Synthesis and Biological Evaluation of Quinuclidine Derivatives Incorporating Phenothiazine Moieties as Squalene Synthase Inhibitors

Tsukasa ISHIHARA,* Hirotoshi KAKUTA, Hiroshi MORITANI, Tohru UGAWA, and Isao YANAGISAWA

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co. Ltd.; 21 Miyukigaoka, Tsukuba, Ibaraki 305–8585, Japan. Received May 25, 2004; accepted July 28, 2004

Squalene synthase inhibitors have the potential to be superior hypocholesterolemic agents. A series of quinuclidine derivatives incorporating phenothiazine systems was synthesized in order to investigate the effects of their structure on the inhibition of hamster liver microsomal enzyme. (±)-3-(10-Methyl-10*H*-phenothiazin-3-ylmethoxy)quinuclidine hydrochloride (19) was the most potent inhibitor in this series with an IC_{50} value of 0.12 μM. Oral dosing of compound 19 to hamsters demonstrated effective reduction of both plasma total cholesterol levels and plasma triglyceride levels. Compound 19 showed a reduced tendency to elevate plasma transaminase levels, an indicator of hepatotoxicity. Enantiomerically pure (−)-19, YM-53546, was found to be more potent than the corresponding (+)-enantiomer.

Key words squalene synthase; hypocholesterolemic agent; quinuclidine; hepatotoxicity

In spite of major advances in pharmaceutical and surgical treatments, coronary heart disease remains a major cause of death in the industrialized world.[1,2] Elevated plasma cholesterol is widely accepted as a risk factor for the disease,[3,4] and thus there have been worldwide research efforts to discover antihypercholesterolemic agents. As over 70% of cholesterol in the body is derived from *de novo* cholesterol biosynthesis, inhibition of this biosynthesis presents an effective approach to reduce plasma cholesterol. In particular, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, the inhibitors of the cholesterol biosynthetic pathway, are currently the most effective therapeutic agents in reducing the levels of plasma cholesterol.[5—7] Treatment of hypercholesterolemia with HMG-CoA reductase inhibitors is reported to reduce the risk of the coronary heart disease by approximately one-third.[8]

However, HMG-CoA reductase inhibitors may also prevent the formation of biologically important isoprenoids such as dolicols, ubiquinones, and isopentenyl *t*-RNA, because the inhibitors suppress the cholesterol biosynthetic pathway early on. Moreover, HMG-CoA reductase inhibitors have little effect on plasma triglyceride levels.[9] A study group from the European Atherosclerosis Society has proposed that hypertriglyceridemia is another risk factor for coronary heart disease.[10] An agent that could reduce both plasma cholesterol levels and plasma triglyceride levels without interruption of isoprenoid biosyntheses would therefore be highly desirable.

Squalene synthase (EC 2.5.1.21), which catalyzes the reductive dimerization of two molecules of farnesyl diphosphate to form squalene *via* intermediate presqualene diphosphate, is involved in the first committed step in cholesterol biosynthesis. Inhibition of squalene synthase does not interfere with the biosyntheses of the above-mentioned isoprenoids, because the enzymatic step occurs after the branches leading to the isoprenoids. Furthermore, squalene synthase inhibitors have been reported to lower plasma triglyceride levels as well as plasma cholesterol levels *in vivo*.[11—13] Consequently, squalene synthase is an attractive therapeutic target for the treatment of not only hyperlipidemia but coronary heart disease.

In our previous paper, we disclosed that phenothiazine derivative **2** was equipotent to 3-(4′-fluorobiphenyl-4-yl)quinuclidin-3-ol **1**, which had been a potent inhibitor of squalene synthase reported by Brown *et al.* (Fig. 1),[14] in the hamster microsomal enzyme assay (IC_{50} values of 0.38 and 0.39 μM for compounds **1** and **2**, respectively).[15] Efforts were thus focused on the further modifications of compound **2**. This paper describes the results of our work on the synthesis, structure–activity relationships, and biological activities of the quinuclidine derivatives containing phenothiazine systems as novel squalene synthase inhibitors.

Chemistry

The synthetic pathway for the preparation of compounds **19**—**24** is shown in Chart 1. 3-Formyl-10-methyl-10*H*-phenothiazine (**9**) was synthesized *via* the method stated in the literature,[16] and the related derivatives **10**—**12** were prepared by identical procedures from the known *N*-alkylated phenothiazines.[17] These aldehydes **9**—**12** were in turn converted into the corresponding alcohols **13**—**16** through reduction with sodium borohydride. Phenothiazine 5-oxide derivative **17** and phenothiazine 5,5-dioxide derivative **18** were prepared by oxidation of the phenothiazine derivative **13** using 1.4 and 3.0 eq of *m*-chloroperbenzoic acid, respectively. Coupling of the phenothiazine moiety with 3-quinuclidinol was achieved through the known method of protecting the quinuclidine ring nitrogen atom as a borane complex.[18] 3-Quinuclidinol-*N*-borane (**4**) was allowed to react with chlorides, which were prepared from the alcohols **13**—**18**, in the presence of sodium hydride. Deprotection of the borane complexes with ethanolic hydrogen chloride solution afforded target compounds **19**—**24**. Enantiomerically pure (+)- and (−)-**19** were obtained from the known (+)- and (−)-3-quinu-

