-chloro-2-methyl-4*H*-3,1-benzoxazin-4-one (2 g, 10.2 mmol) in THF (15 mL) at the rate of 0.25 mL a minute with ice-cooling. After being stirred for 15 min at room temperature, the reaction was quenched with 1N HCl (10 mL). The mixture was extracted with AcOEt, washed with water, dried over Na_2SO_4 and then concentrated. The residue was dissolved in EtOH (20 mL). After addition of 6N HCl (19 mL), the solution was refluxed overnight, neutralized with 1*N* NaOH and extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4, and then concentrated. The residue was chromatographed [eluent: hexane–AcOEt (3:1)] to give **4i** (0.58 g, 2.0 mmol, 20%) as yellow prisms. Mp 91–95 °C. IR ν_{max} (KBr) cm^{-1}: 3470, 3340 (NH), 1635 (C=O). ^1H NMR ($CDCl_3$) δ 3.78 (3H, s), 3.92 (3H, s), 6.39 (2H, br), 6.63–7.27 (6H, m). Anal. ($C_{15}H_{14}ClNO_3$) C, H, N. **4b**,[12] **c**,**e**,[14] **f**,[12] **g**,**h**,**j** were prepared in a similar procedure. **4a**, **d** were commercially available.

Method B. *n*-BuLi hexane solution (1.6 M, 180 mL, 0.29 mol) was added to a solution of veratrol (52 g, 0.376 mol) in THF (200 mL) at the rate of 20 mL a minute with ice-cooling. The mixture was stirred for 30 min and a yellow solid was precipitated. The suspension was added to a solution of 6-chloro-2-methyl-4*H*-3,1-benzoxazin-4-one (49 g, 0.251 mol) in THF (200 mL) at 0 °C. The mixture was stirred for 30 min at 0 °C and concentrated under reduced pressure. The residue was dissolved in EtOH (250 mL) and water (100 mL) and concentrated HCl (140 mL) was added. After being refluxed for 3 h, the mixture was diluted with water (200 mL) and extracted with Et_2O (300 mL×3). The extracts were washed with 1N NaOH and brine, and then concentrated. The residue was dissolved in CH_2Cl_2 (200 mL), dried over Na_2SO_4 and then concentrated under reduced pressure. The residue was chromatographed [eluent: hexane–AcOEt (4:1)] and crystallized with Et_2O–petroleum ether (1:1) to give **4i** (27 g, 92.6 mmol, 37%) as yellow plates. **4k**–**n** were synthesized in a similar procedure.

Table 5. Effect of selected compounds on cholesterol synthesis from [^{14}C] mevalonate in HepG2 cell



Compd	Y	Cholesterol synthesis IC_{50} (μM)[a]	HepG2 enzyme IC_{50} (μM)[b]
1a	2′-Cl	3.2	0.024
2q	2′-OMe	0.91	0.023
2t	2′,3′-diOMe	0.12	0.018
2u	2′,4′-diOMe	0.40	0.020

[a]IC_{50} values were determined by a single experiment run in triplicate.
[b]IC_{50} values were determined by a single experiment run in duplicate.

2-Amino-α-phenylbenzyl alcohol derivatives (5a–n; Table 7). $NaBH_4$ (1.5 g, 39.5 mmol) was added to a suspension of **4i** (5.6 g, 19.2 mmol) in EtOH (150 mL). The mixture was stirred for 4 h, diluted with AcOEt, washed with brine, dried over Na_2SO_4 and then concentrated under reduced pressure to give **5i** (4.9 g, 16.7 mmol, 87%) as colorless powder. Mp 124–126 °C. IR ν_{max} (KBr) cm^{-1}: 3410, 3330 (NH), 3400–3200 (br, OH). ^1H NMR ($CDCl_3$) δ 3.17 (1H, br), 3.83 (3H, s), 3.88 (3H, s), 4.21 (2H, br), 6.03 (1H, br), 6.57-7.11 (6H, m). Anal. ($C_{15}H_{16}ClNO_3$) C, H, N. **5a**,[15] **b**–**h**,**j**–**n** were prepared in a similar procedure.

2-Acetylamino-5-chloro-α-(2-chlorophenyl)benzyl acetate (10a). Ac_2O (8.0 g, 78.1 mmol) and DMAP (0.4 g) was added to a solution of **5a** (9.5 g, 35.4 mmol) in pyridine (100 mL). The mixture was stirred overnight at room temperature. The solvent was removed, and the

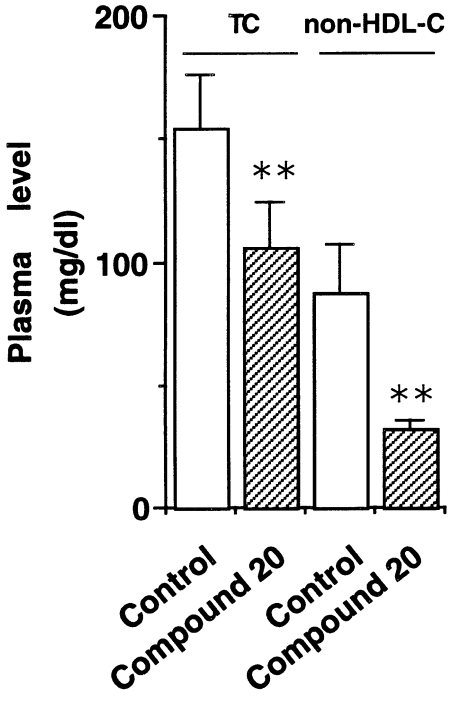


Figure 3. Effect of oral administration (50 mg/kg/day, po, 4 days) of the compound **20** on serum cholesterol levels in common marmosets (male, 19–62 M old). Values are mean ± S.D. ($n = 5$). **$p < 0.01$ vs the control value. TC, total cholesterol; non-HDL-C, non-HDL cholesterol; HDL, high-density lipoprotein.

residue was dissolved in AcOEt. The solution was washed with 1 *N* HCl, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and then concentrated under reduced pressure. The residue was recrystallized from CH_2Cl_2–hexane (1:1) to give **10a** (12 g, 34.1 mmol, 96%) as colorless prisms. Mp 131–133 °C (CH_2Cl_2–hexane). IR ν_{max}(KBr) cm^{-1}: 1735, 1650 (C=O). ^1H NMR ($CDCl_3$) δ 2.20 (3H, s), 2.25 (3H, s), 7.02–7.86 (8H, m), 8.72 (1H, br). Anal. ($C_{17}H_{15}Cl_2NO_3$) C, H, N.

2-Acetyl(methyl)amino-5-chloro-α-(2-chlorophenyl)benzyl acetate (10b). NaH (38 mg, 1.57 mmol) was added to a mixture of **10a** (0.5 g, 1.42 mmol) and MeI (0.22 g, 1.57 mmol) in DMF (5 mL). After stirring for 30 min at room temperature, diluted with AcOEt, washed with water, 5% $KHSO_4$, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and then concentrated under reduced pressure. The residue was chromatographed [eluent: hexane–AcOEt (3:1)] to give **10b** (0.52 g, 1.42 mmol, quant) as a colorless oil. IR ν_{max}(KBr) cm^{-1}: 1747, 1666 (C=O). ^1H NMR ($CDCl_3$) δ 1.21 (1/2×3H, s), 1.88 (1/2×3H, s), 2.16 (1/2×3H, s), 2.17 (1/2×3H, s), 2.75 (1/2×3H, s), 3.27 (1/2×3H, s), 7.07–7.56 (8H, m).

