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Synthesis of 4-[1-(substituted phenyl)-2-oxo-pyrrolidin-4-yl]methyloxybenzoic acids and related compounds, and their inhibitory capacities toward fatty-acid and sterol biosyntheses

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Summary — The synthesis of a series of 4-[1-(substituted phenyl)-2-oxo-pyrrolidin-4-yl]methyloxybenzoic acids and related compounds, and their evaluation for inhibitory capacity toward fatty-acid and sterol biosyntheses using rats' liver slices *in vitro* and rabbits *in vivo*, are described. Among the compounds synthesized, **7e**, **7g**, **7h**, **7i**, **7k**, **7r**, **21**, **23** and **29a**, **b** showed a potent inhibitory activity toward fatty-acid and sterol biosyntheses. Their IC_{50}s were 4.4–6.8 x 10^{-6} M and 6.6–9.8 x 10^{-6} M, respectively. These activities were always superior to those of compounds **I**, **II**, **III** and Clinofibrate as references. The inhibitory activity toward the sterol biosynthesis of these compounds was inferior to that of Pravastatin. The reducing effects of the representative compounds (**7e** and **7l**) toward plasma cholesterols and triglyceride were evaluated in Japanese white rabbits (30 and 100 mg/kg, po) and compared with those of Clinofibrate and Pravastatin. The compounds showed a similar hypocholesterolemic effect to Pravastatin and a more potent hypotriglycemic effect than Clinofibrate and Pravastatin in this animal model. Thus, a dual action of hypolipidemic effects was noted in **7e** and **7l** compared with the references.

fatty-acid biosynthesis / sterol biosynthesis / inhibitory activity / 2-oxo-pyrrolidine / benzoic acid / structure–activity relationship / Clinofibrate / Pravastatin / hypolipidemic effect

Introduction

It is well known that an elevated serum level of total cholesterol is a factor etiologically related to atherosclerotic vascular diseases, particularly coronary heart diseases (CHD) [1–6]. However, the relationship between serum levels of triglyceride and CHD was recently clarified by an epidemiological survey in the Framingham Heart Study [7] and the Helsinki Heart Study [8] of hypertriglyceridemia as a risk factor of CHD. The role of elevated triglyceride levels combined with hypercholesterolemia in the development of CHD is supported by evidence from numerous epidemiological studies and reports [7–11]. A decrease in plasma cholesterol and triglyceride levels is thus important for reducing the incidence of CHD. We were thus prompted to investigate a novel hypolipidemic agent, which reduced both plasma cholesterol and triglyceride levels. Since it is recognized that the liver is the major site of fatty-acid and sterol biosyntheses, a compound with inhibitory capacities toward both of these biosyntheses is attractive as a novel hypolipidemic agent.

4-[2-(4-Isopropylbenzoylamino)ethoxy]benzoic acid (**II**) [12, 13] and 4-(4-chlorobenzyloxy)benzoic acid (**III**) [14, 15] were previously reported as hypolipidemic agents possessing inhibitory effects toward both of these biosyntheses. We have also reported 4-[3-(phenylamino)propyloxy]benzoic acid (**I**) [16], which has a similar mechanism. To develop structurally diverse, yet biologically potent, alternatives to these compounds (**I**, **II** and **III**), efforts were made to replace the linear linkages between the benzene ring on the left-hand side and the benzoic acid moiety of compounds **I**, **II** and **III** with heterocyclic ring components containing a nitrogen atom in the ring as shown in figure 1. We believed that the introduction of cyclic components involving heterocyclic ring systems instead of linear linkages would fix the stereo-structure of the test compound. If this stereo-structure fits the target enzyme then its intro-

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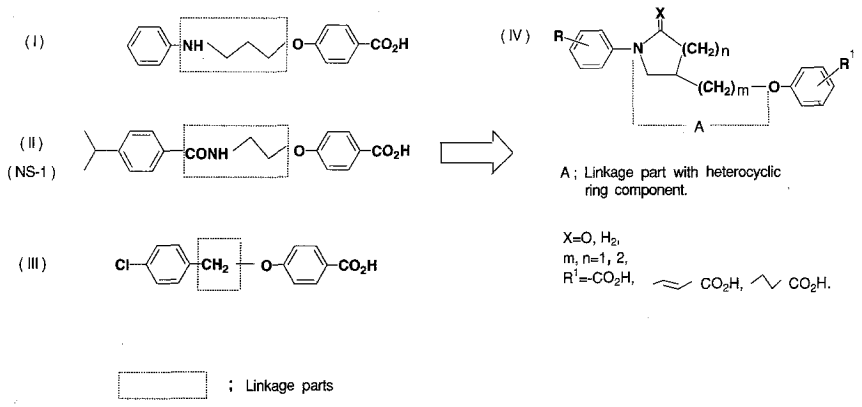


Fig 1. Linkages in compounds **I–IV**.

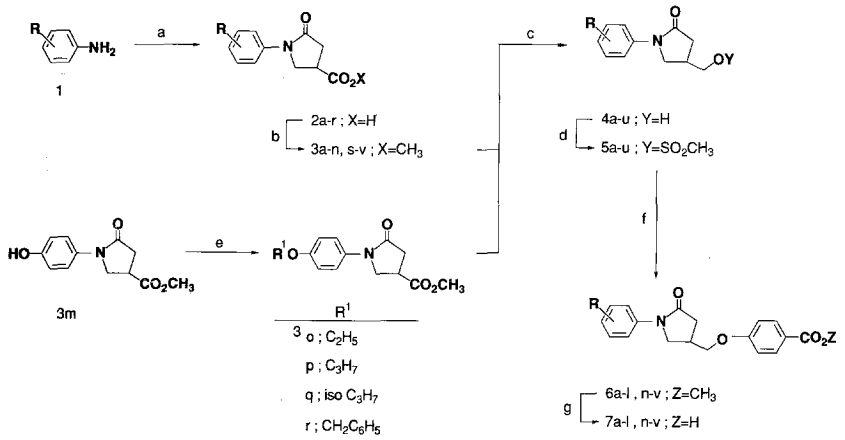
duction would lead to an increase in inhibitory activity. Thus, 2-oxo-pyrrolidine, 2-oxo-piperidine, pyrrolidine and piperidine components were adopted as heterocyclic ring systems in the linkage of compound **IV**. Here we wish to report the syntheses of compound **IV**, which contains heterocyclic ring components as linkages, and their inhibitory activity toward fatty-acid and sterol biosyntheses *in vitro* using rats' liver slices and their hypolipidemic effects (**7e** and **7l**) in rabbits in comparison with Clinofibrate [17] and Pravastatin [18] references.

Chemistry

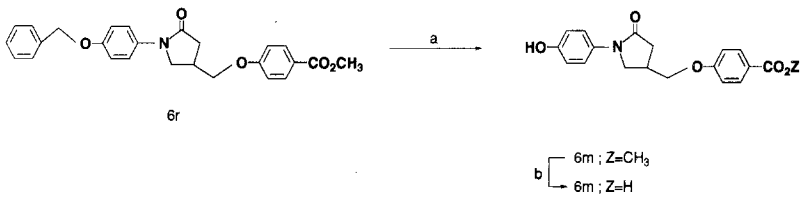
The intermediates and compounds were prepared as racemates according to schemes 1–6 and tables I–II.

The general method used to construct the lactam ring (pyrrolidone and piperidone components) involved Michael additions of methylenesuccinic acid or methyleneglutaric acid [19] with aniline derivatives **1** and subsequent ring closure to lactam according to the method of Paytash [20] (see schemes 1 and 2). Methyl benzoate derivatives, precursors of final target molecules, were prepared by coupling of phenol derivatives with mesylates **5**, **11**, **17** and **27**. Most of the compounds in this study were synthesized as shown in scheme 1.

