Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 2403–2414

BIOORGANIC & MEDICINAL CHEMISTRY

Syntheses and Biological Evaluation of Novel Quinuclidine Derivatives as Squalene Synthase Inhibitors

Tsukasa Ishihara,* Hirotoshi Kakuta, Hiroshi Moritani, Tohru Ugawa, Shuichi Sakamoto, Shin-ichi Tsukamoto and Isao Yanagisawa

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan

Received 25 December 2002; accepted 26 February 2003

Abstract—Squalene synthase (E.C. 2.5.1.21) catalyses the reductive dimerization of two molecules of farnesyl diphosphate to form squalene and is involved in the first committed step in cholesterol biosynthesis. Inhibition of this enzyme is therefore an attractive target for hypocholesterolemic strategies. A series of quinuclidine derivatives incorporating a tricyclic system was synthesized and evaluated for their ability to inhibit squalene synthase in vitro. A 9*H*-fluorene moiety was found to be optimal as the tricyclic system for potent inhibitory activity. Improved activity can be achieved with a conformationally constrained three-atom linkage connecting the tricyclic system with the quinuclidine nucleus. Among these compounds, (*Z*)-3-[2-(9*H*-fluoren-2-yloxy)ethylidene]-quinuclidine hydrochloride **31** was found to be a potent inhibitor of squalene synthase derived from hamster liver and human hepatoma cells with IC_{50} values of 76 and 48 nM, respectively. Oral dosing of compound **31** demonstrated effective reduction of plasma non-HDL cholesterol levels in hamsters.

Introduction

A direct correlation between the levels of plasma low-density lipoprotein (LDL) cholesterol and the incidence of coronary heart disease has been demonstrated.[1] The elevated plasma LDL cholesterol is therefore widely accepted to be a major risk factor for the disease.[2a–d] Over 70% of cholesterol in the body is derived from the de novo cholesterol biosynthesis. Inhibitors of the cholesterol biosynthetic pathway, in particular 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are currently the most effective therapeutic agents for reducing the levels of plasma LDL cholesterol.[3a–c] The Scandinavian 4S clinical study with simvastatin, one of the HMG-CoA reductase inhibitors, has also reported an improvement in the survival of patients with existing coronary heart disease.[4] These investigations have encouraged us to research novel inhibitors of the cholesterol biosynthetic pathway.

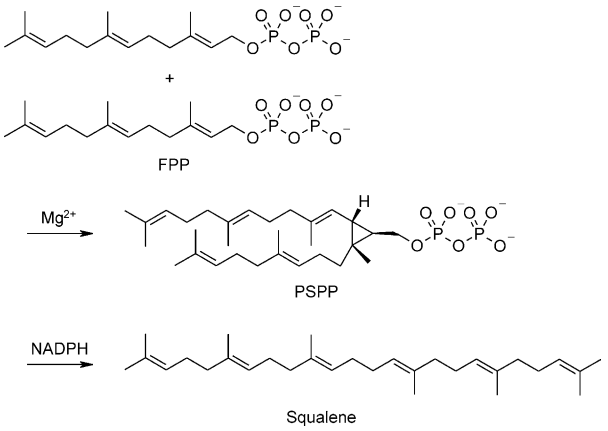
The HMG-CoA reductase inhibitors may also prevent the formation of biologically important isoprenoids such as dolicols, ubiquinones and isopentenyl *t*-RNA because the inhibitors suppress the cholesterol biosynthetic pathway early on. A more selective inhibition of the cholesterol biosynthesis would be accomplished by blocking a step beyond the branches to the isoprenoids in the pathway. Squalene synthase (E.C. 2.5.1.21), which catalyzes the reductive dimerization of two molecules of farnesyl diphosphate (FPP) to form squalene via intermediate presqualene diphosphate (PSPP) (Scheme 1), is involved in the first committed step in the cholesterol biosynthesis. This enzymatic step occurs after the branches to the isoprenoids in the pathway, so inhibition of squalene synthase should minimally affect the biosynthesis of the isoprenoids. A squalene synthase inhibitor is therefore an attractive target for hypercholesterolemia.[5a,b]

Several classes of squalene synthase inhibitors, such as substrate analogues,[6a–g] transition-state analogues,[7a–e] 2,8-dioxabicyclo[3.2.1]octane derivatives,[8a–c] dicarboxylic acid derivatives[9a–e] and quinuclidine derivatives[10a,b] have been reported in the literature. Recently, 4,1-benzoxazepine derivatives[11a–e] and bicyclo[3.2.0]-heptane derivatives[12] have also been described.

Our initial modifications of 3-(4′-fluorobiphenyl-4-yl)-quinuclidin-3-ol **1**, reported by Brown et al. as a potent

*Corresponding author. Tel.: +81-29-852-5111; fax +81-29-852-5387; e-mail: ishihat@yamanouchi.co.jp

0968-0896/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0968-0896(03)00143-3



Scheme 1. The biosynthetic reaction catalyzed by squalene synthase.

squalene synthase inhibitor,[10a] revealed that 3-(dibenzothiophen-2-yl)quinuclidin-3-ol **2** showed comparable inhibitory activity to compound **1** in the hamster microsomal enzyme assay (IC_{50} values of 0.38 and 0.41 μM for compounds **1** and **2**, respectively) (Fig. 1). Efforts were thus focused on the further modifications of compound **2**. In order to improve the inhibitory activity, modifications of the tricyclic system and insertion of various linkages connecting the tricyclic system with the quinuclidine nucleus were carried out.

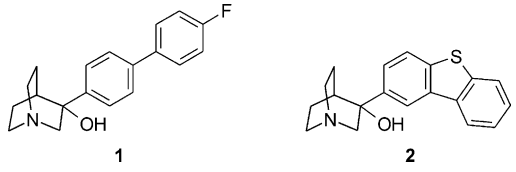
In this paper, we describe the results of our studies on the syntheses and structure–activity relationships of the quinuclidine derivatives containing a tricyclic system as novel squalene synthase inhibitors.

Chemistry

Sonogashira reaction of the known 3-ethynylquinuclidin-3-ol **3**[13] with appropriate aryl bromide produced the desired ethynyl derivatives **4–8** (Scheme 2). *Gem*-dibromovinylidenation[14] of 2-dibenzofurancarboxaldehyde **9**[15] gave the intermediate dibromide **10**, which on treatment with ^{n}BuLi and 3-quinuclidinone yielded the desired ethynyl derivative **11** (Scheme 3). Compound **14** was prepared in a similar procedure from 3-formyl-10-methyl-10*H*-phenothiazine **12**.[16]

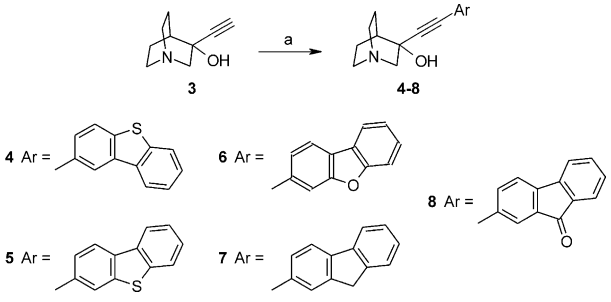
The syntheses of 3-propynylquinuclidin-3-ol derivatives **16** and **19** are shown in Schemes 4 and 5, respectively. Reaction of 2-bromodibenzothiophene **15**[17] with propargyl bromide in the presence of ^{n}BuLi and lithium 2-thienylcyanocuprate[18] followed by reaction with ^{n}BuLi and 3-quinuclidinone produced the desired propynyl derivative **16** (Scheme 4). 2-Hydroxydibenzofuran **17** was reacted with propargyl bromide, and the resulting propyne **18** on treatment with ^{n}BuLi and 3-quinuclidinone produced the desired propynyl derivative **19** (Scheme 5).

Alkylation of a hydroxyl-substituted quinuclidine derivative was achieved with the known methodology[19] of protection for the quinuclidine ring nitrogen atom as a borane complex. 3-(Hydroxymethyl)quinuclidine-*N*-borane[19] was allowed to react with chloride **21**, which was prepared from 2-(hydroxymethyl)dibenzothiophene **20**,[20] in the presence of sodium hydride followed by deprotection of the borane complex with ethanolic hydrogen chloride solution to produce the desired ether **22** (Scheme 6). Methyl 3-hydroxyquinuclidine-3-carboxylate **23**[21] was protected as a borane complex and reduced with borane–tetrahydrofuran complex and a catalytic amount of sodium borohydride[22] to give the intermediate diol **24**. When the resulting diol **24** was treated with chloride **21** in the presence of sodium hydride followed by deprotection of the borane complex

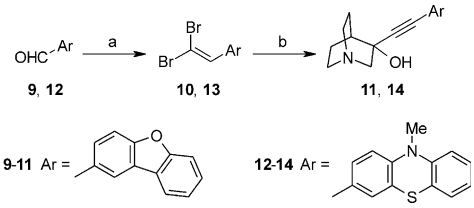


1 2

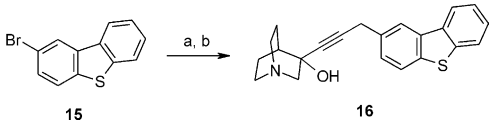
Figure 1. Structures of quinuclidine-based squalene inhibitors.



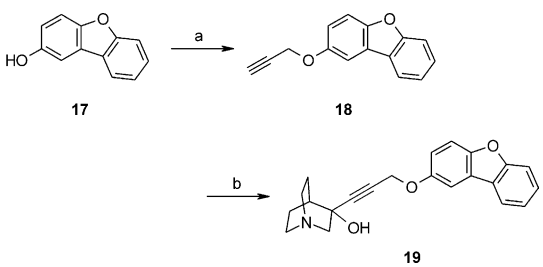
Scheme 2. (a) Aryl bromide, cat. $Pd(PPh_3)_2Cl_2$, cat. CuI, Et_3N, DMF.