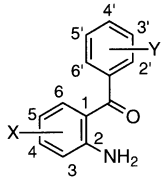
2-Acetyl(ethyl)amino-5-chloro-α-(2-chlorophenyl)benzyl acetate (10c). Compound **10c** (0.54 g, 1.42 mmol, quant) was prepared from **10a** (0.5 g, 1.42 mmol) and EtI (0.24 g, 1.57 mmol) in a similar manner as described for the preparation of **10b** as a colorless oil. IR ν_{max}(KBr) cm^{-1}: 1747, 1666 (C=O). ^1H NMR ($CDCl_3$) δ 1.00 (1/2×3H, t, J = 7.4 Hz), 1.08 (1/2×3H, s), 1.15 (1/2×3H, t, J = 7.4 Hz), 1.86 (1/2 3H, s), 2.14 (1/2×3H, s), 2.17 (1/2×3H, s), 2.31–2.48 (1/2×1H, m), 3.11–3.28 (1/2×1H, m), 4.00–4.18 (1/2×1H, m), 4.39–4.56 (1/2×1H, m), 7.02–7.62 (8H, m).

2-Acylamino-5-chloro-α-(2-chlorophenyl)benzyl alcohol derivatives.[11] A mixture of **5a** (2.0 g, 7.46 mmol), $NaHCO_3$ (0.94 g, 11.2 mmol) and CH_2Cl_2 (50 mL) was added to a solution of cyclopropanecarbonyl chloride (0.82 g, 7.83 mmol) in CH_2Cl_2 (10 mL) at 0 °C at the rate of 0.5 mL a minute. The reaction mixture was stirred at room temperature for 30 min, washed with water, dried over Na_2SO_4, and then concentrated under reduced pressure to give **11a** (2.1 g, 6.25 mmol, 84%) as a colorless powder. **11b** was prepared in a similar procedure. **11a**: mp 115–116 °C. Anal. ($C_{17}H_{15}Cl_2NO_2$) C, H, N. **11b**: mp 109–110 °C. Anal. ($C_{19}H_{21}Cl_2NO_2$) C, H, N.

***N*-Substituted 2-amino-5-chloro-α-phenylbenzyl alcohol derivatives (6a–y; Table 7).** $NaBH_3CN$ (2.0 g, 31.7 mmol) was added to an ice-cooled solution of 2-amino-5-chloro-α-(2,3-dimethoxyphenyl)benzyl alcohol **5i** (6.6 g, 22.5 mmol), pivalaldehyde (2.1 g, 23.9 mmol) and AcOH (3.8 g, 63.4 mmol) in MeOH (70 mL). After being stirred for 1 h at room temperature, the reaction was quenched with 5% $KHSO_4$. The mixture was extracted with AcOEt (100 ml). The extract was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and then concentrated in vacuo to give **6t** (8.2 g, 22.5 mmol, quant) as a pale yellow prisms. Mp 120–121 °C. IR ν_{max} (KBr) cm^{-1}: 3550, (NH), 3500–3200 (br, OH). ^1H NMR ($CDCl_3$) δ 0.93 (9H, s), 2.82 (2H, s), 3.23 (1H, br), 3.83 (3H, s), 3.89 (3H, s), 4.86 (1H, br), 5.99 (1H, s), 6.57–7.13 (5H, m). Anal. ($C_{20}H_{26}ClNO_3$) C, H, N. **6a**,[15] **d–g**,**i–k**,**m–s**,**u–y** were prepared in a similar procedure.

A mixture of **10b** (0.52 g, 1.42 mmol), 1 N NaOH (6 mL) and EtOH (6 mL) was refluxed for 1 h. The reaction mixture was diluted with AcOEt, washed with water

Table 6. Physicochemical properties of 2-aminobenzophenone derivatives **4a–n**



Compd	X	Y	Method	Yield (%)	Mp (°C)	Formula[a]
4a	5-Cl	2′-Cl	(commercially available)			
4b[12]	6-Cl	2′-Cl	A			
4c	4-Cl	2′-Cl	A	78	112–113	$C_{13}H_9Cl_2NO$
4d	5-Cl	H	(commercially available)			
4e[14]	5-Cl	2′-Br	A			
4f[12]	4-Cl	2′-OMe	A			
4g	5-Cl	2′-OMe,4′-F	A	86	106–107	$C_{14}H_{11}ClFNO_2$
4h	5-Cl	2′-OMe,5′-F	A	96	89–90	$C_{14}H_{11}ClFNO_2$
4i	5-Cl	2′,3′-diOMe	A	20	91–95	$C_{15}H_{14}ClNO_3$
			B	37		
4j	5-Cl	2′,4′-diOMe	A	Quant.	102-103	$C_{15}H_{14}ClNO_3$
4k	5-Cl	2′,5′-diOMe	B	22	oil	$C_{15}H_{14}ClNO_3$
4l	5-Cl	2′,6′-diOMe	B	32	172	$C_{15}H_{14}ClNO_3$
4m	5-Cl	2′,4′,6′-triOMe	B	90	188-189	$C_{16}H_{16}ClNO_4$
4n	5-Cl	2′-OMe,3′-Me	B	78	80–81	$C_{15}H_{14}ClNO_2$

[a]Analysis for C, H, N were correct within ±0.4% except for oily compounds.

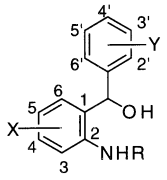
and brine, dried over Na_2SO_4, and then concentrated under reduced pressure. The residue was chromatographed [eluent: hexane–AcOEt (10:1)] to give **6b** (0.38 g, 1.35 mmol, 95%) as a colorless oil. **6c** was similarly synthesized from **10c**.

6b: IR ν_{max}(KBr) cm^{-1}: 3600–3200 (br, NH, OH). ^1H NMR ($CDCl_3$) δ 2.85 (3H, s), 6.12 (1H, s), 6.61 (1H, d, $J=8.8$ Hz), 6.86 (1H, d, $J=2.6$ Hz), 7.18 (1H, dd, $J=2.6$, 8.8 Hz), 7.26–7.48 (4H, m). **6c**: IR ν_{max}(KBr) cm^{-1}: 3600–3200 (br, NH, OH). ^1H NMR ($CDCl_3$) δ: 1.24 (3H, t, $J=7.6$ Hz), 3.13 (2H, q, $J=7.6$ Hz), 6.13 (1H, s), 6.60 (1H, d, $J=8.8$ Hz), 6.84 (1H, d, $J=2.2$ Hz), 7.15 (1H, dd, $J=2.2$, 8.8 Hz), 7.26–7.51 (4H, m).

A solution of **11a** (2.8 g, 8.33 mmol) in Et_2O (20 mL) was added to a suspension of $LiAlH_4$ (0.3 g) in Et_2O (30 mL) over a period of 30 min. The reaction mixture was stirred for 2 h at room temperature. After cooling with ice-bath, the reaction was quenched with water (0.3 mL), 15% NaOH (0.3 mL) and water (1 mL). The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was chromatographed [eluent: hexane–AcOEt (3:1)] to give **6h** (2.1 g, 6.52 mmol, 78%) as a colorless oil. ^1H NMR ($CDCl_3$) δ: 0.1–1.3 (5H, m), 2.92 (2H, d, $J=6.8$ Hz), 6.16 (1H, s), 6.57–7.60 (7H, m). **6l** was similarly synthesized from **11b**.

