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Syntheses of 3-Ethylidenequinuclidine Derivatives as Squalene Synthase Inhibitors. Part 2: Enzyme Inhibition and Effects on Plasma Lipid Levels

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Abstract—Squalene synthase (E.C. 2.5.1.21) is a microsomal enzyme which catalyzes the reductive dimerization of two molecules of farnesyl diphosphate to form squalene, and is involved in the first committed step in cholesterol biosynthesis. It is an attractive target for hypocholesterolemic and hypotriglyceridemic strategies. We synthesized a series of 3-ethylidenequinuclidine derivatives, and evaluated their ability to inhibit squalene synthase in vitro and to lower non-HDL cholesterol levels in hamsters. 3-Ethylidenequinuclidine derivatives incorporating an unsubstituted 9*H*-carbazole moiety reduced plasma non-HDL cholesterol levels and did not affect plasma transaminase levels, indicating a lack of hepatotoxicity. Among the novel compounds, (*Z*)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole hydrochloride **8** (YM-53579) and (*E*)-2-[2-fluoro-2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole hydrochloride **28** (YM-53601) were potent inhibitors of squalene synthase derived from human hepatoma cells, with IC_{50} values of 160 and 79 nM, respectively. They also reduced plasma non-HDL cholesterol levels in hamsters by approximately 50 and 70%, respectively, at an oral dose of 50 mg/kg/day for 5 days.

Introduction

Despite major advances in pharmaceutical and surgical treatments, coronary heart disease remains a major cause of death in the industrialized world.[1] Elevated plasma cholesterol is a key risk factor for the disease,[2] and the most effective approach to reducing plasma cholesterol at present is the inhibition of cholesterol biosynthesis. A number of therapeutic agents are available that block cholesterol formation by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the biosynthesis of mevalonic acid.[3] Treatment of hypercholesterolemia with HMG-CoA reductase inhibitors reduces the risk of coronary heart disease by approximately one-third.[4]

In the hyperlipidemic population there is also a high prevalence of combined hyperlipidemia (elevated plasma cholesterol levels and elevated plasma triglyceride levels). A study group of the European Atherosclerosis Society has recommended that more attention be paid to hypertriglyceridemia as a risk factor for coronary heart disease.[5] However, the HMG-CoA reductase inhibitors have little effect on plasma triglyceride levels.[6] Fibrates are to date the primary drugs for treating hypertriglyceridemia; hence, hyperlipidemia is treated with a combination of a HMG-CoA reductase inhibitor and a fibrate. This therapy is, however, occasionally accompanied by severe adverse effects such as rhabdomyolysis.[7] An agent that could reduce plasma cholesterol and triglyceride levels with less adverse effects would therefore be highly desirable.

Squalene synthase (E.C. 2.5.1.21) catalyzes the reductive dimerization of two molecules of farnesyl pyrophosphate to squalene and is the first committed step in cholesterol biosynthesis. Inhibition of this step does not interfere with the biosyntheses of biologically important isoprenoids such as dolicols, ubiquinones and isopentenyl *t*-RNA, because the step is located after the branch points to the isoprenoids. Furthermore, squalene synthase inhibitors lower triglyceride levels as well

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as plasma cholesterol levels in vivo.[8] Squalene synthase is therefore an attractive target for the treatment of combined hyperlipidemia and coronary heart disease.

Brown et al. reported that 3-(4′-fluorobiphenyl-4-yl)-quinuclidin-3-ol **1** showed potent inhibition against squalene synthase;[9] an analougous quinuclidine derivative was suggested to function as an analogue of carbocationic intermediate in the squalene synthase reaction mechanism, with the hydrophobic side-chain occupying a farnesyl binding site and the charged nitrogen mimicking a carbocation.[10] We previously described a novel type of squalene synthase inhibitor, (*Z*)-3-ethylidenequinuclidine derivative **2** (Fig. 1) that had IC_{50} values of 76 and 48 nM, for the enzymes derived from hamster liver and human hepatoma cells respectively, and also lowered plasma non-HDL cholesterol levels in hamsters by 39% when administered at an oral dose of 50 mg/kg/day for 5 days.[11] The lack of chirality of the 3-ethylidenequinuclidine scaffold, its potent inhibition of squalene synthase and significant non-HDL cholesterol lowering effect made it an attractive template for further optimization.

In this paper, we describe studies on the syntheses and biological activities of 3-ethylidenequinuclidine derivatives as squalene synthase inhibitors.

Chemistry

Compounds **4**–**6** were prepared by reaction of (*Z*)-3-(2-chloroethylidene)quinuclidine-*N*-borane **3**, described previously,[11] with appropriate phenols, in the presence of potassium carbonate, followed by deprotection of the borane complex with ethanolic hydrogen chloride solution (Scheme 1).

The syntheses of 9*H*-carbazole derivatives **8** and **18**–**25** are shown in Scheme 2. Unsubstituted 9*H*-carbazole derivative **8** was obtained by a procedure similar to that described above. Alkylation of unsubstituted 9*H*-carbazole intermediate **7** with an appropriate alkyl halide in the presence of sodium hydride followed by deprotection of the borane complex produced the desired 9*H*-carbazole derivatives **18**–**23** with substituents on the 9-position of the 9*H*-carbazole moiety. Reaction of unsubstituted intermediate **7** with ethyl bromoacetate in the presence of sodium hydride, followed by reduction with diisobutylaluminum hydride, yielded intermediate alcohol **16**; its borane complex was then deprotected, generating the desired alcohol **24**. Mitsunobu condensation of the intermediate alcohol **16** and phthalimide, followed by deprotection of the phthaloyl moiety with hydrazine, produced intermediate amine **17**, which on treatment with ethanolic hydrogen chloride solution yielded the desired amine **25**.