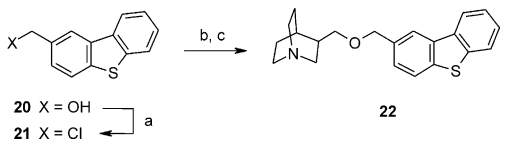
Scheme 3. (a) CBr_4, PPh_3, Zn, CH_2Cl_2; (b) ^{n}BuLi, THF, 3-quinuclidinone.



Scheme 4. (a) ^{n}BuLi, lithium 2-thienylcyanocuprate, THF, propargyl bromide; (b) ^{n}BuLi, THF, 3-quinuclidinone.



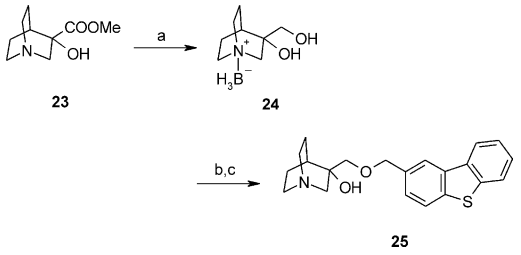
Scheme 5. (a) Propargyl bromide, K_2CO_3, DMF; (b) ^{n}BuLi, THF, 3-quinuclidinone.



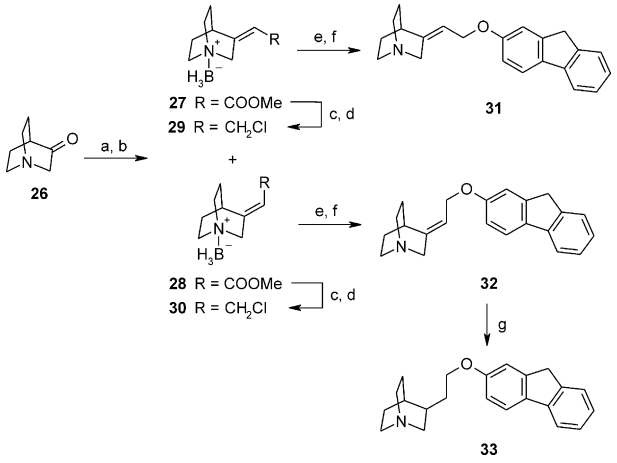
Scheme 6. (a) $SOCl_2$, cat. DMF; (b) 3-(hydroxymethyl)quinuclidine-*N*-borane, NaH, DMF; (c) HCl, EtOH, acetone.

with ethanolic hydrogen chloride solution, this yielded the desired ether **25** (Scheme 7).

The syntheses of 3-ethylidenequinuclidine derivatives **31**–**33** are shown in Scheme 8. A Wadsworth–Emmons reaction of 3-quinuclidinone with trimethyl phosphonoacetate in the presence of sodium hydride followed by protection of the quinuclidine ring nitrogen atom as a borane complex produced the intermediate α,β-unsaturated esters **27** and **28** as a 1:1 mixture of stereoisomers, which were separated by silica gel column chromatography. The stereochemistry of these α,β-unsaturated esters was determined by NMR spectroscopy (Fig. 2). NOE difference experiment on one isomer showed signal enhancement to the methine proton at the 4-position of the quinuclidine nucleus (δ 2.63–2.67) on irradiation of vinyl proton (δ 5.76), thereby establishing its structure as *Z*-isomer **27**. A similar NOE difference experiment on the other isomer revealed its structure as *E*-isomer **28**. Irradiation of vinyl proton (δ 5.68) resulted in signal enhancement to the methylene proton at the 2-position of the quinuclidine nucleus (δ 3.66–3.68). Reduction with diisobutylaluminum hydride of the *Z*-α,β-unsaturated ester **27** followed by chlorination yielded the intermediate chloride **29**. Reaction of the resulting chloride **29** with 2-hydroxy-9*H*-fluorene in the presence of potassium carbonate followed by deprotection of the borane complex with ethanolic hydrogen chloride solution afforded the desired (*Z*)-3-ethylidenequinuclidine derivative **31**. The *E*-α,β-unsaturated ester **28** was similarly treated to give the desired (*E*)-3-ethylidenequinuclidine derivative **32**. Catalytic hydrogenation of the olefin **32** in the presence of rhodium on aluminum[23] yielded the desired ethyl derivative **33**.



Scheme 7. (a) Borane–THF complex, cat. $NaBH_4$, THF; (b) **21**, NaH, DMF; (c) HCl, EtOH, acetone.



Scheme 8. (a) Trimethyl phosphonoacetate, NaH, THF; (b) borane–THF complex, THF; (c) diisobutylaluminum hydride, toluene; (d) methanesulfonyl chloride, lithium chloride, Et_3N, CH_2Cl_2; (e) 2-hydroxy-9*H*-fluorene, K_2CO_3, DMF; (f) HCl, EtOH, acetone; (g) rhodium on alumina, H_2, EtOH.

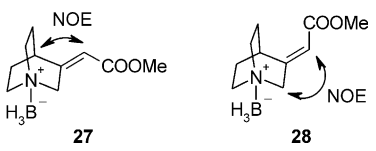
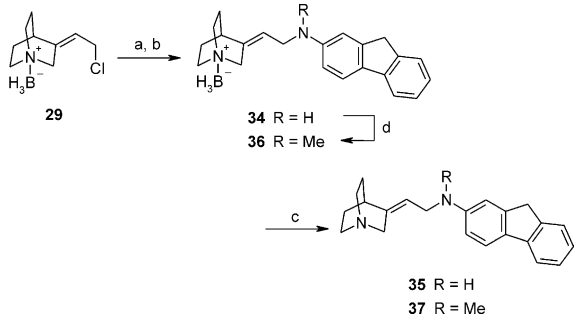


Figure 2. Structures of methyl (3-quinuclidinylidine)acetate derivatives.

The amino analogues **35** and **37** were prepared as follows (Scheme 9). The chloride **29** was allowed to react with 2-trifluoroacetylamino-9*H*-fluorene in the presence of potassium carbonate to provide the intermediate amine **34** after deprotection of the trifluoroacetyl moiety with aqueous potassium carbonate.[24] Deprotection of the borane complex of the resulting amine **34** with ethanolic hydrogen chloride solution gave the desired amine **35**. Reductive amination of formaldehyde with amine **34** using sodium triacetoxyborohydride as the reducing agent afforded the corresponding *N*-methylamino intermediate **36**, which on treatment with ethanolic hydrogen chloride solution gave the desired *N*-methylamine **37**.

Results and Discussion

All compounds were evaluated by 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.[25] The selected compound was evaluated for non-HDL cholesterol lowering effect after oral dosing in hamsters and for the inhibition of squalene synthase derived from human hepatoma (HepG2) cells.

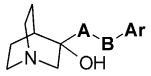


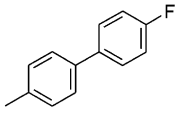
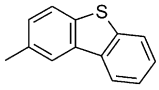
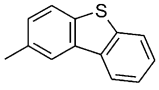
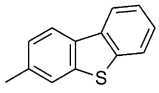
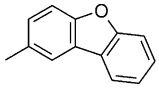
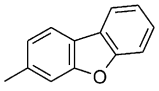
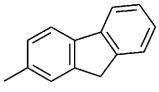
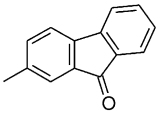
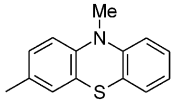
Scheme 9. (a) 2-Trifluoroacetylamino-9*H*-fluorene, K_2CO_3, 2-butanone; (b) K_2CO_3, MeOH, H_2O; (c) HCl, EtOH, acetone; (d) HCHO *aq*, $NaBH(OAc)_3$, AcOH, CH_2Cl_2.

Brown et al. reported that insertion of an ethynyl linkage between the biaryl residue and the quinuclidine nucleus improved the inhibitory activity in a series of squalene synthase inhibitors based on 3-biarylquinuclidine.[10a] This study led us to introduce an ethynyl linkage connecting the dibenzothiophene moiety with the quinuclidine nucleus into the lead compound **2**. As expected, the ethynyl derivative **4** exhibited a 2-fold enhancement in inhibitory activity over compound **2** (Table 1).

It has been suggested that the biaryl moiety of the 3-biarylquinuclidine-based inhibitor functions as a mimic of the farnesyl chain subunit of the intermediate PSPP.[10a] Assuming that the dibenzothiophen-2-ylethynyl moiety of the lead compound **2** acts as a farnesyl chain mimetic and similarly binds to a lipophilic region of the squalene synthase, we explored modifications of the tricyclic system in order to enhance the interactions with the lipophilic region. As shown in Table 1, a variety of structural modifications of the tricyclic system was well tolerated. The dibenzothiophen-3-yl analogue **5** was found to be equipotent to the dibenzothiophen-2-yl derivative **4**. The dibenzofuran analogues **11** and **6** also retained their potent activity. These results reveal that the inhibitory activity is unaffected by the shape of the tricyclic system. The 9*H*-fluoren-2-yl derivative **7** was 2-fold more potent than the dibenzofuran-3-yl derivative **6**, whereas the corresponding 9-oxo-9*H*-fluoren-2-yl derivative **8** showed reduced inhibitory activity relative to compound **6**. A comparison of the activities within these three inhibitors (**7**, **6** and **8**) indicated that the tricyclic system with the smallest topological polar surface area (0, 9.2 and 17.1 Å^2 for 9*H*-fluorene, dibenzofuran and 9-oxo-9*H*-fluorene, respectively)[26] exhibited the greatest potent activity. These results are consistent with the initial hypothesis that the arylethynyl moiety is a mimetic of the flexible and hydrophobic farnesyl chain subunit of the intermediate PSPP. Of all the arylethynyl derivatives, compound **7** incorporating a 9*H*-fluoren-2-yl moiety exhibited the most potent activity (IC_{50} = 82 nM).