Ethyl 3-[*N*-[4-chloro-2-(α-hydroxybenzyl)phenyl]carbamoyl]acrylate (8a–y; Table 8). A solution of fumaric acid chloride monoethyl ester **7** (2.0 g, 12.3 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a solution of **6t** (3.9 g, 10.7 mmol) and $NaHCO_3$ (2.5 g, 30 mmol) in CH_2Cl_2 (100 mL). The reaction mixture was stirred for 2 h at room temperature and filtered. The filtrate was

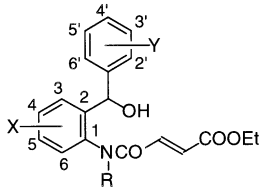
Table 7. Physicochemical properties of 2-amino-α-phenylbenzyl alcohol derivatives **5a–n** and **6a–y**



Compd	X	Y	R	Yield (%)	Mp (°C)	Formula[a]
5a[15]	5-Cl	2′-Cl	H			
5b	6-Cl	2′-Cl	H	94	70–71	$C_{13}H_{11}Cl_2NO$
5c	4-Cl	2′-Cl	H	Quant.	Oil	$C_{13}H_{11}Cl_2NO$
5d	5-Cl	H	H	98	107–109	$C_{13}H_{12}ClNO$
5e	5-Cl	2′-Br	H	83	87–88	$C_{13}H_{11}BrClNO$
5f	5-Cl	2′-OMe	H	87	81–82	$C_{14}H_{14}ClNO_2$
5g	5-Cl	2′-OMe,4′-F	H	98	oil	$C_{14}H_{13}ClFNO_2$
5h	5-Cl	2′-OMe,5′-F	H	92	118–119	$C_{14}H_{13}ClFNO_2$
5i	5-Cl	2′,3′-diOMe	H	87	124–126	$C_{15}H_{16}ClNO_3$
5j	5-Cl	2′,4′-diOMe	H	99	127–128	$C_{15}H_{16}ClNO_3$
5k	5-Cl	2′,5′-diOMe	H	85	177–178	$C_{15}H_{16}ClNO_3$
5l	5-Cl	2′,6′-diOMe	H	72	130–135	$C_{15}H_{16}ClNO_3$
5m	5-Cl	2′,4′,6′-triOMe	H	96	163–164	$C_{16}H_{18}ClNO_4$
5n	5-Cl	2′-OMe,3′-Me	H	95	114–115	$C_{15}H_{16}ClNO_2$
6a[15]	5-Cl	2′-Cl	CH_2Ph			
6b	5-Cl	2′-Cl	Me	95	Oil	$C_{14}H_{13}Cl_2NO$
6c	5-Cl	2′-Cl	Et	93	Oil	$C_{15}H_{15}Cl_2NO$
6d	5-Cl	2′-Cl	Pr	85	Oil	$C_{16}H_{17}Cl_2NO$
6e	5-Cl	2′-Cl	Bu	75	Oil	$C_{17}H_{19}Cl_2NO$
6f	5-Cl	2′-Cl	Pr	96	Oil	$C_{16}H_{17}Cl_2NO$
6g	5-Cl	2′-Cl	CH_2CHEt_2	Quant.	Oil	$C_{19}H_{23}Cl_2NO$
6h	5-Cl	2′-Cl	CH_2-cyclopropyl	78	Oil	$C_{17}H_{17}Cl_2NO_2$
6i	5-Cl	2′-Cl	CH_2-cyclohexyl	91	91–92	$C_{20}H_{23}Cl_2NO$
6j	5-Cl	2′-Cl	$CHEt_2$	87	Oil	$C_{18}H_{21}Cl_2NO$
6k	5-Cl	2′-Cl	$CH_2CH_2CHMe_2$	97	Oil	$C_{18}H_{21}Cl_2NO$
6l	5-Cl	2′-Cl	$CH_2CH_2CMe_3$	83	Oil	$C_{19}H_{23}Cl_2NO$
6m	6-Cl	2′-Cl	CH_2CMe_3	79	Oil	$C_{18}H_{21}Cl_2NO$
6n	4-Cl	2′-Cl	CH_2CMe_3	Quant.	Oil	$C_{18}H_{21}Cl_2NO$
6o	5-Cl	H	CH_2CMe_3	Quant.	Oil	$C_{18}H_{22}ClNO$
6p	5-Cl	2′-Br	CH_2CMe_3	79	99–100	$C_{18}H_{21}BrClNO$
6q	5-Cl	2′-OMe	CH_2CMe_3	84	Oil	$C_{19}H_{24}ClNO_2$
6r	5-Cl	2′-OMe,4′-F	CH_2CMe_3	98	Oil	$C_{18}H_{23}ClFNO_2$
6s	5-Cl	2′-OMe,5′-F	CH_2CMe_3	98	Oil	$C_{18}H_{23}ClFNO_2$
6t	5-Cl	2′,3′-diOMe	CH_2CMe_3	Quant.	120–121	$C_{20}H_{26}ClNO_3$
6u	5-Cl	2′,4′-diOMe	CH_2CMe_3	83	86-87	$C_{20}H_{26}ClNO_3$
6v	5-Cl	2′,5′-diOMe	CH_2CMe_3	64	126-128	$C_{20}H_{26}ClNO_3$
6w	5-Cl	2′,6′-diOMe	CH_2CMe_3	quant.	oil	$C_{20}H_{26}ClNO_3$
6x	5-Cl	2′,4′,6′-triOMe	CH_2CMe_3	72	150-151	$C_{21}H_{28}ClNO_4$
6y	5-Cl	2′-OMe,3′-Me	CH_2CMe_3	73	130-131	$C_{20}H_{26}ClNO_2$ 1/4H_2O

[a]Analysis for C, H, N were correct within ±0.4% except for oily compounds.

Table 8. Physicochemical properties of ethyl 3-[*N*-[2-(hydroxymethyl)phenyl]carbamoyl]acrylate derivatives **8a–y**