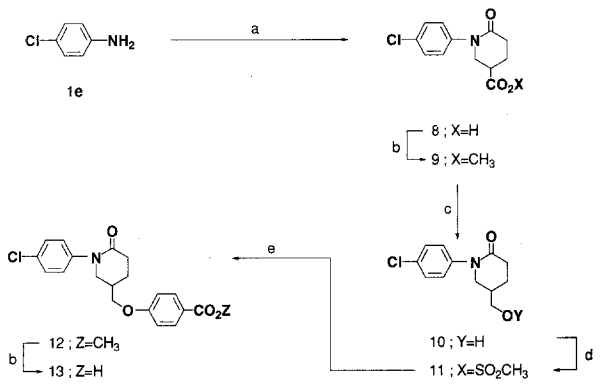
1-Phenylpyrrolidine-3-carboxylic acids **2g–r** and the known compounds, **2a** [21], **2b–g**, **2m**, **2n** and **2q** [20], were prepared by heating a mixture of appropriate aniline with itaconic acid. Acids **2a–r** were esterified in the presence of catalytic conc H_2SO_4 in a solution of methanol and dichloromethane to give the



Scheme 1. Reagents: a) itaconic acid, Δ; b) MeOH, catalytic conc H_2SO_4, CH_2Cl_2; c) $NaBH_4$, THF/MeOH; d) MsCl, Et_3N, CH_2Cl_2; e) R^1Br, K_2CO_3, DMF; f) 4-$HOC_6H_4CO_2CH_3$, K_2CO_3, DMF; g) KOH, MeOH/H_2O.



Scheme 2. Reagents: a) 10% Pd-C, H_2, MeOH/$CHCl_3$; b) KOH, MeOH/H_2O.

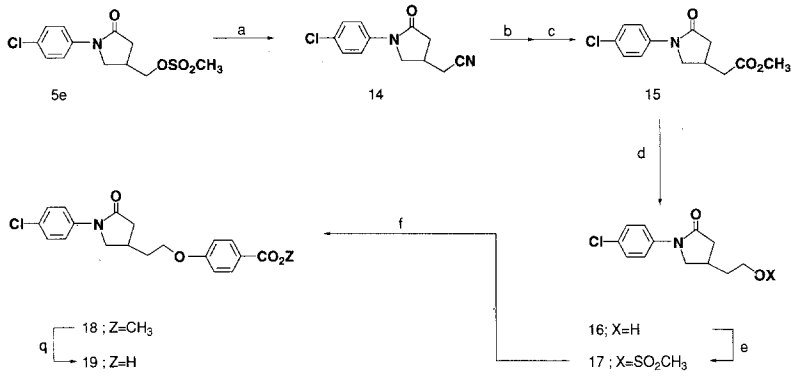


Scheme 3. Reagents: a) $H_2OCC(=CH_2)CH_2$, CH_2CO_2H, Δ; b) MeOH, catalytic conc H_2SO_4, CH_2Cl_2; c) $NaBH_4$, THF/MeOH; d) MsCl, Et_3N, CH_2Cl_2; e) 4-HOC_6H_4-CO_2CH_3, K_2CO_3, DMF; f) KOH, MeOH/H_2O.

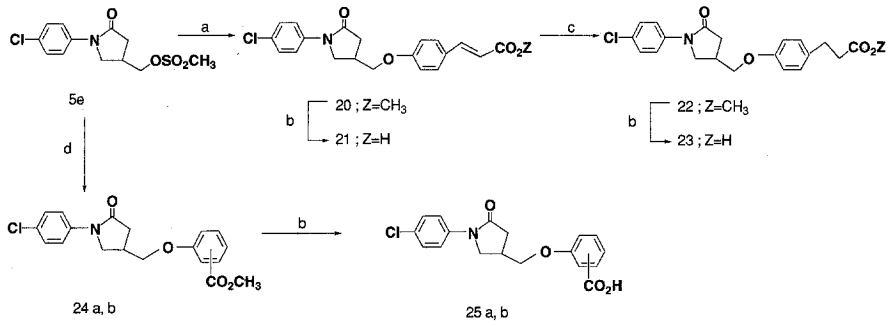
esters **3a–n** and **3s–v**. Methyl 1-(alkoxyphenyl)-5-oxo-pyrrolidine-3-carboxylates **3o–r** were prepared by alkylation with **3m** and appropriate alkyl bromides in the presence of anhydrous potassium carbonate (K_2CO_3) in *N*,*N*-dimethylformamide (DMF). Reduction of methylester **3** by sodium borohydride ($NaBH_4$) gave the hydroxymethyl derivatives **4**, which were esterified by methanesulfonyl (mesyl) chloride in the presence of triethylamine in dichloromethane to afford **5**. Without purifying **5**, its coupling with 4-hydroxybenzoate under anhydrous K_2CO_3 in DMF gave **6a–l** and **6n–v**, which were converted into the target-free acids, **7a–l** and **7n–v** by alkaline hydrolysis (scheme 1). Compound **7m**, which contains the 4-hydroxyphenyl moiety, was obtained through the benzyl deprotection of **6r** by catalytic hydrogenation over 10% palladium-carbon (Pd-C), followed by alkaline hydrolysis of **6m** (scheme 2).

The preparation of target molecule **13** containing the 2-oxo-piperidine moiety is outlined in scheme 3. The condensation of 4-chloroaniline **1e** with 2-methyleneglutaric acid [19] and heating gave 1-(4-chlorophenyl)-6-oxo-piperidine-3-carboxylic acid **8**. Target-free acid **13** was prepared through the general sequence **8** → **9** → **10** → **11** → **12** → **13** in a manner similar to the preparation of **7**.

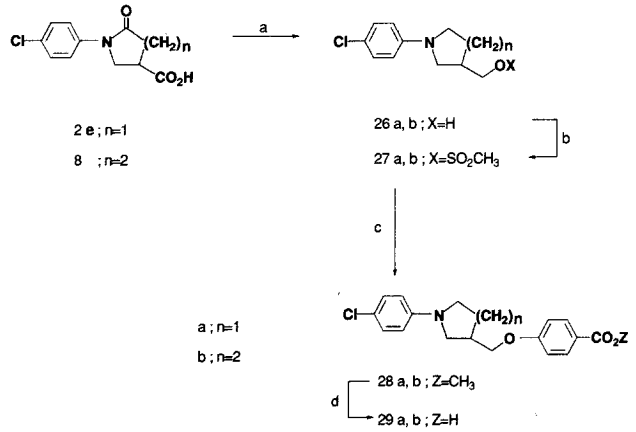
The synthesis of the target molecule, 2-[4-[1-(4-chlorophenyl)-2-oxo-pyrrolidin-4-yl]ethyloxy]benzoic acid **19**, is shown in scheme 4. The condensation of mesylate **5e** with sodium cyanide in DMF afforded



Scheme 4. Reagents: a) NaCN, DMF; b) NaOH, EtOH/H_2O; c) MeOH, catalytic conc H_2SO_4; d) $NaBH_4$, THF/CH_2Cl_2; e) MsCl, Et_3N, CH_2Cl_2; f) *p*-HO/$C_6H_4CO_2CH_3$, K_2CO_3, DMF; g) KOH, MeOH/H_2O.



Scheme 5. Reagents: a) 4-$HOC_6H_4CH{=}CHCO_2CH_3$, K_2CO_3, DMF; b) KOH, $MeOH/H_2O$; c) 10% Pd-C, H_2, AcOEt; d) 2- or 3-$HOC_6H_4CO_2CH_3$, K_2CO_3, DMF. (**a**: $-CO_2CH_3$ at 2-position; **b**: $-CO_2CH_3$ at 3-position).



Scheme 6. Reagents: a) $LiAlH_4$, THF; b) MsCl, Et_3N, CH_2Cl_2; c) 4-$HOC_6H_4CO_2CH_3$, K_2CO_3, DMF; d) KOH, $MeOH/H_2O$.

the cyanomethyl **14**. Alkaline hydrolysis of **14**, and subsequent esterification with methanol gave the methyl acetate **15**. Target-free acid **19** was prepared through the reduction of **15** with $NaBH_4$, methanesulfonylation (mesylation) of **16** with mesyl chloride, coupling of **17** with 4-hydroxybenzoate, and alkaline hydrolysis of **18**.

The preparations of the cinnamic acid analogue **21**, the phenylpropionic acid analogue **23** and the regioisomeric compounds **25a**, **b** with the 4-substituted benzoic acid analogue **7** are outlined in scheme 5. The cinnamic acid analogue **21** was prepared by coupling **5e** with methyl 4-hydroxycinnamate [22] under anhydrous K_2CO_3 in DMF, followed by alkaline hydrolysis of **20**. The phenylpropionic acid analogue **23** was prepared by a catalytic hydrogenation of **20** over 10% Pd-C, followed by alkaline hydrolysis of **22**. Regioisomers, **24a** and **24b** were synthesized by coupling **5e** with methyl 2- or 3-hydroxybenzoates, respectively. Alkaline hydrolysis of **24a** and **24b** gave the target acids **25a** and **25b**, respectively.