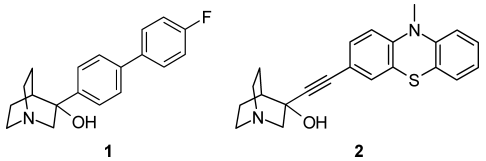

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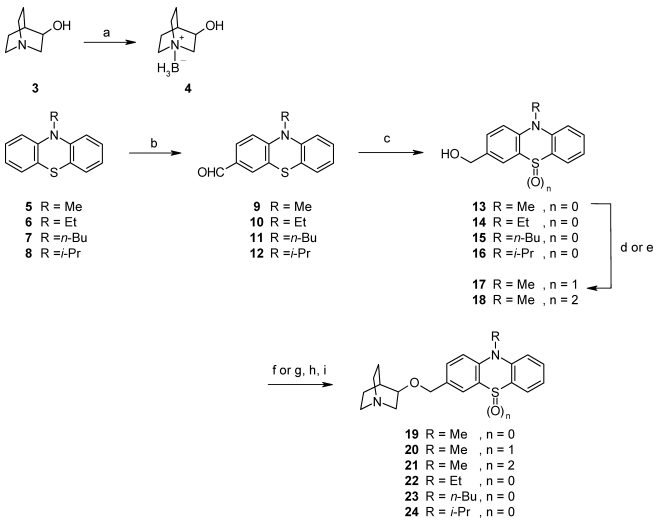
Fig. 1

* To whom correspondence should be addressed. e-mail: ishihat@yamanouchi.co.jp



Reagents: (a) BH_3–THF complex, THF; (b) $POCl_3$, *N*-methylformanilide, 1,2-dichlorobenzene; (c) $NaBH_4$, MeOH; (d) 1.4 eq *m*-CPBA, $CHCl_3$; (e) 3.0 eq *m*-CPBA, CH_2Cl_2; (f) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (g) $SOCl_2$, cat. DMF, $CHCl_3$; (h) NaH, 4, DMF; (i) HCl, EtOH, acetone.

Chart 1. Preparation of Compounds **19**—**24**

clidinol,[19] respectively, in the same manner as the racemic mixture of compound **19**.

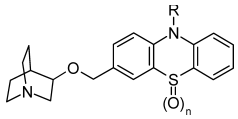
Results and Discussion

All compounds were evaluated by their IC_{50} values for the inhibition of squalene synthase prepared from hamster liver. The inhibitory activities were measured according to the method of Amin *et al.* with a slight modification.[20] The selected compounds were evaluated for lipid lowering effects after oral dosing in hamsters, and their inhibitory activity against squalene synthase derived from human hepatoma (HepG2) cells was also investigated.

The introduction of an ether linkage into the molecule was known to improve its inhibitory activity against squalene synthase for phosphonate-based squalene synthase inhibitors.[21] Also, we had previously reported that a hydroxyl group at the 3-position of the quinuclidine nucleus was unnecessary for the ability to inhibit the enzyme.[15] These two facts were the impetus for our work on an *O*-alkylated 3-quinuclidinol derivative that had an ether linker between the quinuclidine moiety and the tricyclic system without a hydroxy group on the quinuclidine nucleus. Our earlier study also demonstrated that the 10-methyl-10*H*-phenothiazine derivative **2** was a potent inhibitor of squalene synthase,[15] which showed that a 10-methyl-10*H*-phenothiazine system is an attractive moiety for a squalene synthase inhibitor. Using a combination of the results described above, we designed 3-(10-methyl-10*H*-phenothiazin-3-ylmethoxy)quinuclidine (**19**). Compound **19** exhibited a 3-fold enhancement in inhibitory activity over compound **2**, as expected (Table 1).

The improved inhibitory activity observed with compound **19** led us to examine the sensitivity of the structure–activity relationships to structural variations of the phenothiazine moiety. The results are summarized in Table 1. Phenothiazine 5-oxide derivative **20** and phenothiazine 5,5-dioxide derivative **21** sustained at least 8-fold losses in inhibitory activity relative to the phenothiazine derivative **19**. The c-logP values of 10-methyl-10*H*-phenothiazine, 10-methyl-10*H*-phenothiazine 5-oxide, and 10-methyl-10*H*-phenothiazine 5,5-dioxide were 4.49, 1.50, and 1.99, respectively,[22] suggesting that a lipophilic ring system is beneficial for interacting with the enzyme. We therefore explored an incorporation of a bulky substituent into the phenothiazine system in order to increase its lipophilicity. Rather unexpectedly, 10-ethyl-10*H*-phenothiazine derivative **22**, 10-butyl-10*H*-phenothiazine derivative **23**, and 10-(1-methyl)ethyl-10*H*-phenothiazine derivative **24** were less potent inhibitors compared to 10-methyl-10*H*-phenothiazine derivative **19**.[23] These results demonstrated that bulky substituents on the phenothiazine system were undesirable for the inhibition of squalene synthase. The most potent inhibitor in this series was compound **19**.