Compd	X	Y	R	Yield (%)	Mp (°C)	Formula[a]
8a[15]	4-Cl	2′-Cl	CH_2Ph			
8b	4-Cl	2′-Cl	Me	85	128–130	$C_{20}H_{19}Cl_2NO_4$ 1/4H_2O
8c	4-Cl	2′-Cl	Et	Quant.	Oil	$C_{21}H_{21}Cl_2NO_4$
8d	4-Cl	2′-Cl	Pr	78	Oil	$C_{22}H_{23}Cl_2NO_4$
8e	4-Cl	2′-Cl	Bu	96	Oil	$C_{23}H_{25}Cl_2NO_4$
8f	4-Cl	2′-Cl	Pr	87	180-182	$C_{22}H_{23}Cl_2NO_4$
8g	4-Cl	2′-Cl	CH_2CHEt_2	82	Oil	$C_{25}H_{29}Cl_2NO_4$
8h	4-Cl	2′-Cl	CH_2-cyclopropyl	79	Oil	$C_{23}H_{23}Cl_2NO_4$
8i	4-Cl	2′-Cl	CH_2-cyclohexyl	77	Oil	$C_{26}H_{29}Cl_2NO_4$
8j	4-Cl	2′-Cl	$CHEt_2$	82	Oil	$C_{24}H_{27}Cl_2NO_4$
8k	4-Cl	2′-Cl	$CH_2CH_2CHMe_2$	92	Oil	$C_{24}H_{27}Cl_2NO_4$
8l	4-Cl	2′-Cl	$CH_2CH_2CMe_3$	Quant.	Oil	$C_{25}H_{29}Cl_2NO_4$
8m	3-Cl	2′-Cl	CH_2CMe_3	88	Oil	$C_{24}H_{27}Cl_2NO_4$
8n	5-Cl	2′-Cl	CH_2CMe_3	90	152–153	$C_{24}H_{27}Cl_2NO_4$
8o	4-Cl	H	CH_2CMe_3	86	Oil	$C_{24}H_{28}ClNO_4$
8p	4-Cl	2′-Br	CH_2CMe_3	93	168–169	$C_{24}H_{27}BrClNO_4$
8q	4-Cl	2′-OMe	CH_2CMe_3	96	Oil	$C_{25}H_{30}ClNO_5$
8r	4-Cl	2′-OMe,4′-F	CH_2CMe_3	97	Oil	$C_{25}H_{29}ClFNO_5$
8s	4-Cl	2′-OMe,5′-F	CH_2CMe_3	88	Oil	$C_{25}H_{29}ClFNO_5$
8t	4-Cl	2′,3′-diOMe	CH_2CMe_3	92	127–129	$C_{26}H_{32}ClNO_6$
8u	4-Cl	2′,4′-diOMe	CH_2CMe_3	99	137–139	$C_{26}H_{32}ClNO_6$
8v	4-Cl	2′,5′-diOMe	CH_2CMe_3	91	140–142	$C_{26}H_{32}ClNO_6$
8w	4-Cl	2′,6′-diOMe	CH_2CMe_3	68	Oil	$C_{26}H_{32}ClNO_6$
8x	4-Cl	2′,4′,6′-triOMe	CH_2CMe_3	95	Oil	$C_{27}H_{34}ClNO_7$
8y	4-Cl	2′-OMe,3′-Me	CH_2CMe_3	57	Oil	$C_{26}H_{32}ClNO_5$

washed with water, dried over Na_2SO_4, and then concentrated under reduced pressure to give **8t** (4.8 g, 9.8 mmol, 92%) as colorless prisms. Mp 127–129 °C. IR ν_{max} (KBr) cm^{-1}: 3470 (OH); 1725, 1660, 1630 (C=O, C=C). ^1H NMR ($CDCl_3$) δ 0.89 (9H, s), 1.24 (3H, t, $J=7.0$ Hz), 2.81 (1H, d, $J=13.4$ Hz), 3.72 (3H, s), 3.88 (3H, s), 4.15 (2H, q, $J=7.0$ Hz), 4.42 (1H, d, $J=13.4$ Hz), 6.02 (1H, s), 6.78–7.31 (8H, m). Anal. ($C_{26}H_{32}ClNO_6$) C, H, N. The compounds **8a**,[15] **b–s,u–y** were synthesized by acylation similar to that for the preparation of **8t**.

Ethyl (3,5-*trans*)-7-chloro-2-oxo-5-phenyl-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetate derivatives (9a–y; Table 9). A mixture of **8t** (7.2 g, 14.7 mmol) and K_2CO_3 (2 g) in EtOH (100 mL) was stirred for 24 h. The reaction mixture was diluted with AcOEt, washed with water, dried over Na_2SO_4, and then concentrated. The residue was subjected to column chromatography [eluent:hexane–AcOEt (3:1)] and recrystallized from AcOEt–hexane (1:5) to give **9t** (6.5 g, 13.3 mmol, 90%) as colorless prisms. Mp 184–185 °C. IR ν_{max} (KBr) cm^{-1}: 1720, 1680 (C=O). ^1H NMR ($CDCl_3$) δ: 0.95 (9H, s), 1.24 (3H, t, $J=7.2$ Hz), 2.77 (1H, dd, $J=16.4$, 5.8 Hz), 3.04 (1H, dd, $J=16.4$, 7.6 Hz), 3.37 (1H, d, $J=14.0$ Hz), 3.63 (3H, s), 3.89 (3H, s), 4.13 (2H, dq, $J=7.2$, 1.8 Hz), 4.39 (1H, dd, $J=7.6$, 5.8 Hz), 4.52 (1H, d, $J=14.0$ Hz), 6.28 (1H, s), 6.62 (1H, s), 6.96–7.33 (5H, m). Anal. ($C_{26}H_{32}ClNO_6$) C, H, N. **9a**,[15] **b–s,u–y** were prepared from **8a–s,u–y** in a similar procedure.

1-Substituted (3,5-*trans*)-7-chloro-2-oxo-5-phenyl-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetic acid derivatives (2a–y; Tables 1 and 3). A mixture of **9t** (2.5 g, 5.10 mmol), K_2CO_3 (2.8 g, 20.4 mmol), MeOH (70 mL) and water (10 mL) was stirred overnight at room temperature. The reaction mixture was diluted with water, acidified, extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4, and then concentrated under reduced pressure to give **2t** (1.8 g, 3.9 mmol, 76%) as colorless needles. Mp 244–247 °C. IR ν_{max} (KBr) cm^{-1}: 3600–2200 (br, COOH), 1740, 1650 (C=O). ^1H NMR ($CDCl_3$) δ: 0.95 (9H, s), 2.84 (1H, dd, $J=16.6$, 5.6 Hz), 3.08 (1H, dd, $J=16.6$, 7.4 Hz), 3.39 (1H, d, $J=13.8$ Hz), 3.63 (3H, s), 3.89 (3H, s), 4.34 (1H, dd, $J=7.4$, 5.6 Hz), 4.53 (1H, d, $J=13.8$ Hz), 6.28 (1H, s), 6.64 (1H, d, $J=1.6$ Hz), 6.97–7.36 (5H, m). Anal. ($C_{24}H_{28}ClNO_6$) C, H, N. **2a–s,u–y** were synthesized in a similar manner.

(3,5-*trans*)-3-(Aminomethyl)-7-chloro-5-(2-chlorophenyl)-1-neopentyl-1,5-dihydro-4,1-benzoxazepine-2(3*H*)-one hydrochloride (12). DPPA (0.74 mL, 3.44 mmol) and Et_3N (0.48 mL, 3.44 mmol) was added to a solution of **1a** (1.0 g, 2.29 mmol) in DMF (20 mL) at 0 °C. The mixture was stirred for 1 h at room temperature, diluted with water and extracted with AcOEt. The extract was washed with 5% $KHSO_4$ and saturated $NaHCO_3$, dried over $MgSO_4$ and then concentrated. The residue in benzene (100 mL) was refluxed for 1 h. The solvent was removed under reduced pressure. A mixture of the

residue, 6N HCl (50 mL) and dioxane (30 mL) was refluxed for 20 min and then concentrated in vacuo. The residue was recrystallized from EtOH–hexane to give **12** (0.87 g, 1.96 mmol, 86%) as colorless plates.