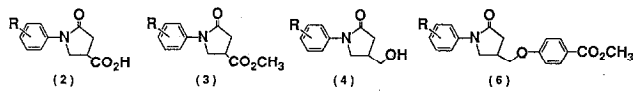
The preparations of compounds **29a** and **29b** are outlined in scheme 6. The reduction of 2 functional groups (carbonyl and carboxyl groups) on **2e** and **8** was performed with lithium aluminum hydride ($LiAlH_4$) in tetrahydrofuran (THF) to give **26a** and **26b**, respectively. Target-free acid **29** was prepared through a mesylation of **26**, followed by coupling of **27** with methyl 4-hydroxybenzoate and alkaline hydrolysis of **28**.

Results and discussion

The target compound, 4-[1-(substituted phenyl)-2-oxo-pyrrolidin-4-yl]methyloxybenzoic acids **7** and related compounds **13**, **19**, **21**, **23**, **25** and **29** were obtained in fair yields as shown in table II and the *Experimental protocols*. The inhibitory activities of these compounds toward fatty-acid and sterol biosyntheses *in vitro* were evaluated by measuring the rate of conversion of [^{14}C]acetate to fatty acids and sterols using rats' liver slices according to Bortz [23] or Tsujita [18]. Biological activities are reported as the IC_{50} values in table III. The structure–activity relationships of the target compounds were studied and compared with compounds **I**, **II**, **III**, Clinofibrate [17] and Pravastatin [18] as references for the inhibitory capacities toward fatty-acid and sterol biosyntheses. Most of the 2-oxo-pyrrolidine analogues showed inhibitory activities of the order of 10^{-5} M to 10^{-6} M (IC_{50}) toward fatty-acid and sterol biosyntheses.

Although the compounds synthesized had only narrow inhibitory activity ranges for fatty-acid and sterol biosyntheses, we determined the following structure–activity relationships from the results obtained. The compounds **7b**, **7e–i** and **7k**, which

Table I. Physical and chemical data of intermediates **2**, **3**, **4** and **6**.



Compound	*R*	*Yield (%)*	*Mp (°C)*	*Formula*	*Anal*[a]
2a	H	93.3	192–194	$C_{11}H_{11}NO_3$	–[b]
2b	4-F	80.6	169–172	$C_{11}H_{10}FNO_3$	–[c]
2c	2-Cl	58.6	143–145	$C_{11}H_{10}ClNO_3$	–[c]
2d	3-Cl	97.7	130–132	$C_{11}H_{10}ClNO_3$	–[c]
2e	4-Cl	93.5	138–139	$C_{11}H_{10}ClNO_3$	–[c]
2f	4-Br	86.0	170–173	$C_{11}H_{10}BrNO_3$	–[c]
2g	4-CH_3	96.9	185–187	$C_{12}H_{13}NO_3$	–[c]
2h	4-C_2H_5	88.3	144–146	$C_{13}H_{15}NO_3$	C, H, N
2i	4-C_3H_7	90.9	129–132	$C_{14}H_{17}NO_3$	C, H, N
2j	4-iC_3H_7	84.6	166–168	$C_{14}H_{17}NO_3$	C, H, N
2k	4-C_4H_9	94.5	133–135	$C_{15}H_{19}NO_3$	C, H, N
2l	4-tC_4H_9	95.1	208–209	$C_{15}H_{19}NO_3$	C, H, N
2m	4-OH	93.5	201–204	$C_{11}H_{11}NO_4$	–[c]
2n	4-OCH_3	94.6	171–172	$C_{12}H_{13}NO_4$	–[c]
2o	3,4-OCH_2O	91.3	171–173	$C_{12}H_{11}NO_5$	C, H, N
2p	3,4-$(Cl)_2$	83.7	165–168	$C_{11}H_9Cl_2NO_3$	C, H, N
2q	2-OCH_3, 5-Cl	65.8	193–195	$C_{12}H_{12}ClNO_4$	–[c]
2r	3,5-$(CH_3)_2$	90.2	172–175	$C_{13}H_{15}NO_3$	C, H, N
3a	H	90.5	68–69	$C_{12}H_{13}NO_3$	C, H, N
3b	4-F	98.2	93–95	$C_{12}H_{12}FNO_3$	C, H, N
3c	2-Cl	94.3	–[d]	$C_{12}H_{12}ClNO_3$	–[e]
3d	3-Cl	90.6	–[d]	$C_{12}H_{12}ClNO_3$	–[e]
3e	4-Cl	92.6	85–87	$C_{12}H_{12}ClNO_3$	C, H, N
3f	4-Br	91.5	66–68	$C_{12}H_{12}BrNO_3$	C, H, N
3g	4-CH_3	95.2	72–74	$C_{13}H_{15}NO_3$	C, H, N
3h	4-C_2H_5	93.2	54–55	$C_{14}H_{17}NO_3 \cdot 1/10H_2O$	C, H, N
3i	4-C_3H_7	96.7	–[d]	$C_{15}H_{19}NO_3$	–[e]
3j	4-iC_3H_7	92.3	–[d]	$C_{15}H_{19}NO_3$	–[e]
3k	4-C_4H_9	95.5	–[d]	$C_{16}H_{21}NO_3$	–[e]
3l	4-tC_4H_9	98.4	114–115	$C_{16}H_{21}NO_3$	C, H, N
3m	4-OH	94.7	–[d]	$C_{12}H_{13}NO_4$	–[e]
3n	4-OCH_3	89.3	84–86	$C_{13}H_{15}NO_4$	C, H, N
3o	4-OC_2H_5	91.4	67–69	$C_{14}H_{17}NO_4 \cdot 1/10H_2O$	C, H, N
3p	4-OC_3H_7	96.1	68–69	$C_{15}H_{19}NO_4$	C, H, N
3q	4-O-iC_3H_7	97.3	–[d]	$C_{15}H_{19}NO_4$	–[e]
3r	4-$OCH_2C_6H_5$	96.0	112–114	$C_{19}H_{19}NO_4$	C, H, N
3s	3,4-OCH_2O-	91.5	144–146	$C_{13}H_{13}NO_5$	C, H, N
3t	3,4-$(Cl)_2$	99.0	110–113	$C_{12}H_{11}Cl_2NO_3$	C, H, N
3u	2-OCH_3, 5-Cl	95.9	–[d]	$C_{13}H_{14}ClNO_4$	–[e]
3v	3,5-$(CH_3)_2$	98.4	74–75	$C_{14}H_{17}NO_3$	C, H, N
4a	H	95.5	91–92	$C_{11}H_{13}NO_2$	C, H, N
4b	4-F	99.0	–[d]	$C_{11}H_{12}FNO_2$	–[e]
4c	2-Cl	98.5	–[d]	$C_{11}H_{12}ClNO_2$	–[e]
4d	3-Cl	99.5	–[d]	$C_{11}H_{12}ClNO_2$	–[e]
4e	4-Cl	99.0	88–87	$C_{11}H_{12}ClNO_2$	C, H, N
4f	4-Br	72.6	79–82	$C_{11}H_{12}BrNO_2 \cdot 1/10H_2O$	C, H, N
4g	4-CH_3	98.3	107–110	$C_{12}H_{15}NO_2 \cdot 1/10H_2O$	C, H, N
4h	4-C_2H_5	81.1	55–57	$C_{13}H_{17}NO_2 \cdot 1/5H_2O$	C, H, N
4i	4-C_3H_7	99.1	–[d]	$C_{14}H_{19}NO_2$	–[e]
4j	4-iC_3H_7	98.3	66–68	$C_{14}H_{19}NO_2 \cdot 1/5H_2O$	C, H, N
4k	4-C_4H_9	99.7	–[d]	$C_{15}H_{21}NO_2$	–[e]

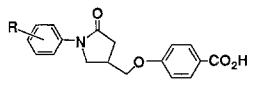
Table I. Continued.