Table 1. *In Vitro* Activities of Compounds **19**—**24**



Compd.	R	*n*	IC_{50} (μM)[a]
2			0.39
19	Me	0	0.12
20	Me	1	>1
21	Me	2	>1
22	Et	0	0.44
23	*n*-Bu	0	0.56
24	*i*-Pr	0	0.37
1			0.38

a) Compounds were tested for their ability to inhibit the conversion of [^{3}H]farnesyl diphosphate to [^{3}H]squalene by squalene synthase derived from hamster liver. IC_{50} values were determined by a single experimental run in duplicate.

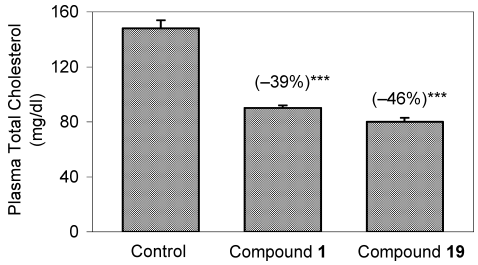


Fig. 2. Effects of Compounds **1** and **19** on Plasma Total Cholesterol Levels after Oral Administration in Hamsters at a Dose of 50 mg/kg/d for 5 d (n=7 and 6, Respectively)

*** $p<0.001$ *versus* control using the Student's *t*-test.

Because of the potent inhibitory activity against squalene synthase, compound **19** was evaluated for its lipid lowering effects; the results are shown in Figs. 2 and 3. Hamsters have plasma lipid compositions very similar to humans,[24] and therefore the animals are suitable for assessments of the potential efficacy of lipid-lowering agents. Compound **19** lowered plasma total cholesterol levels by 46% compared to the control at an oral dose of 50 mg/kg/d for 5 d in hamsters (Fig. 2). Plasma triglyceride levels were also reduced upon oral dosing of compound **19**; a 64% decrease in plasma triglyceride levels was observed (Fig. 3). These results indicated that compound **19** was a potent and orally active squalene synthase inhibitor.

Further investigation of compound **19** revealed an important improvement relative to compound **1**. Compound **1** caused an approximately 3-fold elevation in plasma transami-

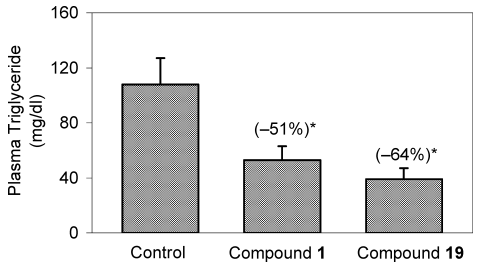


Fig. 3. Effects of Compounds **1** and **19** on Plasma Triglyceride Levels after Oral Administration in Hamsters at a Dose of 50 mg/kg/d for 5 d ($n=7$ and 6, Respectively)

$*p<0.05$ *versus* control using the Student's *t*-test.

Table 2. Effects of Compounds **1** and **19** on Biochemical Parameters in F344 Rats[a)]

Compd.	AST (IU/l)[b)]	ALT (IU/l)[c)]
Vehicle	106	38
19	104	39
1	319*	95

a) Animals were dosed *via* oral gavage at 250 mg/kg/d for 3 d. Data are mean values, and standard errors were less than 30% of the mean ($n=3$). $*p<0.05$ *versus* control using the Student's *t*-test. *b*) Aspartate aminotransferase. *c*) Alanine aminotransferase.

Table 3. *In Vitro* Activities of Enantiomers of Compound **19**

Compd.	IC_{50} (μM)	
	Hamster[a)]	Human[b)]
(+)-**19**	0.20	0.17
(−)-**19**	0.081	0.10

a) Refer to Table 1. *b*) Compounds were tested for their ability to inhibit the conversion of [^{3}H]farnesyl diphosphate to [^{3}H]squalene by squalene synthase from human hepatoma cells. IC_{50} values were determined by a single experimental run in duplicate.

nase levels (AST, ALT) after administration in rats, whereas compound **19** did not show pronounced effects on plasma transaminase levels. Compound **19** did exhibit a reduced acute effect on plasma transaminase levels, an indicator of hepatotoxicity.

Throughout this work, compound **19** was prepared and analyzed as a racemic mixture. It can be assumed that the biological activity of the pure enantiomer could potentially be twice that of the racemic mixture. Thus we performed asymmetric syntheses of both enantiomers of compound **19** and evaluated their inhibitory activities. The (−)-enantiomer of **19** was found to be approximately 2-fold more potent than the corresponding (+)-enantiomer of **19**. The IC_{50} values obtained for the (−)-enantiomer of **19** against hamster and human HepG2 enzymes were 0.081 and 0.10 μM, respectively (Table 3).