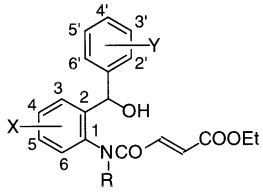
Mp 173–175 °C. IR ν_{max} (KBr) cm^{-1}: 1665 (C=O). ^1H NMR (DMSO-d_6) δ 0.88 (9H, s), 3.0–3.3 (2H, m), 3.68 (1H, d, $J=13.8$ Hz), 4.2–4.3 (1H, m), 4.31 (1H, d, $J=13.8$ Hz), 6.17 (1H, s), 6.36 (1H, d, $J=2.6$ Hz), 7.5–8.1 (6H, m). Anal. ($C_{21}H_{24}Cl_2N_2O_2 \cdot HCl \cdot H_2O$) C, H, N.

(3,5-*trans*)-7-Chloro-5-(2-chlorophenyl)-3-(hydroxymethyl)-1-neopentyl-1,5-dihydro-4,1-benzoxazepin-2(3*H*)-one (13). A solution of $NaNO_2$ (2.0 g) in water (2 mL) was added dropwise to a mixture of **12** (4.0 g, 9.01 mmol), AcOH (24 mL) and water (35 mL) at 0 °C. The mixture was stirred for 2 h at room temperature, poured into water and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was chromatographed [eluent: hexane–AcOEt (4:1)] to give **13** (0.85 g, 2.08 mmol, 23%) as colorless prisms. Mp 169–170 °C. IR ν_{max} (KBr) cm^{-1}: 1670 (C=O). ^1H NMR ($CDCl_3$) δ 0.95 (9H, s), 3.38 (1H, d, $J=13.8$ Hz), 3.8–4.1 (3H, m), 4.55 (1H, d, $J=13.8$ Hz), 6.27 (1H, s), 6.56 (1H, d, $J=2.2$ Hz), 7.3–7.9 (6H, m). Anal. ($C_{21}H_{23}Cl_2NO_3$) C, H, N.

(3,5-*trans*)-7-Chloro-5-(2-chlorophenyl)-3-(2-hydroxyethyl)-1-neopentyl-1,5-dihydro-4,1-benzoxazepin-2(3*H*)-one (14). *N*-Methylmorpholine (4.26 mL, 38.8 mmol) and ClCOOEt (3.71 mL, 38.8 mmol) was added dropwise to a solution of **1a** (14 g, 32.1 mmol) in THF (150 mL) at 0 °C. After stirring for 15 min, $NaBH_4$ (4.1 g, 96.9 mmol) and MeOH (150 mL) were added to the mixture. After being stirred for 2 h at room temperature, the reaction was quenched with 1N HCl. The mixture was diluted with water and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and then concentrated. The residue was chromatographed [eluent: hexane–AcOEt (1:1)] and recrystallized from hexane–AcOEt to give **14** (8.6 g, 20.4 mmol, 64%) as colorless prisms. Mp 157–159 °C. IR ν_{max} (KBr) cm^{-1}: 3460 (OH), 1660 (C=O). ^1H NMR ($CDCl_3$) δ 0.94 (9H, s), 2.15 (2H, q, $J=5.8$ Hz), 2.25 (1H, t, $J=5.3$ Hz), 3.38 (1H, d, $J=14.0$ Hz), 3.71–3.90 (2H, m), 4.15 (1H, t, $J=6.2$ Hz), 4.52 (1H, d, $J=14.0$ Hz), 6.27 (1H, s), 6.53 (1H, d, $J=1.8$ Hz), 7.29–7.49 (5H, m), 7.70–7.77 (1H, m). Anal. ($C_{22}H_{25}Cl_2NO_3$) C, H, N.

(3,5-*trans*)-7-Chloro-3-(2-chloroethyl)-5-(2-chlorophenyl)-1-neopentyl-1,5-dihydro-4,1-benzoxazepin-2(3*H*)-one (15). A mixture of **14** (11 g, 26.0 mmol), $SOCl_2$ (3.65 g, 50 mmol), pyridine (50 mg, 0.63 mmol) and toluene (100 mL) was stirred for 30 min at 90 °C, poured into

Table 9. Physicochemical properties of 1-substituted ethyl (3,5-*trans*)-7-chloro-5-(2-chlorophenyl)-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetate derivatives **9 a–y**



Compd	X	Y	R	Yield (%)	Mp (°C)	Formula[a]
9a[15]	7-Cl	2′-Cl	CH_2Ph			
9b	7-Cl	2′-Cl	Me	68	147–178	$C_{20}H_{19}Cl_2NO_4$
9c	7-Cl	2′-Cl	Et	47	119–120	$C_{21}H_{21}Cl_2NO_4$
9d	7-Cl	2′-Cl	Pr*n*	81	Oil	$C_{22}H_{23}Cl_2NO_4$
9e	7-Cl	2′-Cl	Bu*n*	91	Oil	$C_{23}H_{25}Cl_2NO_4$
9f	7-Cl	2′-Cl	Pr*n*	93	Amorphous	$C_{22}H_{23}Cl_2NO_4$
9g	7-Cl	2′-Cl	CH_2CHEt_2	90	Oil	$C_{25}H_{29}Cl_2NO_4$
9h	7-Cl	2′-Cl	CH_2–cyclopropyl	77	106–107	$C_{23}H_{23}Cl_2NO_4$
9i	7-Cl	2′-Cl	CH_2–cyclohexyl	76	Amorphous	$C_{26}H_{29}Cl_2NO_4$
9j	7-Cl	2′-Cl	$CHEt_2$	30	140–141	$C_{24}H_{27}Cl_2NO_4$
9k	7-Cl	2′-Cl	$CH_2CH_2CHMe_2$	85	Oil	$C_{24}H_{27}Cl_2NO_4$
9l	7-Cl	2′-Cl	$CH_2CH_2CMe_3$	93	Oil	$C_{25}H_{29}Cl_2NO_4$
9m	6-Cl	2′-Cl	CH_2CMe_3	80	Oil	$C_{24}H_{27}Cl_2NO_4$
[a mixture of *cis* and *trans* (1:1)]						
9n	8-Cl	2′-Cl	CH_2CMe_3	86	142–143	$C_{24}H_{27}Cl_2NO_4$
9o	7-Cl	H	CH_2CMe_3	84	Oil	$C_{24}H_{28}ClNO_4$
9p	7-Cl	2′-Br	CH_2CMe_3	82	128–129	$C_{24}H_{27}BrClNO_4$
9q	7-Cl	2′-OMe	CH_2CMe_3	81	173–174	$C_{25}H_{30}ClNO_5$
9r	7-Cl	2′-OMe,4′-F	CH_2CMe_3	83	114–115	$C_{25}H_{29}ClFNO_5$
9s	7-Cl	2′-OMe,5′-F	CH_2CMe_3	86	153–154	$C_{25}H_{29}ClFNO_5$
9t	7-Cl	2′,3′-diOMe	CH_2CMe_3	90	184–185	$C_{26}H_{32}ClNO_6$
9u	7-Cl	2′,4′-diOMe	CH_2CMe_3	80	117–119	$C_{26}H_{32}ClNO_6$
9v	7-Cl	2′,5′-diOMe	CH_2CMe_3	78	165–166	$C_{26}H_{32}ClNO_6$
9w	7-Cl	2′,6′-diOMe	CH_2CMe_3	71	175–177	$C_{26}H_{32}ClNO_6$
9x	7-Cl	2′,4′,6′-triOMe	CH_2CMe_3	80	154–155	$C_{27}H_{34}ClNO_7$
9y	7-Cl	2′-OMe,3′-Me	CH_2CMe_3	88	190–191	$C_{26}H_{32}ClNO_5$

[a]Analysis for C, H, N were correct within ±0.4% except for oily compounds.

saturated $NaHCO_3$ and extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4, and then concentrated under reduced pressure. The residue was subjected to column chromatography [eluent: hexane–AcOEt (7:1)] to give **15** (7.0 g, 15.9 mmol, 61%) as colorless needles. Mp 182–184 °C. ^1H NMR ($CDCl_3$) δ 0.94 (9H, s), 2.13–2.50 (2H, m), 3.39 (1H, d, $J=14.0$ Hz), 3.63–3.84 (2H, m), 4.17 (1H, dd, $J=8.0$, 5.0 Hz), 4.52 (1H, d, $J=14.0$ Hz), 6.27 (1H, s), 6.53 (1H, d, $J=1.8$ Hz), 7.3–7.5 (5H, m), 7.7–7.77 (1H, m). Anal. ($C_{22}H_{24}Cl_3NO_2$) C, H, N.