Table 1. In vitro activities of 3-ethynylquinuclidin-3-ol derivatives



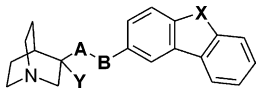
Compd	A–B	Ar	IC_{50} (μM)[a]
1	Direct		0.38
2	Direct		0.41
4	C≡C		0.21
5	C≡C		0.22
11	C≡C		0.15
6	C≡C		0.18
7	C≡C		0.082
8	C≡C		0.41
14	C≡C		0.39

[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.

The increase of the inhibitory activity by the insertion of the ethynyl linkage between the tricyclic system and the quinuclidine nucleus prompted us to investigate alternative linkages in order to improve the inhibitory activity. The exploration of the linking groups was accomplished using derivatives containing a dibenzothiophen-2-yl moiety and also using a derivative incorporating a dibenzofuran-2-yl moiety which was found to provide comparable activity to the corresponding dibenzothiophen-2-yl analogue. The results are summarized in Table 2. The propynyl derivative **16** displayed improved inhibitory activity compared to the ethynyl analogue **4**. However, increased elongation of the tether length (**19**) resulted in a decreased activity comparable to compound **4**. This result suggested that the three-atom linkage produced the suitable length for potent inhibitory activity.

Biller et al. reported a compound with improved activity after the introduction of an ether linkage into the phosphonate-based squalene inhibitor.[6b] We therefore examined related ether derivatives consisting of three

Table 2. In vitro activities of dibenzothiophene-containing-quinuclidine derivatives



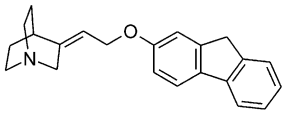
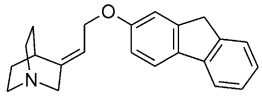
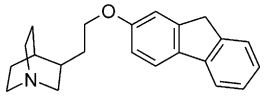
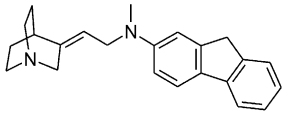
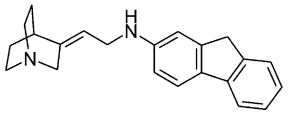
Compd	A–B	X	Y	IC_{50} (μM)[a]
4	C≡C	S	OH	0.21
16	C≡CCH$_2$	S	OH	0.11
19	C≡CCH$_2$O	O	OH	0.27
25	CH$_2$OCH$_2$	S	OH	0.20
22	CH$_2$OCH$_2$	S	H	0.19

[a]Refer to Table 1.

atoms as a linking group between the tricyclic system and the quinuclidine nucleus. Unfortunately, the ether **25** was not found to be more potent than the propynyl derivative **16**. Flexible linkages, such as ethers, are thought to be causing a reduction in the activity of the compounds. The corresponding desoxy analogue **22** displayed comparable inhibitory activity to compound **25**, demonstrating that the hydroxyl group is unnecessary for the ability to inhibit the enzyme. We were convinced that by introducing a three-atom linkage which enforced the desired geometry on the compound we could produce a potent inhibitor. Thus we focused on a 3-ethylidenequinuclidine scaffold which brought no chiral center into the molecule.

When the compounds were prepared with an ethylidene linkage inserted between the tricyclic system and the quinuclidine nucleus, a 9*H*-fluorene-2-yl moiety was chosen as the tricyclic system for all compounds because it was found to be optimal for potent inhibitory activity as examplified by compound **7**. The results of this novel structural series are summarized in Table 3. The *Z*-olefin **31** exhibited potent inhibitory activity, whereas the corresponding *E*-olefin **32** and the saturated ethyl derivative **33** were relatively poor inhibitors (IC_{50} = 76, 150 and 250 nM, respectively). These results indicate that the *E*-ethylidene linkage serves to position the tricyclic system and the quinuclidine nucleus in the appropriate location for efficient enzyme inhibition. The corresponding *N*-methylamino analogue **37** was a less potent inhibitor compared to compound **31**. Removal of the methyl group caused further loss of activity (compound **35**). It can be concluded that a higher lipophilic linking group is desirable for the inhibition. Compound **31** also demonstrated potent inhibitory activity for human enzyme with an IC_{50} value of 48 nM.[27]

Table 3. In vitro activities of 3-ethylidenequinuclidine derivatives

Compd	Structure	IC_{50} (μM)[a]
31		0.076
32		0.15
33		0.25
37		0.27
35		0.56

[a]Refer to Table 1.

Experiments were carried out to investigate the cholesterol-lowering effect of the novel squalene synthase inhibitor based on the (*Z*)-3-ethylidenequinuclidine scaffold. Compound **31**, the most potent inhibitor in this series, reduced plasma non-HDL cholesterol levels by 39% compared to a control at an oral dose of 50 mg/kg/day for 5 days in hamsters which have plasma lipid composition very similar to humans[28] (Fig. 3). We therefore expect the (*Z*)-3-ethylidenequinuclidine derivatives, such as compound **31**, to produce a similar cholesterol-lowering effect in humans.

Conclusion

A novel series of quinuclidine derivatives containing a tricyclic system was synthesized and their inhibitory activities against squalene synthase were measured. Structure–activity relationship studies provided useful information on the structural requirements for the inhibition of the enzyme. A 9*H*-fluorene moiety, which had a smaller topological polar surface area, showed suitability as a tricyclic system for potent inhibitory activity. A three-atom linkage connecting the tricyclic system with the quinuclidine nucleus, which established the geometry of the compound, provided improved activity. Among this novel series, (*Z*)-3-[2-(9*H*-fluoren-2-yloxy)-ethylidene]quinuclidine hydrochloride **31** was found to be a potent inhibitor of squalene synthase derived from hamster liver and human hepatoma cells with IC_{50} values of 76 and 48 nM, respectively. Daily oral dosing of 50 mg/kg/day of compound **31** for 5 days in hamsters resulted in approximately 39% decrease in plasma non-HDL cholesterol. The lack of chirality of the (*Z*)-3-ethylidenequinuclidine scaffold, its high inhibitory activity against squalene synthase and its significant non-HDL cholesterol lowering effect made it an attractive template. (*Z*)-3-ethylidenequinuclidine derivatives represented by compound **31** are promising candidates for a cholesterol-lowering agent. Further work on the more potent analogues will be reported in due course.

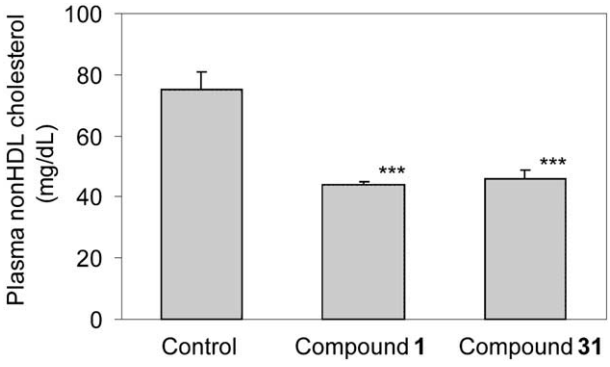


Figure 3. Effects of compound **1** and compound **31** on plasma non-HDL cholesterol levels after oral administration in hamsters at a dose of 50 mg/kg/day for 5 days (n = 4 and 7, respectively). *** $p < 0.001$ versus control by using Student's t-test.

Experimental

Chemistry

^{1}H NMR spectra were measured with a Jeol EX90, LA300, EX400 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. All reagents purchased were used without further purification.

3-(Dibenzothiophen-2-ylethynyl)quinuclidin-3-ol (4). A mixture of 3-ethynylquinuclidin-3-ol **3** (756 mg, 5.00 mmol), 2-bromodibenzothiophene **15** (1.32 g, 5.00 mmol), dichlorobis(triphenylphosphine)palladium (175 mg, 0.25 mmol), copper iodide (95 mg, 0.50 mmol), triethylamine (8.0 mL) and *N*,*N*-dimethylformamide (3.0 mL) was stirred at 60 °C for 14 h. After cooling, saturated aqueous ammonia solution (10 mL) was added and stirred for 1 h at ambient temperature. The precipitate was filtered and washed with saturated aqueous ammonia solution, water and then ether. The resulting precipitate was crystallized from chloroform–ethanol (1:1 by volume) to give the title compound as a colorless crystalline solid (800 mg, 48%): mp 250–252 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30–1.36 (1H, m), 1.56–1.66 (1H, m), 1.90–2.04 (3H, m), 2.68–2.76 (4H, m), 2.88 (1H, d, J = 15.0 Hz), 3.14 (1H, d, J = 15.0 Hz), 5.64 (1H, s), 7.51–7.56 (3H, m), 8.02–8.05 (2H, m), 8.45–8.47 (2H, m); EI-MS m/z 333 (M^+). Anal. calcd for $C_{21}H_{19}NOS{\cdot}0.5H_2O$: C, 73.65; H, 5.89; N, 4.09; S, 9.36. Found: C, 73.52; H, 5.56; N, 3.98; S, 9.46.