Conclusions

A novel series of quinuclidine derivatives containing phenothiazine moieties was synthesized and evaluated for their ability to inhibit squalene synthase. The structure–activity relationships of the prepared compounds provided useful information on the structural requirements for the inhibition of the enzyme. The steric factor of the phenothiazine system is important for activity; replacement of the phenothiazine nucleus with phenothiazine 5-oxide or phenothiazine 5,5-dioxide caused a reduction in *in vitro* potency, and a smaller substituent at the 10-position of the phenothiazine system was preferred for binding to the enzyme. The most potent inhibitor was 10-methyl-10*H*-phenothiazine derivative **19** with an IC_{50} value of 0.12 μM in this series. Compound **19** reduced plasma total cholesterol levels and plasma triglyceride levels by 46% and 64%, respectively, compared to the control, at an oral dose of 50 mg/kg/d for 5 d in hamsters. This compound did not affect plasma transaminase levels despite its good lipid-lowering effects. The (−)-enantiomer of compound **19**, (−)-3-(10-methyl-10*H*-phenothiazin-3-ylmethoxy)quinuclidine hydrochloride (YM-53546), was found to be more potent than the corresponding (+)-enantiomer.

We propose that YM-53546 will be a useful lipid-lowering agent with the potential for the treatment of coronary heart disease.

Experimental

^{1}H-NMR spectra were measured with a JEOL EX90, LA300, or GX500 spectrometer. Chemical shifts are expressed in δ units using tetramethylsilane as the standard (in NMR description, s=singlet, d=doublet, t=triplet, m=multiplet, and br=broad peak). Mass spectra were recorded with a Hitachi M-80 or JEOL JMS-DX300 spectrometer. Melting points were measured with a Yanaco MP-500D melting point apparatus without correction. The optical purity of the optically active compounds were examined using on analytical chiral column (Daicel Chemical Industries. Ltd. CHIRALCEL OJ-H, i.d.=0.46 cm, l=25 cm). The HPLC conditions were as follows: mobile phase, EtOH/*n*-hexane/Et_2NH=500/500/1; flow rate, 0.5 ml/min; detection wavelength, 254 nm. Optical rotation measurements were obtained using a Horiba SEPA-200 polarimeter. All materials and reagents purchased were used without further purification.

(±)-3-Quinuclidinol-*N*-borane (4) A solution of borane–tetrahydrofuran complex in tetrahydrofuran (710 ml, 1.0 M, 710 mmol) was added dropwise to (±)-3-quinuclidinol (**3**) (86.4 g, 680 mmol) in tetrahydrofuran (400 ml) at 0 °C. The reaction mixture was allowed to warm to ambient temperature and stirred for 1 h. The solvent was removed *in vacuo* and the resulting residue was diluted with chloroform. The organic layer was washed with H_2O and then brine, dried over magnesium sulfate, and then concentrated *in vacuo*. The residue was dissolved in diethyl ether (100 ml). To this solution *n*-hexane (400 ml) was added, and the resulting precipitate was filtered to yield the title compound as a colorless solid (60.9 g, 64%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.55—1.72 (2H, m), 1.81—1.91 (1H, m), 2.02—2.21 (2H, m), 2.81—3.14 (5H, m), 3.21—3.29 (1H, m), 4.06—4.12 (1H, m). EI-MS *m/z*: 141 $[M]^+$.

3-Formyl-10-methyl-10*H*-phenothiazine (9) A mixture of 10-methyl-10*H*-phenothiazine (**5**) (9.61 g, 45.1 mmol), *N*-methylformanilide (7.03 g, 52.0 mmol), phosphoryl chloride (7.05 g, 46.0 mmol), and 1,2-dichlorobenzene (10 ml) was stirred at 90 °C for 4 h. After cooling, a solution of sodium acetate (36 g) in H_2O (80 ml) was added dropwise to the reaction mixture. The mixture was extracted with ethyl acetate, and the extract was dried over magnesium sulfate and then concentrated *in vacuo*. The residue was subjected to chromatography over silica gel eluting with *n*-hexane–ethyl acetate (3 : 1 by volume) to give the title compound as a yellow solid (9.19 g, 84%). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 3.41 (3H, s), 6.83 (1H, d, J=5.9 Hz), 6.93—7.29 (4H, m), 7.58—7.70 (2H, m), 9.79 (1H, s). EI-MS *m/z*: 241 $[M]^+$.

3-Hydroxymethyl-10-methyl-10*H*-phenothiazine (13) To a stirred solution of 3-formyl-10-methyl-10*H*-phenothiazine (**9**) (908 mg, 3.76 mmol) in methanol (38 ml) was added sodium borohydride (285 mg, 7.52 mmol) at 0 °C. The reaction mixture was allowed to warm to ambient temperature and stirred for 1 h. The mixture was concentrated *in vacuo*, and the residue was diluted with ethyl acetate. The organic layer was washed with H_2O and then brine, dried over magnesium sulfate, and concentrated *in vacuo* to yield the title compound as a colorless solid (830 mg, 91%). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 3.37 (3H, s), 4.57 (2H, d, J=5.9 Hz), 6.72—7.00 (3H, m), 7.09—7.25 (4H, m). EI-MS *m/z*: 243 $[M]^+$.