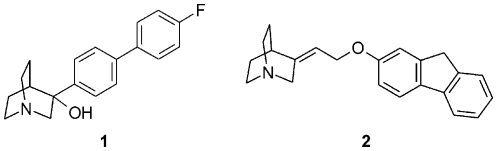
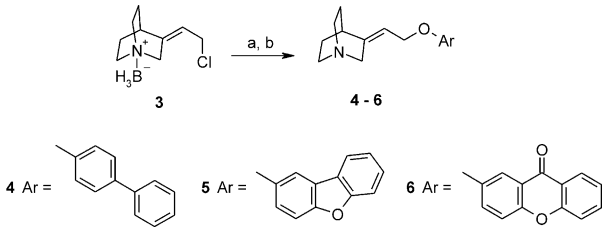
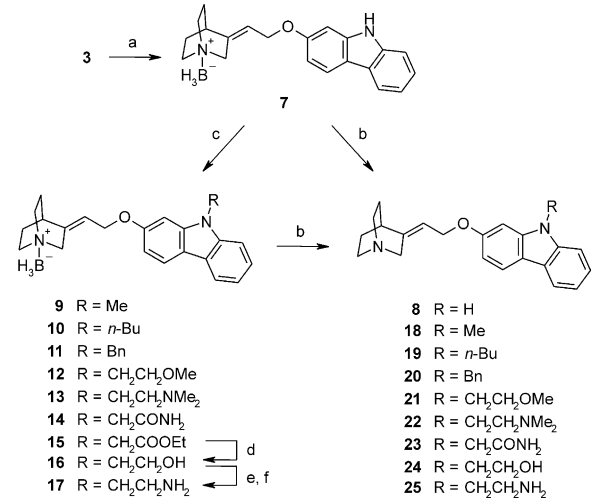


Figure 1. Structures of the quinuclidine-based squalene synthase inhibitors.

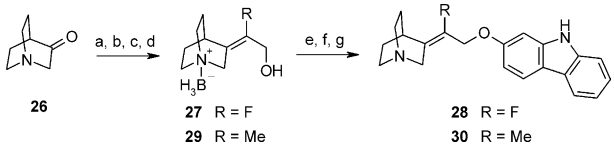
Substituted 3-ethylidenequinuclidine derivatives **28** and **30** were prepared as follows (Scheme 3): Wadsworth–Emmons reaction of 3-quinuclidinone with triethyl 2-fluoro-2-phosphonoacetate and triethyl 2-phosphonopropionate in the presence of sodium hydride followed by protection of the quinuclidine ring nitrogen atom as a borane complex, isomerization of the resulting α,β-unsaturated esters with a catalytic amount of sodium ethoxide, and final reduction with diisobutylaluminum hydride, produced the intermediate allyl alcohols **27** and **29**, respectively. The stereochemistry of allyl alcohol **27** was determined by a NOE difference experiment. Irradiation of the methylene proton at the 2-position of the quinuclidine nucleus (δ 3.67) enhanced the signal of the allylic methylene proton (δ 4.14) and also of the methylene protons at the 6- and 7-positions of the quinuclidine nucleus (δ 3.00–3.15), thereby establishing its structure as *E*-isomer. A similar NOE difference experiment on compound **29** also confirmed its structure as *Z*-isomer (Fig. 2). Chlorination of allyl alcohols **27** and **29** followed by reaction with 2-hydroxy-9*H*-carbazole in the presence of potassium carbonate, and final deprotection of the borane complex, yielded the desired substituted ethylidene derivatives **28** and **30**, respectively.



Scheme 1. (a) ArOH, K_2CO_3, DMF; (b) HCl, EtOH, acetone.

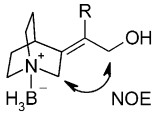


Scheme 2. (a) 2-Hydroxy-9*H*-carbazole, K_2CO_3, DMF; (b) HCl, EtOH, acetone; (c) alkyl halide, NaH, DMF; (d) diisobutylaluminum hydride, toluene; (e) phthalimide, PPh_3, diethyl azodicarboxylate, THF; (f) hydrazine monohydrate, MeOH.



26 **27** R = F **29** R = Me **28** R = F **30** R = Me

Scheme 3. (a) $(EtO)_2P(O)CHRCOOEt$, NaH, THF; (b) NaH, EtOH; (c) borane–THF complex, THF; (d) diisobutylaluminum hydride, toluene; (e) methanesulfonyl chloride, lithium chloride, triethylamine, CH_2Cl_2; (f) 2-hydroxy-9*H*-carbazole, K_2CO_3, DMF; (g) HCl, EtOH, acetone.



27 R = F
29 R = Me

Figure 2. Structures of 3-(2-hydroxyethylidene)quinuclidine derivatives **27** and **29**.

Results and Discussion

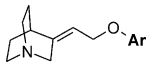
Inhibition by the synthesized compounds of the conversion of [^{3}H]farnesyl diphosphate to [^{3}H]squalene by hamster liver microsomal squalene synthase was measured by the method of Amin et al.[12] with a slight modification. The effect of oral doses of the test compounds on non-HDL cholesterol levels in hamsters was also examined. The selected compounds were also evaluated for lipid lowering effects following oral administration in guinea pigs and for inhibition of squalene synthase derived from human hepatoma (HepG2) cells.

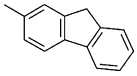
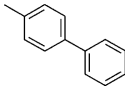
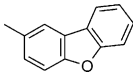
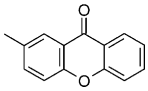
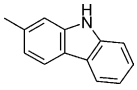
Since 9*H*-fuorene moieties are easily oxidized on their 9-position,[13] we explored alternative aromatic moieties. The corresponding biphenylyl analogue **4** had lower enzyme inhibitory activity than the parent compound **2** and did not reduce plasma non-HDL cholesterol (Table 1), indicating that the tricyclic system in 3-ethylidenequinuclidine derivatives confers potent inhibitory activity and the ability to lower non-HDL cholesterol. Extensive modifications of the tricyclic systems were therefore explored with the aim of improving oral activity, focusing particularly on compounds lacking a benzylic proton in order to avoid oxidative breakdown.

Dibenzofuran derivative **5**, 9*H*-xanthen-9-one derivative **6** and 9*H*-carbazole derivative **8** showed only moderate inhibitory activity aqainst squalene synthase. However, they did lower plasma non-HDL cholesterol after oral administration. The 9*H*-carbazole derivative **8**, in particular, reduced plasma non-HDL cholesterol levels by 51% at a dose of 50 mg/kg/day for 5 days. Introducing a tricyclic system having no benzlic proton positively influenced the pharmacokinetic profile of the 3-ethylidenequinuclidine-based inhibitors.