(3,5-*trans*)-7-Chloro-5-(2-chlorophenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-propionitrile (16). A mixture of **15** (0.3 g, 0.681 mmol), NaCN (0.1 g) and DMSO (6 mL) was stirred for 1 h at 100 °C, diluted with water and extracted with AcOEt. The extract was washed with 5% $KHSO_4$, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was chromatographed [eluent: hexane–AcOEt (4:1)] to give **16** (0.25 g, 0.58 mmol, 85%) as colorless crystals. Mp 194–195 °C. IR ν_{max} (KBr) cm^{-1}: 2240 (CN), 1680 (C=O). ^1H NMR ($CDCl_3$) δ 0.94 (9H, s), 2.0–2.4 (2H, m), 2.59 (2H, t, $J=7.2$Hz), 3.38 (1H, d, $J=13.8$ Hz), 4.05 (1H, dd, $J=7.6$, 5.0 Hz), 4.51 (1H, d, $J=13.8$ Hz), 6.26 (1H, s), 6.54 (1H, d), 7.3–7.8 (6H, m). Anal. ($C_{23}H_{24}Cl_2N_2O_2$) C, H, N.

Ethyl (3,5-*trans*)-7-chloro-5-(2-chlorophenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-propionate (17). A mixture of **16** (0.2 g, 0.464 mmol), 6N HCl (3 mL) and EtOH (3 mL) was refluxed for 6 h, and concentrated under reduced pressure. Water was added to the residue and the resulting mixture was extracted with AcOEt. The extract was washed with saturated $NaHCO_3$, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was chromatographed [eluent:hexane–AcOEt (5:1)] to give **17** (0.18 g, 0.376 mmol, 81%) as colorless crystals. Mp 130–131 °C. IR ν_{max} (KBr) cm^{-1}: 1730, 1680 (C=O). ^1H NMR ($CDCl_3$) δ 0.94 (9H, s), 1.17 (3H, t, $J=7.2$ Hz), 2.0–2.6 (4H, m), 3.37 (1H, d, $J=14.0$ Hz), 3.95–4.11 (3H, m), 4.52 (1H, d, $J=14.0$ Hz), 6.26 (1H, s), 6.52 (1H, d, $J=2.2$ Hz), 7.2–7.8 (6H, m). Anal. ($C_{25}H_{29}Cl_2NO_4$) C, H, N.

(3,5-*trans*)-7-Chloro-5-(2-chlorophenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-carboxylic acid (3a; Table 2). Jones' reagent (1.0 mL) was added to a solution of **13** (0.5 g, 1.22 mmol) in acetone (10 mL). The mixture was stirred for 1.5 h at room temperature, concentrated, dissolved in AcOEt and washed with water. The solvent was removed under reduced pressure. The residue was dissolved in saturated $NaHCO_3$. The solution was washed with Et_2O, acidified and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and then concentrated to give **3a** (0.25 g, 0.592 mmol, 48%) as colorless crystals. Mp 166–167 °C. IR ν_{max} (KBr) cm^{-1}: 1750, 1670, 1630 (C=O). ^1H NMR ($CDCl_3$) δ 0.95 (9H, s), 3.43 (1H, d, $J=13.8$ Hz), 4.53 (1H, d, $J=13.8$ Hz), 4.59 (1H, s), 6.39 (1H, s), 6.59 (1H, d), 7.3–7.9 (6H, m). Anal. ($C_{21}H_{21}Cl_2NO_4$) C, H, N.

(3,5-*trans*)-7-Chloro-5-(2-chlorophenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-propionic acid (3b; Table 2). A mixture of **17** (90 mg, 0.188 mmol), 10% K_2CO_3 (2 mL) and MeOH (5 mL) was refluxed for 1 h, diluted with water, acidified, extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and then concentrated to give **3b** (80 mg, 0.178 mmol, 94%) as colorless crystals. Mp 225–227 °C. IR ν_{max} (KBr) cm^{-1}: 1700, 1680 (C=O). ^1H NMR ($CDCl_3$) δ 0.98 (9H, s), 2.0–2.7 (4H, m), 3.38 (1H, d, $J=14.0$ Hz), 3.96 (1H, dd, $J=7.2$, 5.8 Hz), 4.51 (1H, d, $J=14.0$ Hz), 6.24 (1H, s), 6.52 (1H, s), 7.2–7.8 (6H, m). Anal. ($C_{23}H_{25}Cl_2NO_4$) C, H, N.

(3,5-*trans*)-7-Chloro-5-(2-chlorophenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetamide (3c; Table 2). DEPC (0.46 g) and Et_3N (0.4 mL) was added to a ice-cooled solution of **1a** (1.0 g, 2.29 mmol), NH_4Cl (0.5 g) and Et_3N (0.5 mL) in DMF (8 mL). The mixture was stirred for 30 min at room temperature, poured into water and extracted with AcOEt. The extract was washed with 5% $KHSO_4$, saturated $NaHCO_3$ and water, dried over $MgSO_4$, and then concentrated under reduced pressure to give **3c** (0.65 g, 1.49 mmol, 65%) as colorless crystals. Mp 291–292 °C. IR ν_{max} (KBr) cm^{-1}: 3340, 3200 (NH_2), 1620 (C=O). ^1H NMR ($CDCl_3$) δ 0.96 (9H, s), 2.68 (1H, dd, $J=14.6$, 6.2 Hz), 2.88 (1H, dd, $J=14.6$, 7.0 Hz), 3.37 (1H, d, $J=14.0$ Hz), 4.39 (1H, dd, $J=7.0$, 6.2 Hz), 4.50 (1H, d, $J=14.0$ Hz), 5.37 (1H, br), 5.92 (1H, br), 6.28 (1H, s), 6.61 (1H, d, $J=1.8$ Hz), 6.96–7.33 (6H, m). Anal. ($C_{22}H_{24}Cl_2N_2O_3$) C, H, N.