(±)-3-(10-Methyl-10*H*-phenothiazin-3-ylmethoxy)quinuclidine Hy-

drochloride (19) To a stirred solution of 3-hydroxymethyl-10-methyl-10*H*-phenothiazine (**13**) (11.7 g, 48.1 mmol) and triethylamine (8.71 ml, 62.5 mmol) in dichloromethane (80 ml) was added methanesulfonyl chloride (4.10 ml, 53.0 mmol) at 0 °C. The reaction mixture was allowed to warm to ambient temperature and stirred for 1 h. After the addition of H_2O (4.0 ml), the reaction mixture was extracted with chloroform. The organic layer was washed with saturated aqueous sodium bicarbonate and then brine, dried over magnesium sulfate, and then concentrated *in vacuo* to yield 3-chloromethyl-10-methyl-10*H*-phenothiazine as a yellow solid (7.98 g, 63%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.36 (3H, s), 4.47 (2H, s), 6.71 (1H, d, J=5.9 Hz), 6.77 (1H, d, J=5.9 Hz), 6.91—6.94 (1H, m), 7.10—7.20 (4H, m). FAB-MS *m/z*: 262 $[M+H]^+$.

To a stirred solution of (±)-3-quinuclidinol-*N*-borane (**4**) (3.38 g, 24.0 mmol) in *N*,*N*-dimethylformamide (35 ml) was added sodium hydride (1.16 g, 29.0 mmol, 60% dispersion in mineral oil) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. To this mixture 3-chloromethyl-10-methyl-10*H*-phenothiazine (7.98 g, 30.5 mmol) in *N*,*N*-dimethylformamide (30 ml) was added at 0 °C. The reaction mixture was stirred for 0.5 h, and then allowed to warm to ambient temperature, and stirred for a further 1 h. After the addition of H_2O (200 ml), the reaction mixture was extracted with ethyl acetate. The organic layer was washed with H_2O and then brine, dried over magnesium sulfate, and then concentrated *in vacuo*. The residue was subjected to chromatography over silica gel eluting with *n*-hexane–dichloromethane–ethyl acetate (70 : 15 : 15 by volume) to yield a yellow solid (5.09 g). The resulting solid (5.09 g) in acetone (20 ml) was treated with hydrogen chloride in ethanol (5 M, 10 ml) at 0 °C. The reaction mixture was stirred for 5 min, and then allowed to warm to ambient temperature, and stirred for a further 15 min. The mixture was diluted with diethyl ether (40 ml). The resulting precipitate was filtered, and washed with ethyl acetate and then diethyl ether to yield the title compound as a light green solid (4.48 g, 83%). mp 220—222 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.65—1.70 (2H, m), 1.81—1.88 (1H, m), 1.91—2.01 (1H, m), 2.28—2.32 (1H, m), 2.95—3.15 (5H, m), 3.31 (3H, s), 3.45—3.49 (1H, m), 3.85—3.88 (1H, m), 4.39 (1H, d, J=11.5 Hz), 4.44 (1H, d, J=11.5 Hz), 6.90—7.00 (3H, m), 7.15—7.25 (4H, m) 10.44 (1H, br s). EI-MS *m/z*: 352 $[M]^+$. *Anal.* Calcd for $C_{21}H_{24}N_2OS \cdot HCl$: C, 64.85; H, 6.48; N, 7.20; S, 8.24; Cl, 9.11. Found: C, 64.57; H, 6.43; N, 7.06; S, 8.22; Cl, 9.21.

Identical procedures provided the following two compounds:

(−)-3-(10-Methyl-10*H*-phenothiazin-3-ylmethoxy)quinuclidine Hydrochloride ((−)-19) The title compound was prepared from (−)-3-quinuclidinol[19] as a colorless solid (t_R=21.8 min, >99.5% ee). mp 220—221 °C. $[\alpha]_D^{25}$=−29.2° (c=0.50, DMSO). The ^{1}H-NMR and mass spectra of the title compound were identical to those observed for (±)-**19**. *Anal.* Calcd for $C_{21}H_{24}N_2OS \cdot HCl$: C, 64.85; H, 6.48; N, 7.20; S, 8.24; Cl, 9.11. Found: C, 64.51; H, 6.51; N, 6.93; S, 8.23; Cl, 9.16.

(+)-3-(10-Methyl-10*H*-phenothiazin-3-ylmethoxy)quinuclidine Hydrochloride ((+)-19) The title compound was prepared from (+)-3-quinuclidinol[19] as a colorless solid (t_R=26.4 min, >99.5% ee). mp 215—217 °C. $[\alpha]_D^{25}$=+28.6° (c=0.50, DMSO). The ^{1}H-NMR and mass spectra of the title compound were identical to those observed for (±)-**19**. *Anal*. Calcd for $C_{21}H_{24}N_2OS \cdot HCl$: C, 64.85; H, 6.48; N, 7.20; S, 8.24; Cl, 9.11. Found: C, 64.45; H, 6.47; N, 6.95; S, 8.23; Cl, 9.44.

3-Hydroxymethyl-10-methyl-10*H*-phenothiazine 5-Oxide (17) *m*-Chloroperbenzoic acid (1.66 g, 9.60 mmol) was added to a stirred suspension of 3-hydroxymethyl-10-methyl-10*H*-phenothiazine **(13)** (1.94 g, 8.00 mmol) in chloroform (30 ml) at 0 °C. The mixture was allowed to warm to ambient temperature and stirred for 1 h. Additional *m*-chloroperbenzoic acid (280 mg, 1.60 mmol) was added at ambient temperature, and the reaction mixture was stirred for 0.5 h. The mixture was washed with saturated aqueous sodium bicarbonate, and the organic layer was dried over magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to chromatography over silica gel eluting with chloroform–methanol (100 : 3 by volume) to yield the title compound as a colorless solid (1.95 g, 94%). ^{1}H-NMR (500 MHz, CDCl3) δ: 2.33 (1H, t, J=5.5 Hz), 3.76 (3H, s), 4.71 (2H, d, J=5.5 Hz), 7.24—7.27 (1H, m), 7.35—7.39 (2H, m), 7.60—7.64 (2H, m), 7.89—7.93 (2H, m). FAB-MS *m/z*: 260 $[M+H]^+$.