The effects of substitutions on the 9*H*-carbazole moiety of compound **8** are shown in Table 2. *N*-methyl derivative **18**, *N*-butyl derivative **19** and *N*-benzyl derivative **20** exhibited inhibitory activity for squalene synthase comparable to that of the unsubstituted parent compound **8**. Interestingly, substitution with a lipophilic group, such as a benzyl moiety (**20**), resulted in a dramatic loss in efficacy in lowering non-HDL cholesterol. This led us to introduce a less lipophilic group on the 9*H*-carbazole moiety in order to improve oral activity.

Table 1. In vitro and in vivo activities of 3-ethylidenequinuclidine derivatives



Compd	Ar	IC_{50} (μM)[a]	non-HDL-C% change[b]
2		0.076	−39***
4		0.33	NE[c]
5		0.37	−25
6		0.26	−16
8		0.27	−51***
1		0.38	−44*

[a]Compounds were tested for their ability to inhibit conversion of [^{3}H]farnesyl diphosphate to [^{3}H]squalene by hamster liver squalene synthase. IC_{50} values were determined in duplicate in a single experimental run.
[b]Mean percent change from the respective control value of plasma non-HDL cholesterol levels after oral administration in hamsters (50 mg/kg/day for 5 days, $n=7$). *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus control by Student's *t*-test.
[c]No effect.

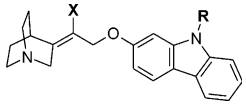
N-(2-Methoxyethyl)-9*H*-carbazole derivative **21** was comparable in both in vitro and in vivo activities to *N*-butyl derivative **19**, whereas the corresponding *N*-(2-hydroxyethyl)-9*H*-carbazole derivative **24** had increased inhibitory activity and oral potency. On the other hand, *N*-(2-dimethylaminoethyl)-9*H*-carbazole derivative **22** and *N*-(aminocarbonylmethyl)-9*H*-carbazole derivative **23** were less potent inhibitors than parent compound **8**. Excellent oral activity was observed for *N*-(2-aminoethyl)-9*H*-carbazole derivative **25**; this compound lowered plasma non-HDL cholesterol levels by 82% at an oral dose of 50 mg/kg/day for 5 days despite its moderate in vitro activity. As expected, introducing a hydrophilic substituent onto the 9*H*-carbazole moiety enhanced oral activity.

Unfortunately, compound **25** elevated plasma transaminase (AST and ALT) levels in rats (Table 3), indicating that it was hepatotoxic. On the other hand, parent compound **8** did not affect the level of plasma transaminase despite its good in vivo activity, suggesting that not all squalene synthase inhibitors are hepatotoxic. We

therefore carried out further modification of unsubstituted 9*H*-carbazole derivatives, hoping that they would possess significant non-HDL cholesterol lowering activity and desirable toxicological profiles.

We have already reported that introduction of a methyl group onto the linkage connecting the tricyclic system with the quinuclidine nucleus increases inhibitory activity.[11] We therefore examined the consequences of adding substituents onto the olefin linkage of compound **8**; the results are shown in Table 2. Methyl-substituted analogue **30** was found to have the same in vitro and in vivo activity as the parent compound **8**. The corresponding fluoro-substituted analogue **28** had improved inhibitory activity and non-HDL cholesterol lowering effect. At an oral dose of 50 mg/kg/day for 5 days, it lowered plasma non-HDL cholesterol levels by 73%. These modifications had interesting effects on rat hepatotoxicity (Table 3): methyl derivative **30** elevated plasma transaminase levels, whereas fluoro analogue **28** did not, indicating that the electron-rich olefine may be the cause of hepatotoxicity.

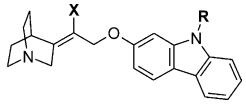
Table 2. In vitro and in vivo activities of carbazole-containing quinuclidine derivatives



Compd	R	X	IC_{50} (μM)[a]	Non-HDL-C% change[a]
8	H	H	0.27	−51***
18	Me	H	0.26	−29*
19	*n*-Bu	H	0.24	−26**
20	Bn	H	0.21	NE[b]
21	CH_2CH_2OMe	H	0.28	−27*
24	CH_2CH_2OH	H	0.14	−35**
22	$CH_2CH_2NMe_2$	H	0.59	NT[c]
25	$CH_2CH_2NH_2$	H	0.23	−82***
23	CH_2CONH_2	H	0.68	NT[c]
30	H	Me	0.30	−47***
28	H	F	0.17	−73***

[a]Refer to Table 1.
[b]No effect.
[c]Not tested.

Table 3. Effects of 3-ethylidenequinuclidine derivatives on biochemical parameters in F344 rats[a]



Compd	R	X	AST (IU/L)[b]	ALT (IU/L)[c]
	Vehicle		106	38
8	H	H	100	43
25	$CH_2CH_2NH_2$	H	165*	112*
30	H	Me	211	61*
28	H	F	103	41
1			319*	95

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

The effects on plasma lipids of the carbazole-based squalene synthase inhibitors were examined in guinea pigs. The guinea pig is the only rodent that responds to administration of HMG-CoA reductase inhibitors[14] and is therefore suitable for comparing the carbazole-based squalene inhibitors and pravastatin, the HMG-CoA reductase inhibitor. Total cholesterol, the sum of HDL cholesterol and non-HDL cholesterol, is known to be a usuful clinical parameter. So we focused on the level of total cholesterol, and determined the each experimental dose that the two lipid lowering agents demonstrated similar effect on total cholesterol levels. At an oral dose of 50 mg/kg/day for 14 days, compound **8** lowered plasma triglyceride levels by 77% (Fig. 3), whereas pravastatin did not affect plasma triglyceride levels. Compound **8** also had a dramatic effect on plasma cholesterol levels (Fig. 4). Pravastatin (25 mg/kg/day, 14 days) lowered plasma non-HDL cholesterol and HDL cholesterol levels by 19 and 22%, respectively, whereas compound **8** (50 mg/kg/day, 14 days) reduced plasma non-HDL cholesterol levels by 50% and increased plasma HDL cholesterol levels by 44%. We have already reported that compound **28** affects plasma lipid levels.[15] Several studies have indicated that plasma