3-(Dibenzothiophen-3-ylethynyl)quinuclidin-3-ol (5). The title compound was obtained from 3-bromodibenzothiophene[29] using the methods described for the synthesis of compound **4** as a colorless crystalline solid (55%): mp 235–236 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29–1.38 (1H, m), 1.57–1.67 (1H, m), 1.89–2.01 (3H, m), 2.69–2.77 (4H, m), 2.87 (1H, d, J = 14.0 Hz), 3.12 (1H, d, J = 14.0 Hz), 5.68 (1H, s), 7.51–7.56 (3H, m), 8.03–8.07 (1H, m), 8.13(1H, s), 8.33–8.38 (2H, m); FAB-MS m/z 334 ($M+H^+$). Anal. calcd for $C_{21}H_{19}NOS{\cdot}0.2H_2O$: C, 74.83; H, 5.80; N, 4.16; S, 9.51. Found: C, 74.91; H, 5.93; N, 4.55; S, 9.64.

3-(Dibenzofuran-3-ylethynyl)quinuclidin-3-ol (6). The title compound was obtained from 3-bromodibenzofuran[30] using the methods described for the synthesis of compound **4** as a colorless crystalline solid (16%): mp 227–228 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42–1.51 (1H, m), 1.66–1.73 (1H, m), 2.01–2.13 (3H, m), 2.82–2.98 (4H, m), 3.10 (1H, d, J = 14.0 Hz), 3.38 (1H, d, J = 14.0 Hz), 7.33–7.37 (1H, m), 7.41 (1H, d, J = 7.6 Hz), 7.45–7.49 (1H, m), 7.57 (1H, d, J = 8.4 Hz), 7.63 (1H, s), 7.87 (1H, d, J = 7.6 Hz), 7.93 (1H, d, J = 7.2 Hz); EI-MS m/z 317 (M^+). Anal. calcd for $C_{21}H_{19}NO_2{\cdot}0.4H_2O$: C, 77.71; H, 6.15; N, 4.32. Found: C, 77.54; H, 5.83; N, 4.24.

3-(9*H*-Fluoren-2-ylethynyl)quinuclidin-3-ol (7). The title compound was obtained from 2-bromo-9*H*-fluorene using the methods described for the synthesis of compound **4** as a colorless crystalline solid (76%): mp 226–228 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.28–1.36 (1H, m), 1.54–1.62 (1H, m), 1.86–2.02 (3H, m), 2.62–2.74 (4H, m), 2.86 (1H, d, J = 14.0 Hz), 3.10 (1H, d, J = 14.0 Hz), 3.93 (2H, s), 5.60 (1H, s), 7.32–7.43 (3H, m), 7.59–7.62 (2H, m), 7.87–7.92 (2H, m); EI-MS m/z 315 (M^+). Anal. calcd for $C_{22}H_{21}NO{\cdot}0.3H_2O$: C, 82.83; H, 6.76; N, 4.39. Found: C, 82.82; H, 6.73; N, 4.44.

2-[(3-Hydroxyquinuclidin-3-yl)ethynyl]-9*H*-fluoren-9-one (8). The title compound was obtained from 2-bromo-9*H*-fluoren-9-one using the methods described for the synthesis of compound **4** as a yellow crystalline solid (57%): mp 199–201 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40–1.50 (1H, m), 1.58–1.68 (1H, m), 1.94–2.12 (3H, m), 2.80–2.96 (4H, m), 3.08 (1H, d, J = 14.5 Hz), 3.35 (1H, d, J = 14.5 Hz), 7.29–7.32 (1H, m), 7.46 (1H, d, J = 7.0 Hz), 7.49–7.54 (3H, m), 7.65–7.68 (2H, m); EI-MS m/z 329 (M^+). Anal. calcd for $C_{22}H_{19}NO_2{\cdot}1.9H_2O$: C, 72.67; H, 6.32; N, 3.93. Found: C, 72.31; H, 6.45; N, 3.93.

2-(2,2-Dibromovinyl)dibenzofuran (10). To a stirred solution of triphenylphosphine (787 mg, 3.00 mmol) in dichloromethane (9.0 mL) was added carbon tetrabromide (995 mg, 3.00 mmol) followed by zinc dust (196 mg, 3.00 mmol) and stirred for 23 h at ambient temperature. 2-Dibenzofurancarboxaldehyde **9** (296 mg, 1.50 mmol) in dichloromethane (3.0 mL) was added and stirred for 5 h at ambient temperature. The reaction mixture was diluted with *n*-hexane and filtered. The filtrate was concentrated in vacuo to give the title compound as a colorless solid (376 mg, 71%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.38 (1H, m), 7.44–7.50 (1H, m), 7.54–7.61 (3H, m), 7.63 (1H, s), 7.95 (1H, d, J = 7.5 Hz), 8.17 (1H, s); EI-MS m/z 352 (M^+).

3-(Dibenzofuran-2-ylethynyl)quinuclidin-3-ol (11). A solution of ^{n}BuLi in *n*-hexane (1.29 mL, 1.71 M, 2.21 mmol) was added dropwise to 2-(2,2-dibromovinyl)dibenzofuran **10** (368 mg, 1.05 mmol) in tetrahydrofuran (3.0 mL) at −78 °C. The reaction mixture was stirred for 1 h, allowed to warm to ambient temperature and then stirred for a further 1 h. A solution of 3-quinuclidinone (145 mg, 1.16 mmol) in tetrahydrofuran (1.0 mL) was added at −78 °C and stirred for 1 h. After addition of H_2O (1.0 mL), the reaction mixture was allowed to warm to ambient temperature. The reaction mixture was extracted with chloroform, and the extract was washed with brine, dried over magnesium sulfate and then concentrated in vacuo. The residue was chromatographed over silica gel eluting with chloroform–methanol-*c*. ammonium hydroxide (100:10:1 by volume). The resulting solid was crystallized from chloroform–ethanol (1:1 by volume) to give the title compound as a colorless crystalline solid (220 mg, 66%): mp 232–233 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.41–1.49 (1H, m), 1.66–1.73 (1H, m), 2.02–2.14 (3H, m), 2.82–2.95 (4H, m), 3.09 (1H, d, J = 14.0 Hz), 3.38 (1H, d, J = 14.0 Hz), 7.33–7.37 (1H,

m), 7.46–7.53 (3H, m), 7.57 (1H, d, $J=8.5$ Hz), 7.93 (1H, d, $J=7.5$ Hz), 8.03 (1H, s); EI-MS m/z 317 (M^+). Anal. calcd for $C_{21}H_{19}NO_2 \cdot 0.2H_2O$: C, 78.58; H, 6.09; N, 4.36. Found: C, 78.48; H, 6.23; N, 4.30.

3-(2,2-Dibromovinyl)-10-methyl-10*H*-phenothiazine (13). The title compound was obtained from 3-formyl-10-methyl-10*H*-phenothiazine **12** using the methods described for the synthesis of compound **10** as a colorless solid (28%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.37 (3H, s), 6.74 (1H, d, $J=4.7$ Hz), 6.80 (1H, d, $J=3.2$ Hz), 6.92–7.01 (1H, m), 7.04–7.17 (1H, m), 7.28–7.40 (3H, m); EI-MS m/z 397 (M^+).

3-[(10-Methyl-10*H*-phenothiazin-3-yl)ethynyl]quinuclidin-3-ol (14). The title compound was obtained from 3-(2,2-dibromovinyl)-10-methyl-10*H*-phenothiazine **13** using the methods described for the synthesis of compound **11** as a colorless crystalline solid (84%): mp 223–224 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39–1.45 (1H, m), 1.62–1.67 (1H, m), 1.95–2.06 (3H, m), 2.82–2.94 (4H, m), 3.04 (1H, d, $J=14.0$ Hz), 3.30 (1H, d, $J=14.0$ Hz), 3.38 (3H, s), 6.69 (1H, d, $J=8.0$ Hz), 6.80 (1H, d, $J=8.0$ Hz), 6.92–6.95 (1H, m), 7.11 (1H, dd, $J=2.0$ Hz, 8.0 Hz), 7.15–7.22 (3H, m); EI-MS m/z 362 (M^+). Anal. calcd for $C_{22}H_{22}N_2OS$: C, 72.90; H, 6.12; N, 7.73; S, 8.85. Found: C, 72.64; H, 5.96; N, 7.68; S, 8.96.

3-[3-(Dibenzothiophen-2-yl)prop-1-yn-1-yl]quinuclidin-3-ol (16). A solution of ^{n}BuLi in *n*-hexane (3.67 mL, 1.71 M, 6.27 mmol) was added dropwise to 2-bromodibenzothiophene **15** (1.50 g, 5.70 mmol) in tetrahydrofuran (12 mL) at −78 °C and stirred for 1 h. A solution of lithium 2-thienylcyanocuprate in tetrahydrofuran (27.4 mL, 0.25 M, 6.84 mmol) was added dropwise at −78 °C and stirred for 0.5 h. A solution of propargyl bromide (1.36 g, 11.4 mmol) in tetrahydrofuran (11 mL) was added dropwise at −78 °C and stirred for 2 h. After addition of saturated aqueous ammonium chloride solution (10 mL), the reaction mixture was allowed to warm to ambient temperature, stirred for 0.5 h and then extracted with ethyl acetate. The extract was washed with saturated aqueous ammonia solution and then brine, dried over magnesium sulfate and then concentrated in vacuo to give a brown oil. The resulting oil was dissolved in tetrahydrofuran (12 mL) and cooled to −78 °C. To this solution was added a solution of ^{n}BuLi in *n*-hexane (2.47 mL, 1.71 M, 4.22 mmol) and stirred for 1 h. A solution of 3-quinuclidinone (428 mg, 3.42 mmol) in tetrahydrofuran (3.4 mL) was added dropwise at −78 °C and stirred for 2 h. After addition of H_2O (3.0 mL), the reaction mixture was allowed to warm to ambient temperature. The reaction mixture was extracted with chloroform, and the extract was washed with brine, dried over magnesium sulfate and then concentrated in vacuo. The residue was chromatographed over silica gel eluting with chloroform - - methanol - concd ammonium hydroxide (100:10:1 by volume). The resulting solid was crystallized from ethyl acetate to give the title compound as a colorless crystalline solid (162 mg, 8.2%): mp 203–205 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38–1.43 (1H, m), 1.52–1.70 (1H, m), 1.98–2.10 (3H, m), 2.81–2.89 (4H, m), 3.03 (1H, d, $J=14.0$ Hz), 3.29 (1H, d, $J=14.0$ Hz), 3.83 (2H, s), 7.41 (1H, d, $J=8.5$ Hz), 7.45–7.47 (2H, m), 7.79 (1H, d, $J=8.5$ Hz), 7.84–7.86 (1H, m), 8.12–8.14 (2H, m); EI-MS m/z 347 (M^+). Anal. calcd for $C_{22}H_{21}NOS \cdot 0.2H_2O$: C, 75.26; H, 6.14; N, 3.99; S, 9.13. Found: C, 74.94; H, 6.11; N, 3.90; S, 9.40.