(±)-10-Methyl-3-[(3-quinuclidinyloxy)methyl]-10*H*-phenothiazine 5-Oxide (20) In a similar procedure to the one described above, 3-chloromethyl-10-methyl-10*H*-phenothiazine 5-oxide was prepared from 3-hydroxymethyl-10-methyl-10*H*-phenothiazine 5-oxide (**17**) as a colorless solid (87%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.77 (3H, s), 4.66 (1H, d, J=12.0 Hz), 4.68 (1H, d, J=12.0 Hz), 7.28 (1H, d, J=8.0 Hz), 7.37—7.40 (2H, m), 7.61—7.65 (2H, m), 7.92—7.95 (2H, m). FAB-MS *m/z*: 278 $[M+H]^+$.

To a stirred solution of (±)-3-quinuclidinol-*N*-borane (**4**) (917 mg, 6.50 mmol) in *N*,*N*-dimethylformamide (13 ml) was added sodium hydride (310 mg, 6.50 mmol, 60% dispersion in mineral oil) at 0 °C. The reaction mixture was allowed to warm to ambient temperature and stirred for 1 h. To this mixture 3-chloromethyl-10-methyl-10*H*-phenothiazine 5-oxide (1.80 g, 6.48 mmol) in *N*,*N*-dimethylformamide (10 ml) was added at 0 °C. The reaction mixture was stirred for 0.5 h, allowed to warm to ambient temperature, and stirred for a further 3 h. The mixture was concentrated *in vacuo*, and the resulting residue was diluted with chloroform. The organic layer was washed with H_2O and then brine, dried over magnesium sulfate, and then concentrated *in vacuo*. The residue was subjected to chromatography over silica gel eluting with chloroform–methanol (100 : 1 by volume) to yield a colorless solid. The resulting solid in acetone (5.0 ml) was treated with hydrogen chloride in ethanol (5 M, 8.0 ml) at 0 °C. The reaction mixture was stirred for 0.5 h, and then allowed to warm to ambient temperature, and stirred for a further 0.5 h. The mixture was concentrated *in vacuo*. The residue was diluted with chloroform and the organic layer was washed with 10% aqueous potassium carbonate. The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The residue was subjected to chromatography over silica gel eluting with chloroform–methanol–*c.* ammonium hydroxide (90 : 10 : 1 by volume) to yield a colorless solid. The resulting solid was recrystallized from chloroform–ethyl acetate–diethyl ether to yield the title compound as a colorless crystalline solid (920 mg, 48%). mp 164—166 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.32—1.46 (2H, m), 1.82—1.86 (1H, m), 1.90—1.96 (1H, m), 2.06—2.12 (1H, m), 2.66—2.74 (1H, m), 2.76—2.86 (3H, m), 2.92—3.00 (1H, m), 3.10—3.16 (1H, m), 3.46—3.60 (1H, m), 3.77 (3H, s), 4.49—4.54 (1H, m), 4.58—4.63 (1H, m), 7.24—7.26 (1H, m), 7.36—7.39 (2H, m), 7.59—7.64 (2H, m), 7.90—7.94 (2H, m). FAB-MS *m/z*: 369 $[M+H]^+$. *Anal.* Calcd for $C_{21}H_{24}N_2O_2S \cdot 0.2H_2O$: C, 67.79; H, 6.61; N, 7.53; S, 8.62. Found: C, 67.78; H, 6.47; N, 7.40; S, 8.85.

3-Hydroxymethyl-10-methyl-10*H*-phenothiazine 5,5-Dioxide (18) *m*-Chloroperbenzoic acid (2.59 g, 15.0 mmol) was added to a stirred suspension of 3-hydroxymethyl-10-methyl-10*H*-phenothiazine (**13**) (1.22 g, 5.00 mmol) in dichloromethane (15 ml) at 0 °C. The mixture was allowed to warm to ambient temperature and stirred for 18 h. The mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate. The organic layer was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was subjected to chromatography over silica gel eluting with ethyl acetate to yield the title compound as a yellow solid (1.23 g, 89%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.70 (3H, s), 4.75 (2H, s), 7.27—7.31 (3H, m), 7.61—7.66 (2H, m), 8.07 (1H, s), 8.11 (1H, d, J=8.0 Hz). FAB-MS *m/z*: 276 $[M+H]^+$.