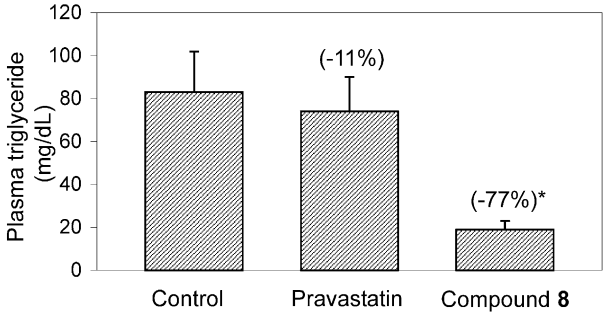


Figure 3. Effects of oral administration of pravastatin and compound **8** on plasma triglyceride levels in guinea pigs. Pravastatin and compound **8** were given for 14 days at 25 mg/kg/day ($n=6$) and 50 mg/kg/day ($n=4$), respectively. The mean percent changes from the respective control value are shown in parentheses. *$p<0.05$ versus control by Student's *t*-test.

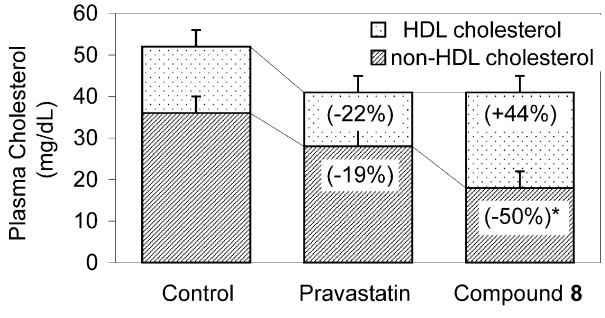


Figure 4. Effect of oral administration of pravastatin and compound **8** on plasma cholesterol levels in guinea pigs. Other data as in the legend to Figure 3.

HDL cholesterol is protective against atherosclerosis and that elevated levels of plasma HDL cholesterol decrease the incidence of coronary heart disease.[16] We therefore expect that the 3-ethylidenequinuclidine-based squalene synthase inhibitors may be superior to the HMG-CoA reductase inhibitors in the treatment of combined hyperlipidemia and coronary heart disease. The mechanism underlying the elevation of plasma HDL cholesterol levels will be considered elsewhere.

Table 4 shows the inhibitory activities of the various compounds for the human enzyme; compounds **8** and **28** were both effective inhibitors with IC_{50} values of 160 and 48 nM, respectively.

Because of their potent inhibition of squalene synthase, significant effect on plasma lipid levels and good safety profiles, compounds **8** (YM-53579) and **28** (YM-53601) were further investigated, and their pharmacokinetic profiles measured in rats after a single oral dose of 50 mg/kg. Plasma concentration–time curves are shown in Fig. 5. It is evident that a subtle change at the linkage connecting the tricyclic system with the quinuclidine nucleus can have a pronounced effect on the overall absorption profile of compounds in this series. Thus, the peak concentration obtained with compound **28** was 0.92 μg/mL compared with 0.44 μg/mL for compound **8**. The areas under the plasma concentration versus time curve of compounds **28** and **8** were 4.1 and 2.6 μg h/mL, respectively. Clearly, the addition of the fluorine atom to the olefin linkage improved the pharmacokinetic parameters.

Table 4. In vitro inhibitory activities of compounds **8** and **28**

Compd	IC_{50} (μM)[a]
8	0.16
28	0.079
1	0.084

[a]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 in duplicate in a single experimental run.

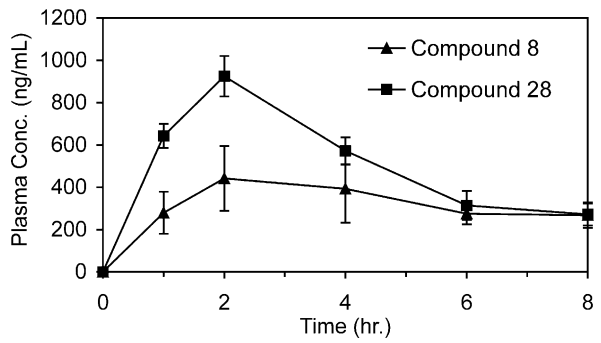


Figure 5. Plasma concentrations of compounds **8** and **28** following oral administration in rats. Compounds **8** and **28** were administered at 50 mg/kg ($n = 6$ and 4, respectively).

Conclusion

We have synthesized a series of novel 3-ethylidenequinuclidine derivatives, and evaluated their ability to inhibit squalene synthase in vitro and to lower non-HDL cholesterol following oral administration in hamsters. The 3-ethylidenequinuclidine derivatives inhibited squalene synthase at submicromolar levels. Compounds incorporating a tricyclic system, such as dibenzofuran, 9*H*-carbazole or 9*H*-xanthen-9-one, reduced plasma non-HDL cholesterol after oral administration whereas their biphenylyl analogues did not, despite inhibiting enzyme activity to a similar extent. Substitution of a hydrophilic moiety, for example a 2-hydroxyethyl or 2-dimethylaminoethyl group, onto the 9-position of the 9*H*-carbazole moiety increased non-HDL cholesterol lowering activity, as did the introduction of a fluorine atom into the olefine linkage.

Of this novel series, (*Z*)-2-[2-(quinuclidin-3-ylidene)-ethoxy]-9*H*-carbazole hydrochloride **8** (YM-53579), and (*E*)-2-[2-fluoro-2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole hydrochloride **28** (YM-53601) lowered plasma non-HDL cholesterol levels by approximately 50 and 70%, respectively, when administered to hamsters at 50 mg/kg/day for 5 days. These two compounds did not affect plasma transaminase levels in rats and were potent inhibitors of squalene synthase from human hepatoma cells, with IC_{50} values of 160 and 79 nM, respectively.