2-(Prop-2-yn-1-yloxy)dibenzofuran (18). A mixture of 2-hydroxydibenzofuran **17** (3.00 g, 16.3 mmol), propargyl bromide (3.88 g, 32.6 mmol), potassium carbonate (4.51 g, 32.6 mmol) and *N*,*N*-dimethylformamide (16 mL) was stirred at ambient temperature for 2 days. The reaction 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 then concentrated in vacuo to give the title compound as a brown oil (3.60 g, 99%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.53 (1H, t, $J=2.4$ Hz), 4.78 (2H, d, $J=2.4$ Hz), 7.10 (1H, dd, $J=2.7$ Hz, 9.0 Hz), 7.21–7.61 (5H, m), 7.86–7.97 (1H, m); EI-MS m/z 222 (M^+).

3-[3-(Dibenzofuran-2-yloxy)prop-1-yn-1-yl]quinuclidin-3-ol (19). A solution of ^{n}BuLi in *n*-hexane (11.0 mL, 1.60 M, 17.6 mmol) was added dropwise to 2-(prop-2-yn-1-yloxy)dibenzofuran **18** (3.55 g, 16.0 mmol) in tetrahydrofuran (32 mL) at −78 °C and stirred for 1.5 h. A solution of 3-quinuclidinone (2.22 g, 16.0 mmol) in tetrahydrofuran (16 mL) was added dropwise at −78 °C and stirred for 1 h. After addition of H_2O (8.0 mL), the reaction mixture was allowed to warm to ambient temperature. The reaction mixture was extracted with chloroform, and the extract was washed with brine, dried over magnesium sulfate and then concentrated in vacuo. The residue was chromatographed over silica gel eluting with chloroform–methanol-concd ammonium hydroxide (100:10:1 by volume). The resulting solid was crystallized from ethyl acetate to give the title compound as a colorless crystalline solid (3.03 g, 55%): mp 188–189 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30–1.37 (1H, m), 1.49–1.56 (1H, m), 1.79–1.84 (1H, m), 1.92–1.97 (2H, m), 2.68–2.82 (4H, m), 2.94 (1H, d, $J=16.0$ Hz), 3.17 (1H, d, $J=16.0$ Hz), 4.83 (2H, s), 7.10 (1H, dd, $J=2.0$ Hz, 8.5 Hz), 7.30–7.34 (1H, m), 7.43–7.56 (4H, m), 7.99 (1H, d, $J=7.0$ Hz); FAB-MS m/z 348 ($M+H^+$). Anal. calcd for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 76.17; H, 6.05; N, 4.02.

2-(Chloromethyl)dibenzothiophene (21). A mixture of 2-(hydroxymethyl)dibenzothiophene **20** (740 mg, 3.45 mmol), two drops of *N*,*N*-dimethylformamide and thionyl chloride (1.2 mL) was stirred at 70 °C for 0.5 h. The reaction 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 then concentrated in vacuo to give the title compound as a yellow solid (799 mg, 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.78 (2H, s), 7.46–7.50 (3H, m), 7.82–7.87 (2H, m), 8.14–8.17 (2H, m); EI-MS m/z 232 (M^+).

3-[(Dibenzothiophen-2-ylmethoxy)methyl]quinuclidine (22). To a stirred solution of 3-(hydroxymethyl)quinuclidine-*N*-borane (490 mg, 3.16 mmol) in *N*,*N*-dimethylformamide (15 mL) was added sodium hydride (139 mg,

3.48 mmol, 60% dispersion in mineral oil) at ambient temperature and stirred for 2 h. 2-(Chloromethyl)dibenzothiophene **21** (772 mg, 3.32 mmol) in *N*,*N*-dimethylformamide (3.0 mL) was added at 0 °C and stirred for 0.5 h. The reaction mixture was allowed to warm to ambient temperature and stirred for a further 15 h. After addition of H_2O (6.0 mL), the reaction mixture was extracted with ethyl acetate. The extract was washed with H_2O and then brine, dried over magnesium sulfate and then concentrated in vacuo. The residue was chromatographed over silica gel eluting with *n*-hexane–ethyl acetate (4:1 by volume) to give a colorless solid. The resulting solid in ethanol–acetone (26 mL, 1:1 by volume) was treated with hydrogen chloride in ethanol (5 M, 1.3 mL) at ambient temperature and stirred for 1 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with 1 M hydrochloric acid. The aqueous layer was washed with ethyl acetate before the addition of excess 5 M sodium hydroxide to pH 10. The mixture was extracted with ethyl acetate, and the extract was dried over magnesium sulfate and then concentrated in vacuo. The resulting solid was crystallized from isopropyl ether to give the title compound as a colorless crystalline solid (630 mg, 59%): mp 118–119 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.36–1.46 (1H, m), 1.54–1.70 (2H, m), 1.86–1.92 (1H, m), 2.02–2.10 (1H, m), 2.38–2.44 (1H, m), 2.76–2.94 (4H, m), 3.04–3.08 (1H, m), 3.44–3.54 (2H, m), 4.66 (1H, d, J = 12.0 Hz), 4.71 (1H, d, J = 12.0 Hz), 7.42–7.47 (3H, m), 8.02–8.05 (2H, m), 8.12 (1H, s), 8.14–8.17 (1H, m); EI-MS m/z 337 (M^+). Anal. calcd for $C_{21}H_{23}NOS{\cdot}0.1H_2O$: C, 74.34; H, 6.89; N, 4.13; S, 9.45. Found: C, 74.29; H, 6.83; N, 4.05; S, 9.48.

3-(Hydroxymethyl)quinuclidin-3-ol-*N*-borane (24). A solution of borane-tetrahydrofuran complex in tetrahydrofuran (6.80 mL, 1.0 M, 6.80 mmol) was added dropwise to methyl 3-hydroxyquinuclidin-3-carboxylate **23** (505 mg, 2.73 mmol) in tetrahydrofuran (14 mL) at −78 °C and stirred for 1 h. Sodium borohydride (5.2 mg, 0.14 mmol) was added at −78 °C and stirred for 0.5 h. The reaction mixture was allowed to warm to ambient temperature and stirred for a further 4 h. Methanol (5.0 mL) was added at 0 °C and stirred for 1 h. The reaction 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 then concentrated in vacuo. The residue was chromatographed over silica gel eluting with *n*-hexane–ethyl acetate (1:2 by volume) to give the title compound as a colorless solid (275 mg, 59%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.61–1.90 (2H, m), 2.07–2.45 (3H, m), 2.79–3.18 (6H, m), 3.54 (1H, d, J = 10.8 Hz), 3.71 (1H, d, J = 10.8 Hz); EI-MS m/z 171 (M^+).

3-[(Dibenzothiophen-2-ylmethoxy)methyl]quinuclidin-3-ol (25). To a stirred solution of 3-(hydroxymethyl)quinuclidin-3-ol-*N*-borane **24** (275 mg, 1.61 mmol) in *N*,*N*-dimethylformamide (8.0 mL) was added sodium hydride (148 mg, 3.70 mmol, 60% dispersion in mineral oil) at ambient temperature and stirred for 1 h. 2-(Chloromethyl)dibenzothiophene **21** (375 mg, 1.61 mmol) in *N*,*N*-dimethylformamide (1.6 mL) was added at 0 °C and stirred for 0.5 h. The reaction mixture was allowed to warm to ambient temperature and stirred for a further 2h. After addition of H_2O (4.0 mL), the reaction mixture was extracted with ethyl acetate. The extract was washed with H_2O and then brine, dried over magnesium sulfate and then concentrated in vacuo. The residue was chromatographed over silica gel eluting with *n*-hexane–ethyl acetate (1:1 by volume) to give a colorless solid. The resulting solid in ethanol–acetone (6.0 mL, 1:1 by volume) was treated with hydrogen chloride in ethanol (5 M, 0.6 mL) at ambient temperature and stirred for 0.5 h. The reaction mixture was concentrated in vacuo and the residue was diluted with 1 M hydrochloric acid. The aqueous layer was washed with ethyl acetate before the addition of excess 5 M sodium hydroxide to pH 10. The mixture was extracted with ethyl acetate, and the extract was dried over magnesium sulfate and concentrated in vacuo. The resulting solid was crystallized from ethyl acetate to give the title compound as a colorless crystalline solid (200 mg, 35%): mp 185–187 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30–1.36 (1H, m), 1.50–1.54 (2H, m), 2.00–2.10 (2H, m), 2.56–2.62 (3H, m), 2.74–2.84 (3H, m), 3.37 (1H, d, J = 9.2 Hz), 3.61 (1H, d, J = 9.2 Hz), 4.71 (1H, d, J = 11.6 Hz), 4.78 (1H, d, J = 11.6 Hz), 7.42–7.49 (3H, m), 7.83–7.87 (2H, m), 8.10 (1H, s), 8.14–8.18 (1H, m); EI-MS m/z 353 (M^+). Anal. calcd for $C_{21}H_{23}NO_2S$: C, 71.36; H, 6.56; N, 3.96; S, 9.07. Found: C, 71.25; H, 6.61; N, 3.86; S, 9.15.