(±)-10-Methyl-3-[(3-quinuclidinyloxy)methyl]-10*H*-phenothiazine 5,5-Dioxide Hydrochloride (21) The title compound was prepared in a procedure similar to that described for the synthesis of compound **19** from 3-hydroxymethyl-10-methyl-10*H*-phenothiazine 5,5-dioxide (**18**) as a colorless solid (62%). mp 274—276 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 1.64—1.74 (2H, m), 1.86—1.94 (1H, m), 1.98—2.06 (1H, m), 2.36—2.40 (1H, m), 3.04—3.22 (5H, m), 3.50—3.54 (1H, m), 3.74 (3H, s), 3.93—3.96 (1H, m), 4.61 (1H, d, J=11.5 Hz), 4.65 (1H, d, J=11.5 Hz), 7.35—7.39 (1H, m), 7.61—7.63 (2H, m), 7.74—7.79 (2H, m), 7.98—8.00 (2H, m), 10.34 (1H, br s). FAB-MS *m/z*: 385 $[M+H]^+$. *Anal.* Calcd for $C_{21}H_{24}N_2O_3S \cdot HCl \cdot 0.4H_2O$: C, 58.91; H, 6.07; N, 6.54; S, 7.49; Cl, 8.28. Found: C, 58.93; H, 5.97; N, 6.70; S, 7.31; Cl, 8.42.

The following compounds were prepared in a similar manner described above:

10-Ethyl-3-formyl-10*H*-phenothiazine (10) The title compound was obtained from 10-ethyl-10*H*-phenothiazine (**6**)[17] as a yellow solid (69%). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.44 (3H, t, J=7.1 Hz), 3.96 (2H, q, J=7.1 Hz), 6.84—7.25 (5H, m), 7.56—7.68 (2H, m), 9.78 (1H, s). EI-MS *m/z*: 255 $[M]^+$.

10-Ethyl-3-hydroxymethyl-10*H*-phenothiazine (14) The title compound was obtained from 10-ethyl-3-formyl-10*H*-phenothiazine (**10**) as a yellow oil (quant.). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.40 (3H, t, J=7.0 Hz), 3.91 (2H, t, J=7.0 Hz), 4.55 (2H, d, J=4.1 Hz), 6.76—6.96 (3H, m), 7.06—7.16 (4H, m). EI-MS *m/z*: 257 $[M]^+$.

(±)-3-(10-Ethyl-10*H*-phenothiazin-3-ylmethoxy)quinuclidine (22) The title compound was prepared from 10-ethyl-3-hydroxymethyl-10*H*-phenothiazine (**14**) as a yellow oil (53%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.36—1.44 (5H, m), 1.66—1.72 (1H, m), 1.89—2.06 (2H, m), 2.66—2.82 (4H, m), 2.91—2.96 (1H, m), 3.06—3.11 (1H, m), 3.53—3.55 (1H, m), 3.92 (2H, q, J=7.0 Hz), 4.32 (1H, d, J=11.5 Hz), 4.42 (1H, d, J=11.5 Hz),

6.82—6.91 (3H, m), 7.10—7.15 (4H, m). EI-MS *m/z*: 366 $[M]^+$. *Anal.* Calcd for $C_{24}H_{26}N_2OS \cdot 0.3H_2O$: C, 71.05; H, 7.21; N, 7.53; S, 8.62. Found: C, 70.99; H, 7.26; N, 7.40; S, 8.86.

10-Butyl-3-formyl-10*H*-phenothiazine (11) The title compound was obtained from 10-butyl-10*H*-phenothiazine (**7**)[17] as a yellow oil (68%). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.95 (3H, t, J=6.7 Hz), 1.26—1.89 (4H, m), 3.89 (2H, t, J=7.3 Hz), 6.84—7.60 (7H, m), 9.78 (1H, s). EI-MS *m/z*: 283 $[M]^+$.

10-Butyl-3-hydroxymethyl-10*H*-phenothiazine (15) The title compound was obtained from 10-butyl-3-formyl-10*H*-phenothiazine (**11**) as a yellow oil (quant.). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 0.93 (3H, t, J=6.7 Hz), 1.20—1.87 (4H, m), 3.84 (2H, t, J=7.1 Hz), 4.56 (2H, s), 6.57—7.34 (7H, m). EI-MS *m/z*: 285 $[M]^+$.

(±)-3-(10-Butyl-10*H*-phenothiazin-3-ylmethoxy)quinuclidine (23) The title compound was prepared from 10-butyl-3-hydroxymethyl-10*H*-phenothiazine (**11**) as a yellow oil (18%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.93 (3H, t, J=7.3 Hz), 1.37—1.49 (4H, m), 1.67—1.81 (3H, m), 1.90—1.98 (1H, m), 2.06—2.08 (1H, m), 2.70—2.83 (4H, m), 2.92—2.95 (1H, m), 3.08—3.12 (1H, m), 3.54—3.56 (1H, m), 3.84 (2H, t, J=7.3 Hz), 4.33 (1H, d, J=11.5 Hz), 4.42 (1H, d, J=11.5 Hz), 6.81—6.91 (3H, m), 7.10—7.15 (4H, m). EI-MS *m/z*: 394 $[M]^+$. *Anal.* Calcd for $C_{24}H_{30}N_2OS \cdot 1.5H_2O$: C, 68.37; H, 7.89; N, 6.64; S, 7.61. Found: C, 68.61; H, 7.77; N, 6.62; S, 7.21.