We propose that carbazole-based derivatives, as represented by compounds **8** and **28**, are promising candidate for lipid-lowering agents with potential in the treatment of coronary heart disease.

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.

(*Z*)-3-[2-(Biphenyl-4-yloxy)ethylidene]quinuclidine hydrochloride (4). A mixture of (*Z*)-3-(2-chloroethylidene)quinuclidine-*N*-borane **3** (880 mg, 4.74 mmol), 4-phenylphenol (807 mg, 4.74 mmol), potassium carbonate (3.28 g, 23.7 mmol) and *N*,*N*-dimethylformamide (15 mL) was stirred at ambient temperature for 13 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 brine, dried over magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel

eluting with *n*-hexane–ethyl acetate (3:1 by volume) to give a colorless solid. The resulting solid was dissolved in ethanol–acetone (30 mL, 1:2 by volume). To this solution was added hydrogen chloride in ethanol (5 M, 2.0 mL) at ambient temperature and stirred for 0.5 h. Ether (10 mL) was added and the resulting precipitate was filtered to give the title compound as a colorless crystalline solid (550 mg, 34%): mp 249–251 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.78–1.84 (2H, m), 1.93–2.00 (2H, m), 2.66–2.68 (1H, m), 3.20–3.30 (4H, m), 4.08 (2H, s), 4.57 (2H, d, J = 6.1 Hz), 5.70–5.74 (1H, m), 7.04–7.05 (2H, m), 7.27–7.33 (1H, m), 7.42–7.45 (2H, m), 7.60–7.62 (4H, m), 10.07 (1H, br s); EI–MS m/z 305 (M^+). Anal. calcd for $C_{21}H_{23}NO{\cdot}HCl$: C, 73.78; H, 7.08; N, 4.10; Cl, 10.37. Found: C, 73.77; H, 7.09; N, 4.06; Cl, 10.27.

(*Z*)-3-[2-(Dibenzofuran-2-yloxy)ethylidene]quinuclidine hydrochloride (5). The title compound was obtained from 2-hydroxydibenzofuran using the methods described for the synthesis of compound **4** as a colorless crystalline solid (68%): mp 206–208 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.80–1.86 (2H, m), 1.92–2.02 (2H, m), 2.70–2.74 (1H, m), 3.12–3.36 (4H, m), 4.13 (2H, s), 4.63 (2H, d, J = 6.0 Hz), 5.76–5.78 (1H, m), 7.13 (1H, dd, J = 2.5 Hz, 9.0 Hz), 7.38–7.41 (1H, m), 7.50–7.53 (1H, m), 7.62 (1H, d, J = 9.0 Hz), 7.67 (1H, d, J = 8.0 Hz), 7.74 (1H, d, J = 2.5 Hz), 8.12 (1H, d, J = 7.0 Hz), 10.55 (1H, br s); EI–MS m/z 319 (M^+). Anal. calcd for $C_{21}H_{21}NO_2{\cdot}HCl{\cdot}0.1H_2O$: C, 70.17; H, 6.28; N, 3.90; Cl, 9.86. Found: C, 69.80; H, 6.29; N, 3.81; Cl, 9.95.

(*Z*)-2-[2-(Quinuclidin-3-ylidene)ethoxy]-9*H*-xanthen-9-one hydrochloride (6). The title compound was obtained from 2-hydroxy-9*H*-xanthen-9-one using the methods described for the synthesis of compound **4** as a colorless crystalline solid (33%): mp 258–260 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.80–1.88 (2H, m), 1.94–2.06 (2H, m), 2.70–2.74 (1H, m), 3.24–3.38 (4H, m), 4.15 (2H, s), 4.67 (2H, d, J = 6.5 Hz), 5.72–5.75 (1H, m), 7.47–7.53 (2H, m), 7.61 (1H, d, J = 3.5 Hz), 7.66–7.69 (2H, m), 7.86–7.90 (1H, m), 8.20–8.22 (1H, m), 10.77 (1H, br s); EI–MS m/z 347(M^+). Anal. calcd for $C_{22}H_{21}NO_3{\cdot}HCl{\cdot}0.5H_2O$: C, 67.26; H, 5.90; N, 3.57; Cl, 9.20. Found: C, 66.92; H, 5.76; N, 3.57; Cl, 9.28.

(*Z*)-2-[2-(Quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane (7). A mixture of (*Z*)-3-(2-chloroethylidene)quinuclidine-*N*-borane **3** (4.67 g, 25.2 mmol), 2-hydroxycarbazole (4.62 g, 25.2 mmol), potassium carbonate (17.4 g, 126 mmol) and *N*,*N*-dimethylformamide (25 mL) was stirred at ambient temperature for 20 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 brine, dried over magnesium sulfate and concentrated in vacuo to give a colorless solid. The resulting solid was crystallized from ethyl acetate to give the title compound as a colorless crystalline solid (5.23 g, 62%): mp 205–207 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.70–1.97 (4H, m), 2.52–2.61 (1H, m), 2.87–3.09 (4H, m), 3.73 (2H, s), 4.59 (2H, d, J = 7.0 Hz), 5.52–5.71 (1H, m), 6.80 (1H, dd, J = 1.8 Hz, 8.1 Hz), 7.01 (1H, d, J = 1.8 Hz), 7.09–7.52 (3H, m), 7.93–8.07 (2H, m), 11.11 (1H, br s); EI–MS m/z 332 (M^+).