{Methyl (*Z*)-(3-quinuclidinylidene)acetate}-*N*-borane (27) and {methyl (*E*)-(3-quinuclidinylidene)acetate}-*N*-borane (28). To a stirred solution of trimethyl phosphonoacetate (9.09 g, 49.9 mmol) in tetrahydrofuran (38 mL) was added sodium hydride (2.00 g, 49.9 mmol, 60% dispersion in mineral oil) at 0 °C and stirred for 0.5 h. The reaction mixture was allowed to warm to ambient temperature and stirred for a further 0.5 h. 3-Quinuclidinone (4.81 g, 38.4 mmol) was added at 0 °C and stirred for 0.5 h. The reaction mixture was allowed to warm to ambient temperature and stirred for a further 12 h. After addition of H_2O (20 mL), the reaction mixture was extracted with ethyl acetate. The extract was washed with brine, dried over magnesium sulfate and then concentrated in vacuo to give a colorless oil. The resulting oil was dissolved in tetrahydrofuran (100 mL) and cooled to −78 °C. To this solution was added a solution of borane-tetrahydrofuran complex in tetrahydrofuran (50.0 mL, 1.0 M, 50.0 mmol) dropwise and stirred for 2 h. After addition of H_2O (5.0 mL), the reaction mixture was allowed to warm to ambient temperature and stirred for 0.5 h. The reaction mixture was extracted with ethyl acetate. The extract was washed with brine, dried over magnesium sulfate and then concentrated in vacuo. The residue was chromatographed over silica gel eluting with *n*-hexane–ethyl acetate (4:1 by volume) to give **28** as a colorless solid (4.41 g, 48%) from the first fraction and **27** as a colorless solid (4.34 g, 47%) from the second fraction.

27. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78–2.02 (4H, m), 2.63–2.67 (1H, m), 2.99–3.20 (4H, m), 3.73 (3H, s), 4.13–4.16 (2H, m), 5.76 (1H, t, J = 2.6 Hz); FAB-MS m/z 194 ($M-H^+$). **28**: ^{1}H NMR (300 MHz, $CDCl_3$) δ

1.79–2.01 (4H, m), 2.97–3.18 (4H, m), 3.66–3.68 (2H, m), 3.72 (3H, s), 4.16–4.20 (1H, m), 5.68 (1H, t, $J=2.0$ Hz); FAB-MS m/z 194 ($M-H^+$).

(*Z*)-3-(2-Chloroethylidene)quinuclidine-*N*-borane (29). A solution of diisobutylaluminum hydride in *n*-hexane (25.4 mL, 0.93 M, 23.6 mmol) was added dropwise to {methyl (*Z*)-(3-quinuclidinylidene)acetate}-*N*-borane **27** (1.84 g, 9.43 mmol) in toluene (27 mL) at −78 °C and stirred for 2.5 h. Methanol (1.70 mL) followed by H_2O (2.83 mL) was added at −78 °C. The reaction mixture was allowed to warm to ambient temperature, stirred for 1.5 h and then filtered through a pad of Celite. The filtrate was concentrated in vacuo to give a colorless oil. To a stirred solution of the resulting oil, lithium chloride (4.00 g, 94.3 mmol) and triethylamine (2.29 g, 22.6 mmol) in dichloromethane (27 mL) was added methanesulfonyl chloride (1.29 g, 11.3 mmol) at 0 °C. The reaction mixture was stirred for 1 h, allowed to warm to ambient temperature and then stirred for a further 1 h. The reaction 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 then concentrated in vacuo to give the title compound as a colorless oil (1.34 g, 77%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.74–2.00 (4H, m), 2.49–2.62 (1H, m), 2.98–3.18 (4H, m), 3.72 (2H, s), 3.96 (2H, d, $J=8.2$ Hz), 5.44–5.68 (1H, m); FAB-MS m/z 184 ($M-H^+$).

(*Z*)-3-[2-(9*H*-Fluoren-2-yloxy)ethylidene]quinuclidine hydrochloride (31). A mixture of (*Z*)-3-(2-chloroethylidene)quinuclidine - *N* - borane **29** (579 mg, 3.12 mmol), 2-hydroxy-9*H*-fluorene (569 mg, 3.12 mmol), potassium carbonate (862 mg, 6.24 mmol) and *N*,*N*-dimethylformamide (6.0 mL) was stirred at ambient temperature for 5 h. The reaction 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 then concentrated in vacuo to give a pale yellow solid. The resulting solid in ethanol–acetone (12 mL, 1:1 by volume) was treated with hydrogen chloride in ethanol (5 M, 3.0 mL) at ambient temperature and stirred for 0.5 h. Ether (9.0 mL) was added and the resulting precipitate was filtered to give the title compound as a colorless crystalline solid (640 mg, 58%): mp 229–231 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.79–1.88 (2H, m), 1.93–2.02 (2H, m), 2.48–2.50 (1H, m), 3.20–3.38 (4H, m), 3.84 (2H, s), 4.10 (2H, s), 4.58 (2H, d, $J=5.9$ Hz), 5.73 (1H, t, $J=5.9$ Hz), 6.99 (1H, dd, $J=2.0$ Hz, 8.0 Hz), 7.21–7.25 (2H, m), 7.32–7.36 (1H, m), 7.54 (1H, d, $J=7.2$ Hz), 7.79–7.81 (2H, m), 10.64 (1H, br s); FAB-MS m/z 318 ($M+H^+$). Anal. calcd for $C_{22}H_{23}NO\cdot HCl\cdot 0.4H_2O$: C, 73.18; H, 6.92; N, 3.88; Cl, 9.82. Found: C, 73.12; H, 6.77; N, 3.81; Cl, 9.89.

(*E*)-3-(2-Chloroethylidene)quinuclidine-*N*-borane (30). The title compound was obtained from {methyl (*E*)-(3-quinuclidinylidine)acetate}-*N*-borane **28** using the methods described for the synthesis of compound **29** as a colorless oil (63%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.79–2.07 (4H, m), 2.91–3.16 (5H, m), 3.63 (2H, s), 4.28 (2H, d, $J=8.2$ Hz), 5.42–5.60 (1H, m); FAB-MS m/z 184 ($M-H^+$).

(*E*)-3-[2-(9*H*-Fluoren-2-yloxy)ethylidene]quinuclidine hydrochloride (32). The title compound was obtained from (*E*)-3-(2-chloroethylidene)quinuclidine-*N*-borane **30** using the methods described for the synthesis of compound **31** as a colorless crystalline solid (32%): mp 240–242 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.76–1.84 (2H, m), 1.94–2.02 (2H, m), 3.16–3.29 (5H, m), 3.87 (2H, s), 3.96 (2H, s), 4.70 (2H, d, $J=7.0$ Hz), 5.67 (1H, t, $J=7.0$ Hz), 6.97 (1H, dd, $J=2.0$ Hz, 8.0 Hz), 7.20–7.25 (2H, m), 7.32–7.35 (1H, m), 7.53 (1H, d, $J=7.5$ Hz), 7.78–7.79 (2H, m), 10.35 (1H, br s); EI-MS m/z 317 (M^+). Anal. calcd for $C_{22}H_{23}NO\cdot HCl\cdot 0.5H_2O$: C, 72.81; H, 6.94; N, 3.86; Cl, 9.77. Found: C, 72.53; H, 6.85; N, 3.86; Cl, 9.70.

3-[2-(9*H*-Fluoren-2-yloxy)ethyl]quinuclidine hydrochloride (33). (*E*)-3-[2-(9*H*-Fluoren-2-yloxy)ethylidene]quinuclidine hydrochloride **32** (140 mg, 0.396 mmol) in ethyl acetate (14 mL) was treated with 1 M sodium hydroxide (14 mL) and stirred for 0.5 h. The organic layer was separated, washed with brine, dried over magnesium sulfate and then concentrated in vacuo to give a colorless solid. To a solution of the resulting solid in ethanol (2.2 mL) was added rhodium on aluminum (5 wt %, 22 mg) and stirred under an atmosphere of hydrogen at ambient temperature for 4 h. The reaction mixture was filtered through a pad of Celite and concentrated in vacuo. The residue was chromatographed over silica gel eluting with chloroform–methanol-concd ammonium hydroxide (100:10:1 by volume) to give a colorless solid. The resulting solid in ethanol–acetone (1.0 mL, 1:1 by volume) was treated with hydrogen chloride in ethanol (5 M, 0.1 mL) at ambient temperature and stirred for 0.5 h. Ether (1.0 mL) was added and the resulting precipitate was filtered to give the title compound as a colorless crystalline solid (32 mg, 23%): mp 231–233 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.68–1.75 (1H, m), 1.80–1.97 (6H, m), 2.19–2.22 (1H, m), 2.85–2.89 (1H, m), 3.11–3.24 (4H, m), 3.41–3.46 (1H, m), 3.87 (2H, s), 4.07 (2H, t, $J=6.1$ Hz), 6.96 (1H, dd, $J=2.0$ Hz, 8.0 Hz), 7.18 (1H, s), 7.21–7.24 (1H, m), 7.32–7.34 (1H, m), 7.53 (1H, d, $J=7.3$ Hz), 7.77–7.79 (2H, m), 10.03 (1H, br s); FAB-MS m/z 320 ($M+H^+$). Anal. calcd for $C_{22}H_{25}NO\cdot HCl$: C, 74.24; H, 7.36; N, 3.94; Cl, 9.96. Found: C, 73.93; H, 7.44; N, 3.92; Cl, 10.28.