Compound	*R*	*Yield (%)*	*Mp (°C)*	*Formula*	*Anal*[a]
4l	4-*t*C_4H_9	98.5	89–90	$C_{15}H_{21}NO_2$	C, H, N
4m	4-OCH_3	98.8	105–107	$C_{12}H_{15}NO_3$	C, H, N
4n	4-OC_2H_5	96.2	104–106	$C_{13}H_{17}NO_3$	C, H, N
4o	4-OC_3H_7	99.3	–[d]	$C_{14}H_{19}NO_3$	–[e]
4p	4-O-*i*C_3H_7	99.0	–[d]	$C_{14}H_{19}NO_3$	–[e]
4q	4-$OCH_2C_6H_5$	94.5	108–110	$C_{18}H_{19}NO_3$	C, H, N
4r	3,4-OCH_2O-	98.9	111–114	$C_{12}H_{13}NO_4$	C, H, N
4s	3,4-$(Cl)_2$	99.0	–[d]	$C_{11}H_{11}Cl_2NO_2$	–[e]
4t	2-OCH_3, 5-Cl	98.2	–[d]	$C_{12}H_{14}ClNO_3$	–[e]
4u	3,5-$(CH_3)_2$	98.5	132–133	$C_{13}H_{17}NO_2$	C, H, N
6a	H	79.5	114–116	$C_{19}H_{19}NO_4 \cdot 1/4H_2O$	C, H, N
6b	4-F	54.9	114–116	$C_{19}H_{18}FNO_4$	C, H, N
6c	2-Cl	80.4	77–78	$C_{19}H_{18}ClNO_4$	C, H, N
6d	3-Cl	81.3	90–91	$C_{19}H_{18}ClNO_4$	C, H, N
6e	4-Cl	61.6	122–124	$C_{19}H_{18}ClNO_4$	C, H, N
6f	4-Br	64.0	130–132	$C_{19}H_{18}BrNO_4$	C, H, N
6g	4-CH_3	89.5	110–112	$C_{20}H_{21}NO_4$	C, H, N
6h	4-C_2H_5	57.4	87–88	$C_{21}H_{23}NO_4$	C, H, N
6i	4-C_3H_7	77.4	97–98	$C_{22}H_{25}NO_4$	C, H, N
6j	4-*i*C_3H_7	87.2	104–105	$C_{22}H_{25}NO_4 \cdot 1/5H_2O$	C, H, N
6k	4-C_4H_9	92.5	83–85	$C_{23}H_{27}NO_4$	C, H, N
6l	4-*t*C_4H_9	89.3[f]	142–144	$C_{23}H_{27}NO_4$	C, H, N
6m	4-OH	93.8[f]	141–143	$C_{19}H_{19}NO_5 \cdot 1/5H_2O$	C, H, N
6n	4-OCH_3	87.7	109–111	$C_{20}H_{21}NO_5$	C, H, N
6o	4-OC_2H_5	79.8	117–118	$C_{21}H_{23}NO_5$	C, H, N
6p	4-OC_3H_7	79.3	104–105	$C_{22}H_{25}NO_5$	C, H, N
6q	4-O-*i*C_3H_7	82.1	99–100	$C_{22}H_{25}NO_5$	C, H, N
6r	4-$OCH_2C_6H_5$	86.3	124–126	$C_{26}H_{25}NO_5$	C, H, N
6s	3,4-OCH_2O-	57.6	157–159	$C_{20}H_{19}NO_6 \cdot 1/5H_2O$	C, H, N
6t	3,4-$(Cl)_2$	68.2	124–127	$C_{19}H_{17}Cl_2NO_4$	C, H, N
6u	2-OCH_3, 5-Cl	88.5	105–106	$C_{20}H_{20}ClNO_5$	C, H, N
6v	3,5-$(CH_3)_2$	89.2	147–148	$C_{21}H_{23}NO_4$	C, H, N

[a]Analytical results were within ± 0.4% of the theoretical values unless otherwise noted; [b]see reference [21]; [c]see reference [20]; [d]compounds obtained as a viscous oil; [e]compounds assigned by ^{1}H-NMR; [f]all compounds **6**, except for **6l** and **6m**, were obtained from **4**; **6l** and **6m** were obtained from **5l** and **6r**, respectively.

have a halogen atom and straight alkyl-chain substituents at the 4-position on the benzene ring, showed potent inhibitory activities toward both fatty-acid and sterol biosyntheses. However, unsubstituted compounds **7a** and halogenated compounds **7c** and **7d** at the 2- or 3-position on the benzene ring tended to decrease slightly the inhibitory activity of the sterol biosynthesis, whereas that of fatty-acid biosynthesis was retained. Moreover, in compounds **7j** and **7l** with bulky alkyl substituents and compounds **7o–q** (except **7n**) with alkoxy substituents, the same tendencies were observed. From these results, it was suggested that the effects of both inhibitory activities did not depend upon an electron-donating or electron-withdrawing nature (for example, **7e–g** and **7e–o**). For the disubstituted compounds (**7s–v**), the introduction of a substituent at the 2-position on the benzene ring decreased both inhibitory activities (see, for example, **7u** and **7v**). Potent inhibitory activity against both fatty-acid and sterol biosyntheses requires a mono-substituent at the 4-position on the benzene ring with the exception of 4-alkoxy substituents, which do not cause activity. Thus, substituents at the 4-position focused on 4-Cl and further structure–activity relationships were studied on several derivatives (**13–29b**). Ring expansion of 2-oxo-pyrrolidine nucleus (**7e**) into 2-oxo-piperidine nucleus (**13**) and extension of the alkyl chain on the 4-position of 2-oxo-pyrrolidine ring in **7e** to compound **19** retained the inhibitory potency of fatty-acid biosynthesis, but considerably reduced the inhibitory activity toward sterol biosynthesis. Replacement of the benzoic acid analogue (**7e**) by cinnamic acid (**21**) or phenylpropionic acid analogues (**23**), or replacement of the 2-oxo-pyrrolidine (**7e**) or 2-oxo-piperidine (**13**) moiety by the pyrrolidine (**29a**)

Table II. Physical and chemical data of compound 7.



Compound	*R*	*Yield (%)*	*Mp (°C)*	*Formula*	*Anal*[a]
7a	H	94.9	198–199	$C_{18}H_{17}NO_4$	C, H, N
7b	4-F	98.8	230–232	$C_{18}H_{16}FNO_4$	C, H, N
7c	2-Cl	83.9	220–222	$C_{18}H_{16}ClNO_4 \cdot 1/2H_2O$	C, H, N
7d	3-Cl	91.1	221–223	$C_{18}H_{16}ClNO_4$	C, H, N
7e	4-Cl	93.5	214–215	$C_{18}H_{16}ClNO_4$	C, H, N
7f	4-Br	90.8	218–220	$C_{18}H_{16}BrNO_4$	C, H, N
7g	4-CH_3	91.7	212–213	$C_{19}H_{19}NO_4$	C, H, N
7h	4-C_2H_5	93.7	195–197	$C_{20}H_{21}NO_4$	C, H, N
7i	4-C_3H_7	95.0	193–195	$C_{21}H_{23}NO_4$	C, H, N
7j	4-iC_3H_7	96.9	209–210	$C_{21}H_{23}NO_4$	C, H, N
7k	4-C_4H_9	93.2	186–188	$C_{22}H_{25}NO_4$	C, H, N
7l	4-tC_4H_9	98.1	258–260	$C_{22}H_{25}NO_4$	C, H, N
7m	4-OH	99.1	247–249	$C_{18}H_{17}NO_5 \cdot 1/4H_2O$	C, H, N
7n	4-OCH_3	85.1	193–195	$C_{19}H_{19}NO_5$	C, H, N
7o	4-OC_2H_5	99.8	218–220	$C_{20}H_{21}NO_5$	C, H, N
7p	4-OC_3H_7	99.0	197–198	$C_{21}H_{23}NO_5$	C, H, N
7q	4-O-iC_3H_7	99.5	186–188	$C_{21}H_{23}NO_5$	C, H, N
7r	4-$OCH_2C_6H_5$	97.3	208–210	$C_{25}H_{23}NO_5$	C, H, N
7s	3,4-OCH_2O-	95.2	236–238	$C_{19}H_{17}NO_6$	C, H, N
7t	3,4-$(Cl)_2$	95.4	240–242	$C_{18}H_{15}Cl_2NO_4$	C, H, N
7u	2-OCH_3, 5-Cl	92.0	172–173	$C_{19}H_{18}ClNO_5$	C, H, N
7v	3,5-$(CH_3)_2$	98.6	215–217	$C_{20}H_{21}NO_4$	C, H, N

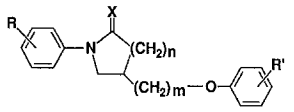
[a]Analytical results were within ± 0.4% of the theoretical values.

or piperidine (**29b**) moiety, respectively, retained both inhibitory activities around IC_{50} 10^{-6} M. Exchanging the benzoic acid moiety at the 4-position (R') on the benzene ring in **7e** for a 2- or 3-position moiety caused a large decrease in both inhibitory activities.