(*Z*)-2-[2-(Quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole hydrochloride (8). (*Z*)-2-[2-(Quinuclidin-3-ylidene)-ethoxy]-9*H*-carbazole-*N*-borane **7** (3.04 g, 9.15 mmol) in ethanol–acetone (23 mL, 1:2 by volume) was treated with hydrogen chloride in ethanol (5 M, 7.5 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 (2.91 g, 90%): mp 251–253 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.80–1.88 (2H, m), 1.93–2.02 (2H, m), 2.70–2.74 (1H, m), 3.22–3.32 (4H, m), 4.14 (2H, s), 4.60 (2H, d, J = 6.0 Hz), 5.74–5.76 (1H, m), 6.79 (1H, dd, J = 2.0 Hz, 8.5 Hz), 6.99 (1H, d, J = 2.0 Hz), 7.09–7.14 (1H, m), 7.27–7.32 (1H, m), 7.62 (1H, d, J = 8.5 Hz), 7.94–8.00 (2H, m), 10.54 (1H, br s), 11.17 (1H, br s); EI–MS m/z 318 (M^+). Anal. calcd for $C_{21}H_{22}N_2O{\cdot}HCl$: C, 71.08; H, 6.53; N, 7.89; Cl, 9.99. Found: C, 70.93; H, 6.51; N, 7.92; Cl, 10.16.

(*Z*)-9-Methyl-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane (9). To a stirred solution of (*Z*)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane **7** (1.06 g, 3.19 mmol) in *N*,*N*-dimethylformamide (9.0 mL) was added sodium hydride (153 mg, 3.83 mmol, 60% dispersion in mineral oil) at ambient temperature and stirred for 0.5 h. Iodomethane (544 mg, 3.83 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 1 h. After addition of H_2O (9.0 mL), the reaction mixture was extracted with ethyl acetate. The extract was washed with H_2O and brine, dried over magnesium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel eluting with *n*-hexane–ethyl acetate (4:1 by volume) to give the title compound as a colorless solid (1.05 g, 95%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.79–2.05 (4H, m), 2.52–2.67 (1H, m), 2.99–3.17 (4H, m), 3.79 (5H, s), 4.56 (2H, d, J = 6.4 Hz), 5.59–5.79 (1H, m), 6.79–6.89 (2H, m), 7.12–7.47 (3H, m), 7.93–8.05 (2H, m); EI–MS m/z 346 (M^+).

(*Z*)-9-Methyl-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole hydrochloride (18). The title compound was obtained from (*Z*)-9-methyl-2-[2-(quinuclidin-3-ylidene)-ethoxy]-9*H*-carbazole-*N*-borane **9** using the methods described for the synthesis of compound **8** as a colorless crystalline solid (99%): mp 242–244 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.82–1.88 (2H, m), 1.94–2.00 (2H, m), 2.71–2.72 (1H, m), 3.21–3.27 (4H, m), 3.84 (3H, s), 4.12 (2H, s), 4.66 (2H, d, J = 6.5 Hz), 5.77–5.79 (1H, m), 6.83 (1H, dd, J = 2.0 Hz, 8.5 Hz), 7.15-7.17 (2H, m), 7.36–7.39 (1H, m), 7.52 (1H, d, J = 8.0 Hz), 8.01–8.04 (2H, m), 10.87 (1H, br s); FAB-MS m/z 333 ($M+H^+$). Anal. calcd for $C_{22}H_{24}N_2O{\cdot}HCl{\cdot}0.4H_2O$: C, 70.26; H, 6.91; N, 7.45; Cl, 9.43. Found: C, 70.18; H, 6.85; N, 7.40; Cl, 9.56.

(*Z*)-9-Butyl-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane (10). The title compound was obtained from iodobutane using the methods described for the

synthesis of compound **9** as a colorless solid (94%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.95 (3H, t, J = 7.5 Hz), 1.20–1.54 (4H, m), 1.67–2.04 (4H, m), 2.54–2.68 (1H, m), 2.99–3.18 (4H, m), 3.80 (2H, s), 4.25 (2H, t, J = 7.3 Hz), 4.56 (2H, d, J = 6.4 Hz), 5.56–5.79 (1H, m), 6.75–6.86 (1H, m), 7.19–7.42 (4H, m), 7.91–8.05 (2H, m); EI–MS m/z 388 (M^+).

(*Z*)-9-Butyl-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole hydrochloride (19). The title compound was obtained from (*Z*)-9-butyl-2-[2-(quinuclidin-3-ylidene)-ethoxy]-9*H*-carbazole-*N*-borane **10** using the methods described for the synthesis of compound **8** as a colorless crystalline solid (84%): mp 202–204 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.89 (3H, t, J = 7.5 Hz), 1.27–1.35 (2H, m), 1.71–1.77 (2H, m), 1.80–1.84 (2H, m), 1.94–2.00 (2H, m), 2.70–2.72 (1H, m), 3.21–3.34 (4H, m), 4.13 (2H, s), 4.35 (2H, t, J = 7.5 Hz), 4.65 (2H, d, J = 6.0 Hz), 5.76–5.78 (1H, m), 6.82 (1H, dd, J = 2.5 Hz, 8.5 Hz), 7.12–7.16 (2H, m), 7.34–7.37 (1H, m), 7.52 (1H, d, J = 8.5 Hz), 8.00–8.03 (2H, m), 10.81 (1H, br s); EI–MS m/z 374 (M^+). Anal. calcd for $C_{25}H_{30}N_2O$·HCl: C, 73.06; H, 7.60; N, 6.82; Cl, 8.63. Found: C, 72.81; H, 7.68; N, 6.76; Cl, 8.67.

(*Z*)-9-Benzyl-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane (11). The title compound was obtained from benzyl bromide using the methods described for the synthesis of compound **9** as a colorless solid (96%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.74–1.95 (4H, m), 2.49–2.63 (1H, m), 2.96–3.14 (4H, m), 3.75 (2H, s), 4.49 (2H, d, J = 6.3 Hz), 5.47 (2H, s), 5.57–5.71 (1H, m), 6.85 (1H, dd, J = 2.2 Hz, 7.9 Hz), 7.10–7.37 (9H, m), 7.94–8.05 (2H, m); EI–MS m/z 422 (M^+).