(*Z*)-3-[2-(9*H*-Fluoren-2-ylamino)ethylidene]quinuclidine-*N*-borane (34). A mixture of (*Z*)-3-(2-chloroethylidene)quinuclidine-*N*-borane **29** (1.30 g, 7.01 mmol), 2-trifluoroacetylamino-9*H*-fluorene (1.94 g, 7.01 mmol), potassium carbonate (2.91 g, 21.0 mmol) and 2-butanone (21 mL) was stirred at 100 °C for 16 h. The reaction mixture was concentrated in vacuo. A mixture of the resulting residue, methanol (15 mL), H_2O (15 mL) and additional potassium carbonate (1.94 g, 14.0 mmol) was stirred under reflux for 1 h. The reaction 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 then concentrated in vacuo to give the title compound

as a colorless solid (1.42 g, 61%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.74–1.96 (4H, m), 2.46–2.60 (1H, m), 2.99–3.17 (4H, m), 3.67–3.81 (6H, m), 5.43–5.49 (1H, m), 6.60 (1H, dd, J = 2.2 Hz, 8.2 Hz), 6.78 (1H, s), 7.04–7.67 (5H, m); EI-MS m/z 330 (M^+).

(*Z*)-3-[2-(9*H*-Fluoren-2-ylamino)ethylidene]quinuclidine hydrochloride (35). (*Z*)-3-[2-(9*H*-Fluoren-2-ylamino)-ethylidene]quinuclidine-*N*-borane **34** (700 mg, 2.12 mmol) in ethanol–acetone (20 mL, 1:1 by volume) was treated with hydrogen chloride in ethanol (5 M, 2.0 mL) at ambient temperature and stirred for 0.5 h. The reaction mixture was concentrated in vacuo to give a colorless solid. The resulting solid was crystallized from acetone to give the title compound as a colorless crystalline solid (741 mg, 90%): mp 191–193 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.72–1.82 (2H, m), 1.90–2.00 (2H, m), 2.66–2.68 (1H, m), 3.10–3.20 (2H, m), 3.22–3.32 (2H, m), 3.82 (2H, d, J = 7.3 Hz), 3.96 (2H, s), 4.14 (2H, s), 5.58–5.62 (1H, m), 7.31–7.38 (3H, m), 7.41–7.68 (2H, m), 7.89–7.96 (2H, m), 10.97 (3H, br s); FAB-MS m/z 317 ($M+H^+$). Anal. calcd for $C_{22}H_{24}N_2{\cdot}2HCl{\cdot}0.6H_2O$: C, 66.03; H, 6.85; N, 7.00; Cl, 17.72. Found: C, 65.90; H, 6.73; N, 6.98; Cl, 17.54.

(*Z*)-3-{2-[*N*-(9*H*-Fluoren-2-yl)-*N*-methylamino]ethylidene}quinuclidine-*N*-borane (36). A mixture of (*Z*)-3-[2-(9*H*-fluoren-2-ylamino)ethylidene]quinuclidine-*N*-borane **34** (700 mg, 2.12 mmol), formaldehyde in H_2O (1.82 mL, 37 wt %, 24.3 mmol) and dichloromethane (20 mL) was treated acetic acid (1.3 mL) followed by sodium triacetoxyborohydride (900 mg, 4.24 mmol). The reaction mixture was stirred at ambient temperature for 0.5 h. The reaction mixture was washed with saturated aqueous sodium bicarbonate solution and then brine, dried over magnesium sulfate and then concentrated in vacuo. The residue was chromatographed over silica gel eluting with *n*-hexane–ethyl acetate (3:1 by volume) to give the title compound as a colorless solid (280 mg, 38%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.71–1.96 (4H, m), 2.42–2.58 (1H, m), 2.95–3.18 (7H, m), 3.74–3.88 (6H, m), 5.31–5.59 (1H, m), 6.77 (1H, dd, J = 1.8 Hz, 8.1 Hz), 6.91 (1H, s), 7.08–7.67 (5H, m); EI-MS m/z 344 (M^+).

(*Z*)-3-[2-[*N*-(9*H*-Fluoren-2-yl)-*N*-methylamino]ethylidene]quinuclidine fumarate (37). (*Z*)-3-{2-[*N*-(9*H*-Fluoren-2-yl)-*N*-methylamino]ethylidene}quinuclidine-*N*-borane **36** (251 mg, 0.73 mmol) in ethanol–acetone (9.0 mL, 1:1 by volume) was treated with hydrogen chloride in ethanol (5 M, 3.0 mL) at ambient temperature and stirred for 0.5 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with 1 M hydrochloric acid. The aqueous layer was washed with ethyl acetate before the addition of excess 5 M sodium hydroxide to pH 10. The mixture was extracted with ethyl acetate, and the extract was dried over magnesium sulfate and then concentrated in vacuo to give a pale pink powder. To a solution of the resulting powder in acetone (9.0 mL) was added fumaric acid (93 mg, 0.80 mmol) at ambient temperature and the mixture was stirred for 0.5 h. The resulting precipitate was filtered and dried over Mg_2SO_4 to give the title compound as a colorless crystalline solid (310 mg, 95%): mp 192–194 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.67–1.75 (2H, m), 1.80–1.90 (2H, m), 2.47–2.50 (1H, m), 2.92 (3H, s), 3.04–3.16 (4H, m), 3.82 (2H, s), 3.86–3.92 (4H, m), 5.31–5.39 (1H, m), 6.56 (2H, s), 6.77 (1H, dd, J = 1.8 Hz, 8.5 Hz), 6.96 (1H, s), 7.13–7.16 (1H, m), 7.26–7.29 (1H, m), 7.47 (1H, d, J = 7.3 Hz), 7.65–7.67 (2H, m); FAB-MS m/z 331 ($M+H^+$). Anal. calcd for $C_{23}H_{26}N_2{\cdot}C_4H_4O_4$: C, 72.62; H, 6.77; N, 6.27. Found: C, 72.88; H, 6.75; N, 6.09.

Preparation of microsomes from hamster liver and HepG2 cells. Microsomes were prepared from the livers of hamsters and from HepG2 cells, a human hapatoma cell line previously described.[31] 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 100,000*g* for 60 min at 4 °C. The microsome precipitates were suspended in HEPES buffer (1–5 mg/mL). Protein was assayed by the method of Lowry.[32]

Assay of squalene synthase inhibitory activity. Squalene synthase activities of these microsomes were assayed using the technique of Amin with a slight modification. The test compounds were dissolved in DMSO and the assay 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 μM), [^{3}H]-FPP (0.017 μM, 15 Ci/mmol), NB-598 (10 μM) and sodium pyrophosphate decahydrate (1 mM). After preincubation of these components at 30 °C for 5 min, the reaction was started 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 solution of 40% KOH–ethanol (100 μL, 1:1 by volume). Synthesized [^{3}H]-squalene was extracted in petroleum ether after the saponification at 60 °C for 30 min and counted in Aquasol-2 using a Beckman liquid scintillation counter.

Plasma non-HDL cholesterol lowering effect 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 h. The animals were fed a standard low cholesterol diet (CE-2) and water was provided ad libitum. Animals were orally given test compound at dose of 50 mg/kg of body weight once a day for 5 days. 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 compound dose from animals which had fasted 18 h. All plasma samples were analyzed for non-HDL cholesterol which was subtracted HDL cholesterol from total cholesterol using a Hitachi 7250 Automatic analyzer (Tokyo, Japan).

Acknowledgements

The authors deeply acknowledge Dr. Koyo Matsuda and Dr. Masahiko Isaka for useful discussion. We would like to express our gratitude to Dr. Minoru Okada and Mr. Fukushi Hirayama for helpful support in the preparation of this paper and are also grateful to the staff of the Division of Analytical Science Laboratories for the elemental analysis and spectral measurements.