Among all the compounds, **7e**, **7g–i**, **7k**, **7r**, **21**, **23** and **29a** showed the most potent activities with a good balance between the inhibition of fatty-acid and sterol biosyntheses. The IC_{50} of these compounds were on average 10^{-6} M, slightly higher than that of **I**, **II** and **III**. Moreover, these compounds were more potent than Clinofibrate. However, the inhibitory activity of these compounds toward sterol biosynthesis was less potent than that of Pravastatin. The inhibitory activities toward sterol and fatty-acid biosyntheses of all of the synthesized compounds were first measured using rats' liver slices. Furthermore, the inhibitory activities of a representative compound (**7l**) toward partially isolated HMG-CoA reductase and acyl-CoA carboxylase were recently identified in our laboratory. (These inhibitory activities and the details of the mechanism of action against these enzymes will be presented in another communication in the near future.) In further investigations, in order to certify the hypolipidemic effects *in vivo*, the representative compounds, **7e** and **7l**, were selected as the high and middle activity compounds *in vitro*, respectively. It is generally known [24] that in the case of the *in vivo* evaluation of the hypolipidemic activities of compounds based on the inhibition of sterol biosynthesis, the use of rabbits or dogs is preferable to the use of rodents. Thus, the evaluation of the hypolipidemic effect of **7e** and **7l** was carried out at 2 doses (30 and 100 mg/kg) in rabbits according to the method of Okada [25] and compared with Clinofibrate (100 mg/ kg) or Pravastatin (30 mg/kg). These data are shown in table IV.

When **7e** and **7l** were orally administered at 100 mg/kg/d, plasma cholesterol and triglyceride levels were reduced on day 10 by about 30–44% and 34–35%, respectively. However, the hypolipidemic effects at 30 mg/kg were poor on day 10. The hypocholesterolemic activity of these compounds at

Table III. Biological data of compounds **7**, **13**, **19**, **21**, **23**, **25** and **29**.



Compound	*R*	*X*	*m*	*n*	*R'*	*Inhibition of biosynthesis (μM)*[a]	
						Fatty acids	*Sterols*
7a	H	0	1	1	4-CO_2H	8.1	19.1
7b	4-F	0	1	1	4-CO_2H	8.0	9.5
7c	2-Cl	0	1	1	4-CO_2H	9.1	24.1
7d	3-Cl	0	1	1	4-CO_2H	8.6	21.2
7e	4-Cl	0	1	1	4-CO_2H	6.2	7.5
7f	4-Br	0	1	1	4-CO_2H	6.2	9.3
7g	4-CH_3	0	1	1	4-CO_2H	6.7	7.9
7h	4-C_2H_5	0	1	1	4-CO_2H	6.4	8.6
7i	4-C_3H_7	0	1	1	4-CO_2H	4.2	7.8
7j	4-*i*C_3H_7	0	1	1	4-CO_2H	7.6	15.5
7k	4-C_4H_9	0	1	1	4-CO_2H	5.9	7.5
7l	4-*t*C_4H_9	0	1	1	4-CO_2H	5.3	12.0
7m	4-OH	0	1	1	4-CO_2H	6.0	19.2
7n	4-OCH_3	0	1	1	4-CO_2H	17.9	34.2
7o	4-OC_2H_5	0	1	1	4-CO_2H	8.6	18.2
7p	4-OC_3H_7	0	1	1	4-CO_2H	8.6	19.4
7q	4-O-*i*C_3H_7	0	1	1	4-CO_2H	7.3	31.1
7r	4-$OCH_2C_6H_5$	0	1	1	4-CO_2H	5.9	9.8
7s	3, 4-OCH_2O-	0	1	1	4-CO_2H	9.4	24.7
7t	3,4-$(Cl)_2$	0	1	1	4-CO_2H	7.7	10.5
7u	2-OCH_3, 5-Cl	0	1	1	4-CO_2H	25.6	43.4
7v	3,5-$(CH_3)_2$	0	1	1	4-CO_2H	16.5	30.6
13	4-Cl	0	1	2	4-CO_2H	8.9	23.0
19	4-Cl	0	2	1	4-CO_2H	8.5	> 50
21	4-Cl	0	1	1	4-$CH{=}CHCO_2H$	4.4	6.6
23	4-Cl	0	1	1	4-$CH_2CH_2CO_2H$	6.0	7.4
25a	4-Cl	0	1	1	2-CO_2H	> 50	> 50
25b	4-Cl	0	1	1	3-CO_2H	44.6	> 50
29a	4-Cl	H_2	1	1	4-CO_2H	6.7	8.6
29b	4-Cl	H_2	1	2	4-CO_2H	6.0	9.6
I						8.2	19.1
II						7.1	32.8
III						15.8	33.1
Pravastatin						> 500	0.2
Clinofibrate						81.4	> 500

[a]See *Experimental protocols.*

Table IV. Hypolipidemic effects on Japanese white rabbits of **7e** and **7l** in comparison with Pravastatin and Clinofibrate.

Compound	*Dose*[a] *(mg/kg)*	*% of reduction from control value*[b]			
		3 d		*10 d*	
		TC[c]	*TG*[d]	*TC*[c]	*TG*[d]
7e	30	12.1	12.1	2.1	− 7.4
	100	34.2	24.5	44.2	33.9
7l	30	24.0	14.3	18.1	18.2
	100	29.7**	40.2**	29.1**	35.4*
Pravastatin	30	39.2*	− 0.2	46.4*	− 14.2
Clinofibrate	100	6.6	− 1.3	1.5	27.9

[a]Orally administered once a day for 10 d; [b]the student's *t*-test was used for the statistical calculations, statistically significant at $*P < 0.05$, $**P < 0.01$; [c]total cholesterol in plasma; [d]triglyceride in plasma.

100 mg/kg was similar to that of Pravastatin at 30 mg/kg and the hypotriglycemic activity of these compounds was better than that of Clinofibrate at the same dose. The inhibitory activities of **7e** and **7l** were lower than Pravastatin by a factor of 1/60–1/37 (IC_{50} = 0.2 x 10^{-6} M), but the hypocholesterolemic activity of these compounds was nearly the same. The reason for this is suggested by the pharmacokinetics as follows. The plasma and liver concentrations of **7l** in rabbits (n = 3) were measured simultaneously during an *in vivo* study, before feeding and after final oral administration (dose: **7l**; 100 mg/kg, Pravastatin; 30 mg/kg, for 10 d) of the compounds. The plasma and liver concentrations were 3.4 ± 1.5 μg/ml, 23.0 ± 1.2 μg/g for **7l** and < 0.1 μg/ml, < 0.1 μg/g for Pravastatin, respectively. As can be seen from the above data, the plasma and liver concentrations of **7l** were higher than that of Pravastatin. Therefore, in spite of the lower inhibitory activity *in vitro* of **7l** toward sterol biosynthesis in comparison with Pravastatin, the cholesterol-lowering effect of **7l** *in vivo* was nearly equal to that of Pravastatin.

Conclusions

4-[1-(Substituted phenyl)-2-oxo-pyrrolidin-4-yl]methyl-oxybenzoic acids and related compounds were prepared and evaluated for their abilities to inhibit fatty-acid and sterol biosyntheses using rats' liver slices *in vitro*. The compounds **7e**, **7g–i**, **7k**, **7r**, **21**, **23** and **29a** showed potent inhibitory activities for fatty-acid and sterol biosyntheses. These compounds were also slightly more potent than compounds **I**, **II** and **III**, and more potent than the reference Clinofibrate. Compound **21** was the most potent, having IC_{50} of 4.4 x 10^{-6} M and 6.6 x 10^{-6} M for the inhibition of fatty-acid and sterol biosyntheses, respectively. Several compounds possessing inhibitory capacities toward both biosynthetic pathways *in vitro* were found in this series. Moreover, from the results of the *in vivo* study on **7e** and **7l**, we found that these compounds had dual action, reducing both plasma cholesterols and triglycerides, a feature not possessed by Clinofibrate or Pravastatin. Toxicological evaluation of the potent compounds *in vitro* and *in vivo* has led us to select compound **7l** as a candidate for further development.