(*Z*)-9-Benzyl-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole hydrochloride (20). The title compound was obtained from (*Z*)-9-benzyl-2-[2-(quinuclidin-3-ylidene)-ethoxy]-9*H*-carbazole-*N*-borane **11** using the methods described for the synthesis of compound **8** as a colorless crystalline solid (82%): mp 220–222 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.74–1.82 (2H, m), 1.90–1.98 (2H, m), 2.66–2.70 (1H, m), 3.16–3.24 (2H, m), 3.28–3.34 (2H, m), 4.10 (2H, s), 4.60 (2H, d, J = 6.0 Hz), 5.63 (2H, s), 5.72–5.74 (1H, m), 6.85 (1H, dd, J = 2.5 Hz, 8.5 Hz), 7.15–7.34 (8H, m), 7.52 (1H, d, J = 8.5 Hz), 8.04–8.07 (2H, m), 10.48 (1H, br s); EI–MS m/z 408 (M^+). Anal. calcd for $C_{28}H_{28}N_2O$·HCl·0.5H_2O: C, 74.07; H, 6.66; N, 6.17; Cl, 7.81. Found: C, 74.05; H, 6.78; N, 6.18; Cl, 7.88.

(*Z*)-9-(2-Methoxyethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane (12). The title compound was obtained from 2-methoxyethyl chloride using the methods described for the synthesis of compound **9** as a colorless solid (93%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.78–2.00 (4H, m), 2.52–2.63 (1H, m), 2.99–3.15 (4H, m), 3.30 (3H, s), 3.70–3.85 (4H, m), 4.19 (2H, t, J = 5.4 Hz), 4.56 (2H, d, J = 6.3 Hz), 5.60–5.79 (1H, m), 6.76–6.95 (2H, m), 7.15–7.41 (3H, m), 7.89–8.05 (2H, m); EI–MS m/z 390 (M^+).

(*Z*)-9-(2-Methoxyethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole (21). (*Z*)-9-(2-Methoxyethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane **12** (1.16 g, 2.97 mmol) in ethanol–acetone (15 mL, 1:2 by volume) was treated with hydrogen chloride in ethanol (5 M, 5.0 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 reaction mixture was extracted with ethyl acetate and the extract was dried over magnesium sulfate and concentrated in vacuo. The resulting 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 was crystallized from ether to give the title compound as a colorless crystalline solid (258 mg, 23%): mp 90–91 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.73–1.76 (4H, m), 2.42–2.44 (1H, m), 2.85–2.97 (4H, m), 3.31 (3H, s), 3.64 (2H, s), 3.76 (2H, t, J = 6.5 Hz), 4.42 (2H, t, J = 6.5 Hz), 4.59 (2H, d, J = 6.0 Hz), 5.55–5.57 (1H, m), 6.88 (1H, dd, J = 2.5 Hz, 8.5 Hz), 6.94 (1H, d, J = 2.5 Hz), 7.18–7.21 (1H, m), 7.36–7.39 (2H, m), 7.94 (1H, d, J = 8.5 Hz), 7.98 (1H, d, J = 8.0 Hz); FAB-MS m/z 377 ($M+H^+$). Anal. calcd for $C_{24}H_{28}N_2O_2$·0.1H_2O: C, 76.20; H, 7.51; N, 7.41. Found: C, 76.08; H, 7.65; N, 7.43.

(*Z*)-9-(2-Dimethylaminoethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane (13). To a stirred solution of (*Z*)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane **7** (970 mg, 2.92 mmol) in *N*,*N*-dimethylformamide (15 mL) was added sodium hydride (257 mg, 6.42 mmol, 60% dispersion in mineral oil) at ambient temperature and stirred for 0.5 h. 2-Dimethylaminoethyl chloride hydrochloride (463 mg, 3.21 mmol) was added at 0 °C and stirred for 0.5 h. The reaction mixture was allowed to warm to ambient temperature, stirred for a further 15 h. After addition of H_2O (15 mL), the reaction mixture was extracted with ethyl acetate. The extract was washed with H_2O and brine, dried over magnesium sulfate and concentrated in vacuo. The resulting residue was chromatographed over silica gel eluting with chloroform–methanol–concd ammonium hydroxide (100:10:1 by volume) to give the title compound as a colorless solid (830 mg, 70%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.70–1.95 (4H, m), 2.32 (6H, s), 2.49–3.12 (7H, m), 3.78 (2H, s), 4.31 (2H, t, J = 8.1 Hz), 4.51 (2H, d, J = 7.2 Hz), 5.52–5.70 (1H, m), 6.82 (1H, dd, J = 1.8 Hz, 7.7 Hz), 7.12–7.40 (3H, m), 7.79–8.00 (3H, m); FAB-MS m/z 404 ($M+H^+$).

(*Z*)-9-(2-Dimethylaminoethyl)-2-[2-(quinuclidin-3-ylidene)-ethoxy]-9*H*-carbazole hydrochloride (22). The title compound was obtained from (*Z*)-9-(2-dimethylaminoethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane **13** using the methods described for the synthesis of compound **8** as a colorless crystalline solid (51%): mp 214–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.80–1.90 (2H, m), 1.94–2.04 (2H, m), 2.71–2.73 (1H, m), 2.85 (6H, s), 3.26–3.33 (4H, m), 3.40 (2H, t, J = 8.0 Hz), 4.15 (2H, s), 4.72 (2H, d, J = 6.0 Hz), 4.85 (2H, t, J = 8.0 Hz), 5.78–5.81 (1H, m), 6.87 (1H, dd, J = 2.0 Hz, 8.4 Hz), 7.18–7.22 (1H, m), 7.38–7.42 (1H, m), 7.51 (1H, d, J = 2.0 Hz), 7.72 (1H, d, J = 8.0 Hz), 8.03–8.06 (2H, m),

10.76 (1H, br s), 11.67 (1H, br s); FAB-MS *m*/*z* 390 ($M+H^+$). Anal. calcd for $C_{25}H_{31}N_3O{\cdot}2HCl{\cdot}0.8H_2O$: C, 62.97; H, 7.31; N, 8.81; Cl, 14.87. Found: C, 63.09; H, 7.47; N, 8.69; Cl, 14.88.