References and Notes

1. *J. Am. Med. Assoc.* **1990**, *264*, 3053.
2. (a) *J. Am. Med. Assoc.* **1984**, *251*, 351. (b) *J. Am. Med. Assoc.* **1984**, *251*, 365. (c) Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. *N. Engl. J. Med.* **1989**, *320*, 915. (d) Steinberg, D.; Witztum, J. L. *J. Am. Med. Assoc.* **1990**, *264*, 3047.
3. (a) Shepherd, J.; Packard, C. J. *Trends Pharmacol. Sci.* **1988**, *9*, 326. (b) Alberts, A. W. *Drug Invest.* **1990**, *2*, 9. (c) Maher, V. M. G.; Thompson, G. R. *Quart. J. Med.* **1990**, *74*, 165.
4. Pedersen, T. R.; Kjekshus, J.; Berg, K.; Haghfelt, T.; Fargeman, O.; Thorgeirsson, G.; Pyorala, K.; Mertinen, T.; Wilhelmsmen, L.; Olsson, A. G.; Wedel, H. *Lancet* **1994**, *344*, 1383.
5. (a) For reviews, see: (a) Abe, I.; Tomesch, J. C.; Wattanasin, S.; Prestwich, G. D. *Nat. Prod. Rep.* **1994**, *11*, 279. (b) Biller, S. A.; Neuenschwander, K.; Ponpipom, M. M.; Poulter, C. D. *Curr. Pharm. Des.* **1996**, *2*, 1.
6. (a) Biller, S. A.; Forster, C.; Gordon, E. M.; Harrity, T.; Rich, L. C.; Marretta, J.; Ciosek, C. P., Jr. *J. Med. Chem.* **1991**, *34*, 1912. (b) Biller, S. A.; Sofia, M. J.; DeLange, B.; Forster, C.; Gordon, E. M.; Harrity, T.; Rich, L. C.; Ciosek, C. P., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 8522. (c) Biller, S. A.; Abt, J. W.; Pudzianowski, A. T.; Rich, L. C.; Slusarchyk, D. A.; Ciosek, C. P., Jr. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 595. (d) Ciosek, C. P., Jr.; Magnin, D. R.; Harrity, T. W.; Logan, J. V. H.; Dickson, J. K., Jr.; Gordon, E. M.; Hamilton, K. A.; Jolibois, K. G.; Kunselman, L. K.; Lawrence, R. M.; Mookhtiar, K. A.; Rich, L. C.; Slusarchyk, D. A.; Sulsky, R. B.; Biller, S. A. *J. Biol. Chem.* **1993**, *268*, 24832. (e) Magnin, D. R.; Biller, S. A.; Dickson, J. K., Jr.; Logan, J. V.; Lawrence, R. M.; Chen, Y.; Sulsky, R. B.; Ciosek, C. P., Jr.; Harrity, T. W.; Jolibois, K. G.; Kunselman, L. K.; Rich, L. C.; Slusarchyk, D. A. *J. Med. Chem.* **1995**, *38*, 2596. (f) Magnin, D. R.; Biller, S. A.; Chen, Y.; Dickson, J. K., Jr.; Fryszman, O. M.; Lawrence, R. M.; Logan, J. V.; Sieber-McMaster, E. S.; Sulsky, R. B.; Traeger, S. C.; Hsieh, D. C.; Lan, S.-J.; Rinehart, J. K.; Harrity, T. W.; Jolibois, K. G.; Kunselman, L. K.; Rich, L. C.; Slusarchyk, D. A.; Ciosek, C. P., Jr. *J. Med. Chem.* **1996**, *39*, 657. (g) Dickson, J. K.; Biller, S. A.; Magnin, D. R.; Petrillo, E. W., Jr.; Hillyer, J. W.; Hsieh, D. C.; Lan, S. J.; Rienhart, J. K.; Gregg, R. E.; Harrity, T. W.; Jolibois, K. G.; Kalinowski, S. S.; Kunselman, L. K.; Mookhtiar, K. A.; Ciosek, C. P., Jr. *J. Med. Chem.* **1996**, *39*, 661.
7. (a) Sandifer, R. M.; Thompson, M. D.; Gaughan, R. G.; Poulter, C. D. *J. Am. Chem. Soc.* **1982**, *104*, 7376. (b) Poulter, C. D.; Capson, T. L.; Thompson, M. D.; Bard, R. S. *J. Am. Chem. Soc.* **1989**, *111*, 3734. (c) Oehlschlager, A. C.; Singh, S. M.; Sharma, S. *J. Org. Chem.* **1991**, *56*, 3856. (d) Steiger, A.; Pyun, H.-J.; Coates, R. M. *J. Org. Chem.* **1992**, *57*, 3444. (e) Prashad, M.; Kathawala, F. G.; Scallen, T. *J. Med. Chem.* **1993**, *36*, 1501.
8. (a) For reviews, see: (a) Koert, U. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 773. (b) Kelly, M. J.; Roberts, S. M. *Nature* **1995**, *373*, 192. (c) Nadin, A.; Nicolaou, K. C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1622.
9. (a) Iwasawa, Y.; Hayashi, M.; Nomoto, T.; Shibata, J.; Mitsuya, M.; Hirota, K.; Yonemoto, M.; Kamei, T.; Miura, K.; Tomimoto, K. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1989. (b) Iwasawa, Y.; Shibata, J.; Mitsuya, M.; Masaki, H.; Hayashi, M.; Kanno, T.; Sawasaki, Y.; Hisaka, A.; Kamei, T.; Tomimoto, K. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 463. (c) Fung, A. K. L.; Baker, W. R.; Fakhoury, S.; Stein, H. H.; Cohen, J.; Donner, B. G.; Garvey, D. S.; Spina, K. P.; Rosenberg, S. H. *J. Med. Chem.* **1997**, *40*, 2123. (d) Shen, W.; Garvey, D. S.; Cohen, J.; Stein, H. H.; Rosenberg, S. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 891. (e) Watanabe, S.; Hirai, H.; Kambara, T.; Kojima, Y.; Nishida, H.; Sugiura, A.; Yamauchi, Y.; Yoshikawa, N.; Harwood, H. J., Jr.; Huang, L. H. *J. Antibiot.* **2001**, *54*, 1025.
10. (a) Brown, G. R.; Clarke, D. S.; Foubister, A. J.; Freeman, S.; Harrison, P. J.; Johnson, M. C.; Mallion, K. B.; McCormick, J.; McTaggart, F.; Reid, A. C.; Smith, G. J.; Taylor, M. J. *J. Med. Chem.* **1996**, *39*, 2971. (b) Brown, G. R.; Foubister, A. J.; Freeman, S.; McTaggart, F.; Mirrless, D. J.; Reid, A. C.; Smith, G. J.; Taylor, M. J.; Thomason, D. A.; Whittamore, P. R. O. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 597.
11. (a) Miki, T.; Kori, M.; Fujishima, A.; Mabuchi, H.; Tozawa, R.; Nakamura, M.; Sugiyama, Y.; Yukimasa, H. *Bioorg. Med. Chem.* **2002**, *10*, 385. (b) Miki, T.; Kori, M.; Mabuchi, H.; Banno, H.; Tozawa, R.; Nakamura, M.; Itokawa, S.; Sugiyama, Y.; Yukimasa, H. *Bioorg. Med. Chem.* **2002**, *10*, 401. (c) Miki, T.; Kori, M.; Tozawa, R.; Nakamura, M.; Sugiyama, Y.; Yukimasa, H. *Chem. Pharm. Bull.* **2002**, *50*, 53. (d) Tarui, N.; Nakahama, K.; Nagano, Y.; Izawa, M.; Matsumoto, K.; Kori, M.; Nagata, T.; Miki, T.; Yukimasa, H. *Chem. Pharm. Bull.* **2002**, *50*, 59. (e) Miki, T.; Kori, M.; Mabuchi, H.; Tozawa, R.; Nishimoto, T.; Sugiyama, Y.; Teshima, K.; Yukimasa, H. *J. Med. Chem.* **2002**, *45*, 4571.
12. Fairlamb, I. J. S.; Dickinson, J. M.; O'connor, R.; Higson, S.; Grieveson, L.; Marin, V. *Bioorg. Med. Chem.* **2002**, *10*, 2641.
13. Maillard, J.; Langlois, M.; Tri, V. V.; Chenu, J.; Eskenazi, P.; Benharkate, M.; Morin, R.; Pautas, C.; Parisy, D. *Chim. Ther.* **1971**, *6*, 257.
14. Corey, E. J.; Fuchs, P. L. *Tetrahedron Lett.* **1972**, 3769.
15. Campaigne, E.; Ashby, J. *J. Heterocycl. Chem.* **1969**, *6*, 875.
16. Buu-Hoi, N. P.; Hoan, N. *J. Chem. Soc.* **1951**, 1834.
17. Acheson, R. M.; Harrison, D. R. *J. Chem. Soc. (C)* **1970**, 1764.
18. For a review, see:Normant, J. F. *Synthesis* **1972**, 63.
19. Stotter, P. L.; Friedman, M. D.; Dorsey, G. O.; Shiely, R. W.; Williams, R. F.; Minter, D. E. *Heterocycles* **1987**, *25*, 251.
20. Campaigne, E.; Ashby, J. *J. Heterocycl. Chem.* **1969**, *6*, 517.
21. Grob, C. A.; Renk, E. *Helv. Chim. Acta* **1954**, *37*, 1689.
22. Saito, S.; Hasegawa, T.; Inaba, M.; Nishida, R.; Fujii, T.; Nomizu, S.; Moriwake, T. *Chem. Lett.* **1984**, *8*, 1389.
23. Oikawa, Y.; Tanaka, T.; Horita, K.; Noda, I.; Nakajima, N.; Kakusawa, N.; Hamada, T.; Yonemitsu, O. *Chem. Pharm. Bull.* **1987**, *35*, 2184.
24. Okada, M.; Yoden, T.; Kawaminami E.; Shimada, Y.; Ishihara T.; Kudou, M. PCT Int. Appl. WO9504723, 1995; *Chem. Abstr.* **1995**, *123*, 228181d.
25. Amin, D.; Cornell, S. A.; Gustafson, S. K.; Needle, S. J.; Ullrich, J. W.; Bilder, G. E.; Perrone, M. H. *J. Lipid. Res.* **1992**, *33*, 1657.
26. Etrl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
27. The compound was tested for its ability to inhibit the conversion of farnesyl diphosphate to squalene by squalene

synthase derived from HepG2 cells. The IC_{50} value was determined by a single experimental run in duplicate.
28. Spady, D. K.; Dietschy, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4526.
29. Illuminati, G.; Nobis, J. F.; Gilman, H. *J. Am. Chem. Soc.* **1951**, *73*, 5887.
30. Cullinane, N. M.; Padfield, H. J. H. *J. Chem. Soc.* **1935**, 1131.
31. Lindsey, S.; Harwood, H. J., Jr. *J. Biol. Chem.* **1995**, *270*, 9083.
32. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.