Experimental protocols

Chemistry

Melting points were obtained on a Yanagimoto micromelting apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Hitachi R-90H spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed as δ values (ppm). Elemental analysis were carried out with a Yanagimoto C, H, N Corder MT-2.

1-(4-t-Butylphenyl)-5-oxo-pyrrolidine-3-carboxylic acid ***2l***

The title compound **2l** was prepared from itaconic acid (9.1 g, 70 mmol) and 4-*t*-butylaniline (10.4 g, 70 mmol) according to the method of Paytash [20] and Anschütz [21]. Yield: 17.3 g, 95.1%; mp: 208–209°C. The other compounds **2a–k** and **2m–r** were prepared by the same method as **2l** using appropriately substituted anilines. Physical and chemical data are listed in table I.

*Methyl 1-(4-t-butylphenyl)-5-oxo-pyrrolidine-3-carboxylate **3l***
Catalytic conc H_2SO_4 (0.3 ml) was added to a solution of **2l** (16.1 g, 61.7 mmol) in dichloromethane (80 ml) and methanol (25 ml). The reaction mixture was refluxed for 14 h and evaporated *in vacuo*. The residue was triturated with water, the resulting precipitate was collected and washed with sodium bicarbonate solution to give the ester **3l** (16.7 g, 98.4%) as a white solid; mp: 114–115°C; ^{1}H-NMR ($CDCl_3$) δ 1.31 (9H, s), 2.81–2.92 (2H, m), 3.21–3.57 (1H, m), 3.77 (3H, s), 4.00–4.11 (2H, m), 7.31–7.56 (4H, m); anal $C_{16}H_{21}NO_3$ (C, H, N).

The other compounds **3a–k**, **3m**, **3n** and **3s–v** were prepared by the same method as **3l**. In the cases of **3c**, **3d**, **3i–3k**, **3n** and **3u**, after the trituration with water, the whole was extracted with chloroform, the organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by column chromatography to give the ester **3**. Physical and chemical data are listed in table I.

*Methyl 1-(4-ethoxyphenyl)-5-oxo-pyrrolidine-3-carboxylate **3o***
A mixture of **3m** (5.0 g, 21.3 mmol), ethyl bromide (3.0 g, 27.5 mmol) and potassium carbonate (4.5 g, 32.6 mmol) in DMF (50 ml) was stirred for 12 h at 80°C. The reaction mixture was evaporated *in vacuo*. The residue was triturated with ice-water, the resulting precipitate was collected and washed with water to give **3o** (5.1 g, 91.4%) as a white solid; mp: 67–69°C; ^{1}H-NMR($CDCl_3$) δ 1.40 (3H, t, J = 6.9 Hz), 2.85–2.91 (2H, m), 3.30–3.42 (1H, m), 3.78 (3H, s), 3.96–4.11 (4H, m), 6.89 (2H, d, J = 9.0 Hz), 7.45 (2H, d, J = 9.0 Hz); anal $C_{14}H_{17}NO_4$ (C, H, N).

The other compounds **3p–r** were prepared by the same method as **3o**. Physical and chemical data are listed in table I.

*1-(4-t-Butylphenyl)-4-hydroxymethyl-2-oxo-pyrrolidine **4l***
Methanol (17.6 ml) was added dropwise to a suspension of **3l** (16.5 g, 60 mmol) and sodium borohydride ($NaBH_4$) (2.5 g, 66 mmol) in THF (100 ml) under refluxing. The reaction mixture was stirred for 2 h at the same reaction temperature and evaporated *in vacuo*. The residue was triturated with ice-water, the resulting precipitate was collected and washed with water to give **4l** (14.6 g, 98.5%) as a white solid; mp: 89–90°C; ^{1}H-NMR ($CDCl_3$) δ 1.30 (9H, s), 2.83–2.93 (5H, m), 3.53–4.05 (4H, m), 7.39 (2H, d, J = 9.2 Hz), 7.49 (2H, d, J = 9.2 Hz); anal $C_{15}H_{21}NO_2$ (C, H, N).

The other compounds **4a–k** and **4m–v** were prepared by the same method as **4l**. In the cases of **4b–d**, **4i**, **4k**, **4o**, **4p**, **4s** and **4t**, after the trituration with water, the resulting residue was extracted with chloroform. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by column chromatography to give the alcohol **4**. Physical and chemical data are listed in table I.

*Methyl 4-[1-(4-t-butylphenyl)-2-oxo-pyrrolidin-4-yl]-methyloxybenzoate **6l***
A solution of mesyl chloride (5.8 g, 11.1 mmol) in dichloromethane (20 ml) was added dropwise to a solution of **4l** (25.0 g, 10.1 mmol) and triethylamine (16.8 ml) in dichloromethane (150 ml) under ice cooling. The reaction mixture was stirred at the same temperature for 1 h and evaporated *in vacuo*. The resulting residue was extracted with chloroform and then washed with water. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. The crude product was purified by a column chromatography to give **5l** (32.5 g, 98.7%) as a viscous oil; ^{1}H-NMR($CDCl_3$) δ 1.31 (9H, s), 2.40–2.50 (1H, m), 2.76–3.00 (2H, m), 3.05 (3H, s), 3.68–3.80 (1H, m), 3.94–4.08 (1H, m), 4.20–4.30 (2H, m), 7.39 (2H, d, J = 8.9 Hz), 7.49 (2H, d, J = 8.9 Hz).

A mixture of **5l** (9.0 g, 27.7 mmol), methyl 4-hydroxybenzoate (4.2 g, 27.7 mmol) and potassium carbonate (5.7 g, 41.5 mmol) in DMF (100 ml) was stirred for 8 h at 80°C. The reaction mixture was evaporated *in vacuo*. The residue was triturated with ice-water, the resulting precipitate was collected and washed with water to give **6l** (8.9 g, 89.3%) as a white solid; mp: 142–144°C; ^{1}H-NMR($CDCl_3$) δ 1.27 (9H, s), 2.55 (1H, d), 2.80–3.10 (2H, m), 3.78–3.88 (1H, m), 3.34 (3H, s), 4.00–4.20 (3H, m), 7.04 (2H, d, J = 8.6 Hz), 7.38 (2H, d, J = 8.6 Hz), 7.56 (2H, d, J = 8.6 Hz), 7.88 (2H, d, J = 8.6 Hz); anal $C_{23}H_{27}NO_4$ (C, H, N).

The other compounds **6a–k** and **6n–v** were prepared by the same method as **6l**. All compounds **6** except for **6l** were used immediately without purification of **5** in the next step. Physical and chemical data are listed in table I.

*4-[1-(4-t-Butylphenyl)-2-oxo-pyrrolidin-4-yl]methyloxybenzoic acid **7l***
A suspension of **6l** (22.5 g, 59 mmol), 2 N KOH (45 ml) in ethanol (150 ml) was refluxed for 16 h. The reaction mixture was concentrated up to about 50 ml *in vacuo* and acidified with conc HCl under ice cooling. The resulting precipitate was collected and washed with water to give **7l** (21.3 g, 98.1%) as a white solid; mp: 258–260°C; ^{1}H-NMR($CDCl_3$) δ 1.27 (9H, s), 2.55 (1H, d), 2.65–3.00 (2H, m), 3.68–3.80 (1H, m), 3.95–4.20 (3H, m), 7.04 (2H, d, J = 8.6 Hz), 7.38 (2H, d, J = 8.6 Hz), 7.57 (2H, d, J = 8.6 Hz), 7.89 (2H, d, J = 8.9 Hz); anal $C_{22}H_{25}NO_4$ (C, H, N).

The other compounds **7a–k** and **7m–v** were prepared by the same method as **7l**. Physical and chemical data are listed in table II.