(3,5-*trans*)-7-Chloro-5-(2-chlorophenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetaldehyde (3d; Table 2). A solution of DMSO (4.73 mL, 66.6 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a solution of $(COCl)_2$ (3.87 mL, 44.4 mmol) in CH_2Cl_2 (110 mL) at −78 °C. After stirring for 10 min at −78 °C, a solution of **14** (9.4 g, 22.3 mmol) in CH_2Cl_2 (60 mL) was added dropwise. The mixture was stirred for 1.5 h at −70 °C. After addition of Et_3N (15.5 mL, 0.11 mol) at −60 °C, the mixture was stirred for 5 min at −78 °C and for 3 h at room temperature. The mixture was poured into water and extracted with CH_2Cl_2. The extract was washed with water, dried over $MgSO_4$, and then concentrated. The residue was chromatographed [eluent: hexane–AcOEt (3:1)] and recrystallized from hexane-AcOEt to give **3d** (8.6 g, 20.5 mmol, 92%) as colorless prisms. Mp 173–176 °. IR ν_{max} (KBr) cm^{-1}: 1720, 1680 (C=O). ^1H NMR ($CDCl_3$) δ 0.94 (9H, s), 2.89 (1H, ddd, $J=17.6$, 5.5, 1.5 Hz), 3.11 (1H, ddd, $J=17.6$, 6.4, 1.0 Hz), 3.41 (1H, d, $J=14.0$ Hz), 4.48 (1H, t, $J=6.1$ Hz), 4.51 (1H, d, $J=14.0$ Hz), 6.28 (1H, s), 6.54 (1H, s), 7.31–7.49 (5H, m), 7.67–7.74 (1H, m), 9.83 (1H, s). Anal. ($C_{22}H_{23}Cl_2NO_3$) C, H, N.

Methyl *N*-[(3*R*,5*S*)-7-chloro-5-(2,3-dimethoxyphenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetyl]-L-leucinate (18a) and methyl *N*-[(3*S*,5*R*)-7-chloro-5-(2,3-dimethoxyphenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetyl]-L-leucinate (18b). DEPC (0.45 g, 2.6 mmol) was added to an ice-cooled solution of **2t** (1.0 g, 2.16 mmol) and methyl L-leucinate

hydrochloride (0.47 g, 2.6 mmol) in DMF (20 mL), followed by addition of Et_3N (0.75 mL, 5.41 mmol). The mixture was stirred for 30 min at room temperature, poured into water and extracted with AcOEt. The extract was washed with diluted HCl and saturated $NaHCO_3$, dried over $MgSO_4$, and then concentrated. The residue was chromatographed [eluent: hexane–AcOEt (2:1)] to give **18a** (0.52 g, 0.883 mmol, 41%) as colorless prisms from the first fraction and **18b** (3*S*,5*R*, 0.55 g, 0.934 mmol, 43%) as a colorless oil from the second fraction.

18a: mp 150–151 °C. IR ν_{max} (KBr) cm^{-1}: 1740, 1670 (C=O). ^1H NMR ($CDCl_3$) δ 0.8–1.0 (15H, m), 1.95–2.2 (1H, m), 2.68 (1H, dd, $J=14.4$, 5.8 Hz), 2.91 (1H, dd, $J=14.4$, 7.0 Hz), 3.35 (1H, d, $J=14.0$ Hz), 3.62 (3H, s), 3.70 (3H, s), 3.89 (3H, s), 4.3–4.4 (1H, m), 4.49 (1H, d, $J=14.0$ Hz), 4.5–4.65 (1H, m), 6.2–6.4 (2H, m), 6.62 (1H, d, $J=2.2$ Hz), 6.9–7.4 (5H, m). Anal. ($C_{31}H_{41}ClN_2O_7\cdot 1/2H_2O$) C, H, N. **18b**: IR ν_{max} (neat) cm^{-1}: 1740, 1670, 1660 (C=O). ^1H NMR ($CDCl_3$) δ 0.8–1.0 (15H, m), 1.5–1.75 (1H, m), 2.70 (1H, dd, $J=14.4$, 6.0 Hz), 2.89 (1H, dd, $J=14.4$, 6.6 Hz), 3.37 (1H, d, $J=14.0$ Hz), 3.62 (3H, s), 3.71 (3H, s), 3.89 (3H, s), 4.37 (1H, t, $J=6.3$ Hz), 4.52 (1H, d, $J=14.0$ Hz), 4.5–4.7 (1H, m), 6.28 (1H, m), 6.41 (1H, brd, $J=8.4$ Hz), 6.61 (1H, d, $J=1.6$ Hz), 6.9–7.4 (5H, m).

(3*R*,5*S*)-7-Chloro-5-(2,3-dimethoxyphenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetic acid (19a) and (3*S*,5*R*)-7-chloro-5-(2,3-dimethoxyphenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetic acid (19b). A mixture of **18a** (0.4 g, 0.679 mmol), dioxane (6 mL), MeOH (10 mL) and concentrated HCl (5 mL) was refluxed overnight, cooled to room temperature, poured into water and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$ and then concentrated. The residue was dissolved in DMF (5 mL), and MeI (0.042 mL, 0.68 mmol) and K_2CO_3 (0.094 g, 0.68 mmol) was added. The reaction mixture was stirred for 20 min at room temperature, poured into water, and extracted with AcOEt. The extract was washed with diluted HCl and saturated $NaHCO_3$, dried over $MgSO_4$ and then concentrated. The residue was chromatographed [eluent: hexane–AcOEt (5:1)] to give methyl ester of **19a** (85 mg, 0.179 mmol, 26%). A mixture of this compound, K_2CO_3 (50 mg, 0.36 mmol), MeOH (2 mL) and water (2 mL) was refluxed for 2 h, diluted with water, acidified and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and then concentrated to give **19a** (60 mg, 0.13 mmol, 73%) as colorless crystals. Mp 218–222 °C. $[\alpha]_D^{24}$ −246.8° (*c* 0.43, MeOH). Anal. ($C_{24}H_{28}ClNO_6\cdot 3/4H_2O$) C, H, N.%ee 99.4% [HPLC analysis; ULTRON ES-OVM (4.6ID×150; Shinwa Chemical Industries, Ltd); Eluent, EtOH: 0.02 M KH_2PO_4 (pH 3.5) = 35:65; flow rate, 0.5 mL/min; retention time, 19.2 min for **19a** and 10.3 min for **19b**].

19b was similarly synthesized from **18b**. Mp 227–230 °C. $[\alpha]_D^{24}$ + 242.7° (*c* 0.40, MeOH). Anal. ($C_{24}H_{28}ClNO_6\cdot 1/2H_2O$) C, H, N.%ee 98.1% (HPLC analysis).

Sodium (3*R*,5*S*)-7-chloro-5-(2,3-dimethoxyphenyl)-1-neopentyl-2-oxo-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-acetate (20). A solution of **19a** (30 g, 64.9 mmol) and 1 N NaOH (64.9 mL, 64.9 mmol) in MeOH (400 mL) was concentrated under reduced pressure. AcOEt was added to the residue and the solvent was removed. The residue was washed with AcOEt to give **20** (31.8 g, 65.7 mmol, quant) as a colorless powder. Mp > 300°. $[\alpha]_D^{23}$ −235.1° (*c* 0.60, MeOH). IR ν_{max} (KBr) cm^{-1}: 1660, 1580 (C=O). ^1H NMR ($CDCl_3$) δ 0.95 (9H, s), 2.59 (1H, dd, $J=15.4$, 6.4 Hz), 2.79 (1H, dd, $J=15.4$, 7.2 Hz), 3.53 (1H, d, $J=14.0$ Hz), 3.58 (3H, s), 3.88 (3H, s), 4.39 (1H, dd, $J=7.2$, 6.4 Hz), 4.44 (1H, d, $J=14.0$ Hz), 6.21 (1H, s), 6.51 (1H, d, $J=2.4$ Hz), 7.08 (1H, dd, $J=7.8$, 2.0 Hz), 7.19 (1H, t, $J=7.8$ Hz), 7.27 (1H, dd, $J=7.8$, 2.0 Hz), 7.40 (1H, dd, $J=8.4$, 2.4 Hz), 7.55 (1H, d, $J=8.8$ Hz). Anal. ($C_{24}H_{28}ClNNaO_6$) C, H, N.