3-Formyl-10-(1-methylethyl)-10*H*-phenothiazine (12) The title compound was obtained from 10-(1-methylethyl)-10*H*-phenothiazine (**8**)[17] as a yellow oil (29%). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.68 (6H, d, J=6.9 Hz), 4.22—4.54 (1H, m), 6.57—7.66 (7H, m), 9.79 (1H, s). EI-MS *m/z*: 269 $[M]^+$.

3-Hydroxymethyl-10-(1-methylethyl)-10*H*-phenothiazine (16) The title compound was obtained from 3-formyl-10-(1-methylethyl)-10*H*-phenothiazine (**12**) as a yellow oil (quant.). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.61 (6H, d, J=6.8 Hz), 4.12—4.43 (1H, m), 4.56 (2H, s), 6.57—7.25 (7H, m). EI-MS *m/z*: 271 $[M]^+$.

(±)-3-{10-(1-Methylethyl)-10*H*-phenothiazin-3-ylmethoxy}quinuclidine (24) The title compound was prepared from 3-hydroxymethyl-10-(1-methylethyl)-10*H*-phenothiazine (**16**) as a yellow oil (7.1%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.34—1.48 (2H, m), 1.62 (6H, d, J=6.5 Hz), 1.66—1.76 (1H, m), 1.89—1.95 (1H, m), 2.06—2.10 (1H, m), 2.70—2.86 (4H, m), 2.94—3.00 (1H, m), 3.10—3.16 (1H, m), 3.35—3.57 (1H, m), 4.24—4.30 (1H, m), 4.33 (1H, d, J=11.5 Hz), 4.43 (1H, d, J=11.5 Hz), 6.89—6.92 (1H, m), 7.00—7.04 (2H, m), 7.08—7.14 (4H, m); EI-MS *m/z*: 380 $[M]^+$; *Anal.* Calcd for $C_{23}H_{28}N_2OS \cdot 1.4H_2O$: C, 68.08; H, 7.65; N, 6.90; S, 7.90. Found: C, 68.00; H, 7.28; N, 6.84; S, 7.93.

Preparation of Microsomes from Hamster Liver and HepG2 Cells Microsomes were prepared from the livers of hamsters and from HepG2 cells, a human hepatoma cell line described previously.[25] The tissues or harvested cells were homogenized in HEPES buffer (50 mM) using a glass homogenizer. Homogenates were centrifuged at 500×***g*** for 5 min at 4 °C, and the resulting supernatants were further centrifuged at 8000×***g*** for 15 min at 4 °C. Microsomes were then isolated from this second supernatant by ultra-centrifugation at 100000×***g*** for 60 min at 4 °C. The microsome precipitates were suspended in HEPES buffer (1—5 mg/ml). Protein was assayed by the Lowry method.[26]

Assay of Squalene Synthase Inhibitory Activity The squalene synthase activities of these microsomes were assayed using the Amin technique with modifications. The test compounds were dissolved in DMSO and the assay was carried out in HEPES buffer (50 mM, pH 7.5) containing: NaF (11 mM), $MgCl_2$ (5.5 mM), dithiothreitol (3 mM), NADPH (1 mM), FPP (5 mM), [^{3}H]-FPP (0.017 mM, 15 Ci/mmol), NB-598 (10 mM), and sodium pyrophosphate decahydrate (1 mM). After pre-incubation of these components at 30 °C for 5 min, the reaction was initiated by the addition of microsomes (10 μg protein). The reaction was carried out at 30 °C for 20 min and then terminated by the addition of 40% KOH–ethanol solution (100 μl, 1 : 1 by volume). Synthesized [^{3}H]-squalene was extracted in petroleum ether after saponification at 60 °C for 30 min and counted in Aquasol-2 using a Beckman liquid scintillation counter.

Plasma Total Cholesterol and Triglyceride Lowering Effects in Hamsters Male Syrian golden hamsters were purchased from Hamri (Ibaraki, Japan). At the start of the study, the 8-week-old animals weighed approximately 140 g. They were kept for a week under reverse diurnal light cycles with the lights off from 07:30 to 20:30. The animals were fed a standard low cholesterol diet (CE-2), and water was provided *ad libitum*. Animals were given test compound orally at a dose of 50 mg/kg of body weight once a day for 5 d. The test compound was suspended in a 0.5% methylcellulose vehicle solution. The no-treatment control group was given an equal volume of the 0.5% methylcellulose vehicle solution. Blood specimens were obtained 2 h after the last dose from animals that had been fasted for 18 h. All plasma samples were analyzed for total cholesterol and triglyceride using a Hitachi 7250 Automatic Analyzer (Tokyo, Japan).

Acute Toxic Study in Rats Five-week-old male F344 rats (from SLC, Shizuoka, Japan) were fed CE-2 diet (CLEA Japan, Inc., Tokyo, Japan) and water *ad libitum*. Rats were given test compounds orally at a dose of 250 mg/kg of body weight once a day for 3 d. The test compound was suspended in a 0.5% methylcellulose vehicle solution. The no-treatment control group was given an equal volume of the 0.5% methylcellulose vehicle solution. In all experiments, blood specimens were obtained from the animals 24 h after the last dose. All plasma samples were analyzed for aspartate aminotransferase and alanine aminotransferase using a Hitachi 7250 Automatic Analyzer (Tokyo, Japan).

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