Methyl 4-[1-(4-hydroxypheny]-2-oxo-pyrrolidin-4-yl]methyloxybenzoate **6m**
A suspension of **6r** (2.0 g, 4.6 mmol), 10% Pd-C (0.2 g) in methanol (20 ml) and chloroform (50 ml) was stirred at atmospheric pressure under a hydrogen atmosphere at room temperature for 22 h. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was triturated with 50% aqueous methanol, the resulting precipitate was collected and washed with water to give **6m** (1.48 g, 93.8%) as a white solid; mp: 141–143°C; anal $C_{19}H_{19}NO_5 \cdot 1/5H_2O$ (C, H, N).

*1-(4-Chlorophenyl)-6-oxo-piperidine-3-carboxylic acid **8***
Compound **8** was prepared from 4-chloroaniline (15.0 g, 11.8 mmol) and methyleneglutaric acid [19] (18.4 g, 11.8 mmol) by the same method as **2l**. Yield 22.5 g, 75.6%; mp: 184–187°C; anal $C_{12}H_{12}ClNO_3$ (C, H, N).

*Methyl 1-(4-chlorophenyl)-6-oxo-piperidine-3-carboxylate **9***
Compound **9** was prepared from **8** (10.0 g, 39.4 mmol) by the same method as **3l**. Yield: 9.5 g, 90.0%; viscous oil; ^{1}H-NMR ($CDCl_3$) δ 2.15–2.20 (2H, m), 2.50–2.70 (2H, m), 2.95–3.10 (1H, m), 3.76 (3H, s), 3.85–3.98 (2H, m), 7.20 (2H, d, J = 8.9 Hz), 7.35 (2H, d, J = 8.9 Hz).

*1-(4-Chlorophenyl)-5-hydroxymethyl-2-oxo-piperidine **10***
Compound **10** was prepared from **9** (9.5 g, 35.5 mmol) by the same method as **4l**. Yield 4.5 g, 53.3%; viscous oil; ^{1}H-NMR ($CDCl_3$) δ 1.48–2.94 (6H, m), 3.36–3.81 (4H, m), 7.13–7.40 (4H, m).

*Methyl 4[1-(4-chlorophenyl)-2-oxopiperidin-5-yl]methyloxybenzoate **12***
Compound **12** was prepared from **10** (4.0 g, 16.8 mmol) *via* **11** by the same method as **6l**. Yield 4.0 g, 63.6%; mp: 148–150°C;

^{1}H-NMR ($CDCl_3$) δ 1.78 (2H, m), 2.57–2.74 (3H, m), 3.49–4.24 (7H, m), 6.89 (2H, d, J = 9.0 Hz), 7.22 (2H, d, J = 9.0 Hz), 7.33 (2H, d, J = 9.0 Hz), 7.99 (2H, d, J = 9.0 Hz); anal $C_{20}H_{20}ClNO_4$ (C, H, N).

4-[1-(4-Chlorophenyl)-2-oxo-piperidin-5-yl]methyloxybenzoic acid ***13***
Compound **13** was prepared from **12** (4.0 g, 10.7 mmol) by the same method as **7l**. Yield 2.4 g, 62.3%; mp: 195–197°C; anal $C_{19}H_{18}ClNO_4$ (C, H, N).

1-(4-Chlorophenyl)-4-cyanomethyl-2-oxo-pyrrolidine ***14***
A suspension of mesylate **5e** (8.0 g, 26.4 mmol) and sodium cyanide (1.6 g, 32.7 mmol) in DMF (40 ml) was stirred at 100°C for 22 h. The reaction mixture was evaporated *in vacuo*, the residue was extracted with ethyl acetate, and then washed with water. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo*. The residue was triturated with 20% aqueous methanol, the resulting precipitate was collected and washed with water to give **14** (5.2 g, 84.1%) as a white solid; mp: 92–94°C; ^{1}H-NMR ($CDCl_3$) δ 2.28 (5H, m), 3.56–3.73 (1H, m), 7.34 (2H, d, J = 9.3 Hz), 7.52 (2H, d, J = 9.3 Hz); anal $C_{12}H_{11}ClN_2O$ (C, H, N).

Methyl 1-(4-chlorophenyl)-5-oxo-pyrrolidine-3-acetate ***15***
A mixture of **14** (5.0 g, 21.3 mmol) and NaOH (2.6 g, 65 mmol), ethanol (2.5 ml) in water (15 ml) was refluxed for 8 h. The reaction mixture was acidified with conc HCl under ice cooling and extracted with dichloromethane. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo* to give a carboxylic acid as a crude oily product. A catalytic conc H_2SO_4 (0.11 ml) was added to a solution of the obtained crude carboxylic acid in methanol (50 ml). The reaction mixture was refluxed for 15 h and evaporated *in vacuo*. The residue was extracted with ethyl acetate and washed with water. The organic layer was dried ($MgSO_4$) and evaporated *in vacuo* to give **15** (5.4 g, 94.7%) as a white solid; mp: 65–67°C; ^{1}H-NMR ($CDCl_3$) δ 2.17–3.00 (5H, m), 3.47–3.71 (4H, m), 3.96–4.16 (1H, m), 7.32 (2H, d, J = 9.2 Hz), 7.54 (2H, d, J = 9.2 Hz); anal $C_{13}H_{14}ClNO_3$ (C, H, N).

1-(4-Chlorophenyl)-4-hydroxyethyl-2-oxo-pyrrolidine ***16***
Compound **16** was prepared from **15** (5.2 g, 19.4 mmol) by the same method as **4l**. Yield 4.2 g, 90.2%; viscous oil; ^{1}H-NMR ($CDCl_3$) δ 1.69–1.89 (3H, m), 2.15–2.91 (3H, m), 3.45–4.05 (4H, m), 7.31 (2H, d, J = 9.0 Hz), 7.53 (2H, d, J = 9.0 Hz).

Methyl 4-[2-[1-(4-chlorophenyl)-2-oxo-pyrrolidin-4-yl]ethyloxy]-benzoate ***18***
Compound **18** was prepared from **16** (4.2 g, 17.5 mmol) *via* **17** by the same method as **6l**. Yield 5.4 g, 82.4%; mp: 126–128°C; ^{1}H-NMR($CDCl_3$) δ 1.96–2.15 (2H, m), 2.34–2.96 (3H, m), 6.90 (2H, d, J = 8.8 Hz), 7.32 (2H, d, J = 8.8 Hz), 7.54 (2H, d, J = 8.8 Hz), 7.99 (2H, d, J = 8.8 Hz); anal $C_{20}H_{20}ClNO_4$ (C, H, N).

4-[2-[1-(4-Chlorophenyl)-2-oxo-pyrrolidin-4-yl]ethyloxy]-benzoic acid ***19***
Compound **19** was prepared from **18** (5.2 g, 13.9 mmol) by the same method as **7l**. Yield 4.8 g, 96.0%; mp: 228–229°C; anal $C_{19}H_{18}ClNO_4 \cdot 1/2H_2O$ (C, H, N).

Methyl 4-[1-(4-chlorophenyl)-2-oxo-pyrrolidin-4-yl]methyloxy cinnamate ***20***
Compound **20** was prepared from **5e** (3.0 g, 10 mmol) and methyl 4-hydroxycinnamate [22] (1.8 g, 10 mmol) by the same method as **6l**. Yield 3.3 g, 85.6%; mp: 137–138°C; ^{1}H-NMR ($CDCl_3$) δ 2.34–3.13 (3H, m), 3.70–4.13 (7H, m), 6.31 (2H, d, J = 6.0 Hz), 6.89 (2H, d, J = 8.8 Hz), 7.26–7.73 (7H, m); anal $C_{21}H_{20}ClNO_4$ (C, H, N).

4-[1-(4-Chlorophenyl)-2-oxo-pyrrolidin-4-yl]methyloxycinnamic acid ***21***
Compound **21** was prepared from **20** (3.0 g, 7.8 mmol) by the same method as **7l**. Yield 2.5 g, 86.5%; mp: 206–207°C; anal $C_{20}H_{18}ClNO_4$ (C, H, N).