Crystallographic data

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 164792 (**19a**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Animals and materials

Animals were supplied by Clea, Japan, Inc. unless otherwise mentioned. Male Wistar rats were free access to standard rodent chow (CE-2 in pellet form, Clea Japan). RS-[2-^{14}C] mevalonolactone and [1-^{3}H]-farnesyl pyrophosphate were purchased from New England Nuclear. [2-^{14}C] mevalonic acid was synthesized from [2-^{14}C] mevalonolactone by saponification with potassium hydroxide. [2-^{14}C] Sodium acetate was purchased from Amersham. Farnesyl pyrophosphate was synthesized by the method described by V. J. Davisson and coworkers[16] (Nemoto & Co.). HepG2 cells were supplied by ATCC. Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from GIBCO. Human lipoprotein deficient serum (human LPDS) was purchased from Sigma. All other reagents were supplied by Wako Pure Chemical Industries.

Preparation of rat squalene synthase

An SD male rat (6 weeks old) was killed by bleeding, and its liver was excised. About 10 g of the liver was washed with a saline solution cooled with ice, which was then homogenized in 15 mL of an ice-cooled buffer solution [100 mM potassium phosphate (pH 7.4), 15 mM nicotinamide, 2 mM $MgCl_2$], followed by centrifugation for 20 min at 10,000*g* (4 °C). The supernatant layer was separated and subjected to further centrifugation for 90 min at 105,000*g* (4 °C). The sediment was then suspended in an ice-cooled 100 mM potassium phosphate buffer solution (pH 7.4), which was again subjected to centrifugation for 90 min at

105,000 g (4 °C). The sediment thus obtained (microsome fraction) was suspended in an ice-cooled 100 mM potassium phosphate buffer (pH 7.4) (about 40 mg/mL protein concentration, determined using BCA protein assay kit of Pierce Co., Ltd.). This suspension was used as the enzyme solution.

Preparation of human squalene synthase

HepG2 cells (about 1×10^9 cells) obtained by incubation (37 °C in the presence of 5% CO_2) in a DMEM contains 10% FBS, penicillin G (100 units/mL) and streptomycin (10 g/mL) were suspended in 10 mL of ice-cooled buffer solution [100 mM potassium phosphate buffer (pH 7.4), 30 mM nicotinamide and 2.5 mM $MgCl_2$]. The cells were crashed by means of ultrasonication (for 30 s, twice). From the sonicate thus obtained, the microsome fraction was obtained by the same procedure as in preparation of rat-derived enzyme, which was suspended in an ice-cooled 100 mM potassium phosphate buffer (pH 7.4) (about 4 mg/mL protein concentration). This suspension was used as the enzyme solution.

Assay of squalene synthase inhibitory activity

Squalene synthase activity was monitored by the formation of [^{3}H]squalene from [1-^{3}H]FPP. Fifty microliter of assay mixture included 5 μM[1-^{3}H]FPP (25 μCi/mol), 1 mM NADPH, 5 mM $MgCl_2$, 6 mM glutathione, 100 mM buffer solution of potassium phosphate (pH 7.4), the test compound dissolved in DMSO (a final concentration of DMSO was 2%) and enzyme solution prepared from rat or HepG2 cells (protein content 0.8 g). The assay ran 45 min at 37 °C and stopped by adding 150 μL of $CHCl_3$–MeOH (1:2) containing 0.2% cold squalene as carrier. Aqueous solution of 3 N NaOH (50 μM) and $CHCl_3$ (50 μM) were added to the mixture. The chloroform layer containing the reaction mixture having squalene as the principal component and 3 mL of toluene-based liquid scintillator were mixed, and its radioactivity was determined by means of a liquid scintillation counter. The squalene synthase inhibitory activity was expressed in terms of the concentration of the test compound inhibiting by 50% the radioactivity taken into the chloroform layer [IC_{50}, molar concentration (M)].

Inhibition of cholesterol synthesis in HepG2 cells

HepG2 cells were cultured in 24-well cell culture plates (10^5 cells/well) in a DMEM contains 10% FBS, penicillin G (100 units/mL) and streptomycin (10 g/mL) for 6 days and preincubated overnight in a DMEM containing 10% human LPDS (Sigma). The medium was replaced with the medium containing the test compounds that previously had been dissolved in DMSO (a final concentration of DMSO was 0.4% or less). After incubation for 1 h at 37 °C, 10 ml of 25 mM [^{14}C] mevalonic acid (2 μCi/ μmol) 10 μL was added, and further incubated for 2 h. The cells were washed with phosphate-buffered saline (twice), dissolved in 15% aqueous solution of potassium hydroxide (100 μ) at 37 °C. The cell lysates were saponified for 1 h at 75 °C by adding 400 μL of 15% potassium hydroxide in 80% ethanol, followed by adding 300 μL of distilled water. Non-saponifiables were extracted with *n*-hexane (800 μL). The hexane layer (400 μL) was concentrated under reduced pressure. The residue was dissolved in the 0.1% solution of cholesterol in acetone–ethanol (1:1) (200 μL). The 0.5% solution of digitonin in 50% ethanol (400 μL) was added to the solution. After leaving at room temperature, cholesterol fraction was obtained as digitonin precipitate. The radioactivity taken into digitonin precipitate was measured.

Cholesterogenesis in the liver in Wistar rats

Six-week-old male Wistar rats were orally administered with a test compound 0.5%–methylcellulose emulsion, and were intravenously injected with [^{14}C] acetate (10 μCi/rat) 1 h after administration. Animals were killed 1 h after the injection. The livers were removed, saponified, extracted with petroleum ether and then dried under nitrogen vapor. The residue was dissolved in the ethanol–acetone (1:1) (3 mL). The 0.5% solution of digitonin in 50% ethanol (2 mL) was added to the solution. After leaving at room temperature for 4 h, cholesterol fraction was obtained as digitonin precipitate. The radioactivity taken into digitonin precipitate was measured.

Effect on plasma cholesterol levels in marmosets

By a stomach tube, a test compound 0.5%–methylcellulose emulsion was administered to male Common marmosets (19–62 months old, purchased from Japan EDM) for 4 days. Under non-fasted condition, blood was taken from the femoralvain and the plasma total cholesterol, HDL (high-density lipoprotein)-cholesterol, and triglyceride were enzymatically measured using an assay kit (Wako Pure Chemical Industries). The non-HDL-cholesterol was calculated by subtracting HDL-cholesterol from total cholesterol.

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References and Notes

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8. Removal of the 7-chloro atom of 1-isobutyl derivative **1b** caused a significant loss of activity (40% inhibition at 10 μM against rat-derived enzymes). The 7-chloro atom was found to be essential to exhibit inhibitory activity (unpublished data).
9. The assay conditions for the sodium salt **20** are the same as those for the free acid **19a**. Inhibitory activity of **20** is 2-fold more potent than the racemate **2t**. Although activity of **19a** does not reach such a 2-fold increase over **2t**, **19a** is 1.5–1.7-fold more potent than **2t**. It is assumed that **19a** would be as potent an inhibitor as **20**.
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