Methyl 3-[4-[1-(4-chlorophenyl)-2-oxo-pyrrolidin-4-yl]methyl-oxyphenyl] propionate ***22***
A suspension of **20** (3.0 g, 7.8 mmol), 10% Pd-C (0.5 g) in ethyl acetate (50 ml) was stirred at atmospheric pressure under hydrogen atmosphere at room temperature for 12 h. The reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was triturated with 10% aqueous methanol and the resulting precipitate was collected and washed with water to give **22** (2.5 g, 83.0%) as a white solid; mp: 113–115°C; ^{1}H-NMR($CDCl_3$) δ 2.39–3.04 (7H, m), 3.66–4.12 (7H, m), 6.81 (2H, d, J = 8.8 Hz), 7.12 (2H, d, J = 8.8 Hz), 7.31 (2H, d, J = 9.2 Hz), 7.59 (2H, d, J = 9.2 Hz); anal $C_{21}H_{22}ClNO_4$ (C, H, N).

3-[4-[1-(4-Chlorophenyl)-2-oxo-pyrrolidin-4-yl]methyloxy-phenyl]propionic acid ***23***
Compound **23** was prepared from **22** (2.0 g, 5.2 mmol) by the same method as **7l**. Yield 1.6 g, 83.0%; mp: 122–123°C; anal $C_{20}H_{20}ClNO_4$ (C, H, N).

Methyl 2-[1-(4-chlorophenyl)-2-oxo-pyrrolidin-4-yl]methyloxy-benzoate ***24a***
Compound **24a** was prepared from **5e** (3.0 g, 10 mmol) and methyl 2-hydroxybenzoate (1.6 g, 10.5 mmol) by the same method as **6l**. Yield 3.5 g, 98.5%; viscous oil; ^{1}H-NMR ($CDCl_3$) δ 2.34–3.17 (3H, m), 3.83–4.24 (7H, m), 6.91–7.09 (2H, m), 7.26–7.36 (3H, m), 7.61 (2H, d, J = 9.2 Hz), 7.75–7.84 (1H, m).

Compound **24b**: yield: 77.5%; mp: 106–107°C; ^{1}H-NMR ($CDCl_3$) δ 2.36–3.14 (3H, m), 3.57–4.12 (7H, m), 7.04–7.71 (8H, m); anal $C_{19}H_{18}ClNO_4$ (C, H, N).

2-[1-(4-Chlorophenyl)-2-oxo-pyrrolidin-4-yl]methyloxybenzoic acid ***25a***
Compound **25a** was prepared from **24a** (3.0 g, 8.3 mmol) by the same method as **7l**. Yield 1.5 g, 52.0%; mp: 155–156°C; anal $C_{18}H_{16}ClNO_4$ (C, H, N).

Compound **25b**: yield: 84.5%; mp: 156–158°C; anal $C_{18}H_{16}ClNO_4$ (C, H, N).

1-(4-Chlorophenyl)-3-hydroxymethylpyrrolidine ***26a***
Lithium aluminum hydride (2.93 g, 77.1 mmol) was added in several portions to a suspension of **2e** (4.6 g, 19.2 mmol) in THF (90 ml) under ice cooling and the reaction mixture was gently refluxed for 20 h. A mixture of small amount of water and ethyl acetate (100 ml) was then added and the reaction mixture was filtered. The filtrate was evaporated *in vacuo* and the crude product was purified by a column chromatography to give **26a** (4.0 g, 99.0%) as a viscous oil; ^{1}H-NMR ($CDCl_3$) δ 1.63–2.71 (4H, m), 2.99–3.49 (4H, m), 3.66 (2H, d, J = 6.4 Hz), 6.45 (2H, d, J = 8.9 Hz), 7.14 (2H, d, J = 8.9 Hz).

Compound **26b**: yield: 97.3%; viscous oil; ^{1}H-NMR ($CDCl_3$) δ 1.15–1.98 (6H, m), 2.43–2.87 (2H, m), 3.37–3.73 (4H, m), 6.87 (2H, d, J = 9.1 Hz), 7.17 (2H, d, J = 9.1 Hz).

Methyl 4-[1-(4-chlorophenyl)pyrrolidin-3-yl]methyloxybenzoate ***28a***

Compound **28a** was prepared from **26a** (3.0 g, 14.2 mmol) *via* **27a** by the same method as **6l**. Yield: 2.6 g, 55.1%; mp: 89–90°C; ^{1}H-NMR ($CDCl_3$) δ 1.82–2.43 (2H, m), 2.70–3.61 (5H, m), 3.88 (3H, s), 4.03 (2H, d, J = 6.6 Hz), 6.48 (2H, d, J = 9.0 Hz), 6.91 (2H, d, J = 9.0 Hz), 7.16 (2H, d, J = 9.0 Hz), 7.99 (2H, d, J = 9.0 Hz); anal $C_{19}H_{20}ClNO_3 \cdot 1/5H_2O$ (C, H, N).

Compound **28b**: yield: 54.5%; mp: 150–151°C; anal $C_{20}H_{22}ClNO_3$ (C, H, N).

4-[1-(4-Chlorophenyl)pyrrolidin-3-yl]methyloxybenzoic acid ***29a***

The title compound **29a** was prepared from **28a** (2.0 g, 5.8 mmol) by the same method as **7l**. Yield: 1.7 g, 88.6%; mp: 200–203°C; anal $C_{18}H_{18}ClNO_3 \cdot 1/2H_2O$ (C, H, N).

Compound **29b**: yield: 80.7%; mp: 214–217°C; anal $C_{19}H_{20}ClNO_3$ (C, H, N).

Biology

Inhibitory activities toward sterol and fatty-acid biosyntheses using rats' liver slices in vitro

Male Wistar rats (weighing about 200 g) were killed, their livers were taken out, perfused with cold Krebs-Ringer bicarbonate (KRB) solution and cut into small slices. Using the small liver slices, the test was carried out according to the methods of Bortz [23] and Tsujita [18]. Small liver slices (100 mg) were weighed and added into the KRB (1 ml) containing [^{14}C]acetic acid (2 μCi/2 μmol) and the prescribed amount of test compounds, and the mixture was reacted with shaking at 37°C for 2 h under an atmosphere of 95% O_2/5% CO_2. Thereafter, to the reaction mixture was added 15% solution of potassium hydroxide in ethanol (1 ml), and further heated at 75°C for 2 h. After cooling, petroleum ether (2 ml) was added to the mixture, and it was shaken and separated into layers. The organic layer (upper layer) was extracted and concentrated to dryness. Digitonin solution (1 ml) was then added and sterols were collected in the resulting precipitation fraction. This fraction was washed with diethyl ether and dissolved in acetic acid (1 ml), and the radioactivity of the sample was measured to determine the inhibitory activity toward sterol biosynthesis. On the basis of the value obtained in the control test, in which the above procedure was repeated except that no test compound was used, the concentration (μM) of the test compound inhibiting 50% inhibitory concentration (IC_{50}) was obtained. On the other hand, hydrochloric acid was added to the lower layer obtained by extraction with petroleum ether in the above procedure, and the mixture was extracted with petroleum ether under acidic conditions and the organic layer was concentrated and then the radioactivity was measured likewise to determine the inhibitory activity toward fatty-acid biosynthesis. In the same way as above, on the basis of the inhibitory activity for fatty-acid biosynthesis obtained in the control test, the 50% inhibitory concentration (IC_{50}) toward fatty-acid biosynthesis of the test compounds was determined. The results obtained are shown in table III.

Hypolipidemic effects in Japanese white rabbits in vivo

This test was carried out according to the method of Okada [25] and male Japanese white rabbits (weighing 1.9–2.1 kg) were fed a normal commercial chaw pallet (CR-2, Clea Japan Inc) alone or the same chaw containing an added test compound. The rabbits were used after they had been subjected to 2 weeks prefeeding. The test compounds were dissolved in chloroform/methanol (3:1) and the mixture was homogeneously blended into CR-2 solid feed at a percentage of 0.25%, and then the solvent was removed. The rabbits were divided into groups (3 rabbits per each group) and were fed with 100–120 g feed (100 g/less than 2.5 kg body weight, 110 g/2.5–3.0 kg body weight, 120 g/more than 3.0 kg body weight) at 9 am each day for 10 d. Before the feeding, blood was taken from ear venula with the lapse of time and at the same time their weight and the uptake of feed were measured. The plasma lipids (total cholesterols and triglyceride) were determined by enzyme assay with an automatic analyzer using commercial Kits, Kyowa Medex (total cholesterol; Determiner-TC 555, triglyceride; Determiner-TG-S). After administration of the compound for 10 d, the plasma lipids of rabbits were measured using the same procedure as described above. The reducing effects of plasma cholesterols and triglyceride were revealed as the changing rate (%) from the control value.

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