(*Z*)-{2-[2-(Quinuclidin-3-ylidene)ethoxy]-9*H*-carbazol-9-yl}acetamide-*N*-borane (14). The title compound was obtained from chloroacetamide using the methods described for the synthesis of compound **9** as a colorless solid (54%): ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.72–1.98 (4H, m), 2.72–2.74 (1H, m), 2.89–3.04 (4H, m), 3.71 (2H, s), 4.59 (2H, d, $J=6.1$ Hz), 4.94 (2H, s), 5.53–5.78 (1H, m), 6.85 (1H, dd, $J=1.8$ Hz, 8.1 Hz), 7.09–7.71 (5H, m), 7.95–8.05 (3H, m); EI–MS *m*/*z* 389 (M^+).

(*Z*)-{2-[2-(Quinuclidin-3-ylidene)ethoxy]-9*H*-carbazol-9-yl}acetamide hydrochloride (23). The title compound was obtained from (*Z*)-{2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazol-9-yl}acetamide-*N*-borane **14** using the methods described for the synthesis of compound **8** as a colorless crystalline solid (62%): mp 252–254 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.78–1.88 (2H, m), 1.92–2.00 (2H, m), 2.68–2.72 (1H, m), 3.12–3.35 (4H, m), 4.12 (2H, s), 4.64 (2H, d, $J=6.5$ Hz), 4.96 (2H, s), 5.70–5.76 (1H, m), 6.84 (1H, dd, $J=2.0$ Hz, 8.5 Hz), 7.10 (1H, d, $J=2.0$ Hz), 7.12–7.18 (1H, m), 7.27 (1H, br s), 7.33–7.36 (1H, m), 7.43 (1H, d, $J=8.0$ Hz), 7.73 (1H, br s), 8.00–8.03 (2H, m), 10.73 (1H, br s); FAB-MS *m*/*z* 376 ($M+H^+$). Anal. calcd for $C_{23}H_{25}N_3O_2{\cdot}HCl{\cdot}0.1H_2O$: C, 66.77; H, 6.38; N, 10.16; Cl, 8.57. Found: C, 66.68; H, 6.62; N, 9.98; Cl, 8.59.

[Ethyl (*Z*)-{2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazol-9-yl}acetate]-*N*-borane (15). The title compound was obtained from ethyl bromoacetate using the methods described for the synthesis of compound **9** as a brown oil (85%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.24 (3H, t, $J=7.3$ Hz), 1.76–1.98 (4H, m), 2.51–2.65 (1H, m), 2.86–3.19 (4H, m), 3.78 (2H, s), 4.21 (2H, q, $J=7.3$ Hz), 4.54 (2H, d, $J=6.4$ Hz), 4.93 (2H, s), 5.57–5.78 (1H, m), 6.79–6.88 (2H, m), 7.21–7.34 (3H, m), 7.90–8.04 (2H, m); EI–MS *m*/*z* 418 (M^+).

{(*Z*)-9-(2-Hydroxyethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole}-*N*-borane (16). A solution of diisobutylaluminum hydride in *n*-hexane (53.6 mL, 0.98 M, 52.5 mmol) was added dropwise to a solution of [ethyl (*Z*)-{2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazol-9-yl}acetate]-*N*-borane **15** (7.33 g, 17.5 mmol) in dichloromethane (86 mL) at −78 °C and stirred for 4 h. Methanol (4.41 mL) followed by H_2O (7.35 mL) was added at −78 °C. The reaction mixture was allowed to warm to ambient temperature, stirred for a further 1.5 h and filtered through a pad of Celite. The filtrate was concentrated in vacuo to give the title compound as a colorless solid (5.92 g, 90%). ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.76–1.98 (4H, m), 2.56–2.66 (1H, m), 2.97–3.15 (4H, m), 3.77 (2H, s), 4.04 (2H, t, $J=5.0$ Hz), 4.42 (2H, t, $J=5.0$ Hz), 4.55 (2H, d, $J=6.1$ Hz), 5.57–5.78 (1H, m), 6.77–6.95 (2H, m), 7.12–7.43 (3H, m), 7.90–8.03 (2H, m); EI–MS *m*/*z* 376 (M^+).

(*Z*)-9-(2-Hydroxyethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole (24). The title compound was obtained from {(*Z*)-9-(2-hydroxyethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole}-*N*-borane **16** using the methods described for the synthesis of compound **21** as a colorless crystalline solid (42%): mp 146–148 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.64–1.67 (4H, m), 2.35–2.36 (1H, m), 2.62–2.68 (2H, m), 2.75–2.81 (2H, m), 3.56 (2H, s), 4.01 (2H, t, $J=5.5$ Hz), 4.39 (2H, t, $J=5.5$ Hz), 4.49 (2H, d, $J=6.0$ Hz), 5.47–5.49 (1H, m), 6.84 (1H, dd, $J=2.5$ Hz, 8.5 Hz), 6.96 (1H, d, $J=2.5$ Hz), 7.18–7.21 (1H, m), 7.36–7.43 (2H, m), 7.94 (1H, d, $J=8.5$ Hz), 7.98 (1H, d, $J=8.0$ Hz); FAB-MS *m*/*z* 363 ($M+H^+$). Anal. calcd for $C_{23}H_{26}N_2O_2{\cdot}0.2H_2O$: C, 75.46; H, 7.27; N, 7.65. Found: C, 75.58; H, 7.24; N, 7.64.

(*Z*)-9-(2-Aminoethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane (17). To a stirred solution of (*Z*)-9-(2-hydroxyethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane **16** (3.60 g, 9.57 mmol), phthalimide (1.83 g, 12.4 mmol) and triphenylphosphine (3.26 g, 12.4 mmol) in tetrahydrofuran (19 mL) at ambient temperature was added diethyl azodicarboxylate (2.16 g, 12.4 mmol). After stirring at ambient temperature for 15 h, the reaction mixture was concentrated in vacuo. The residue was chromatographed over silica gel eluting with chloroform–methanol (10:1 by volume) to give a pale yellow solid. A mixture of the resulting solid, hydrazine monohydrate (403 mg, 8.07 mmol) and ethanol (80 mL) was stirred under reflux for 1.5 h. The reaction mixture was cooled to ambient temperature and filtered. The filtrate was concentrated in vacuo and the residue was chromatographed over silica gel eluting with chloroform–methanol–concd ammonium hydroxide (100:10:1 by volume) to give the title compound as a colorless solid (1.52 g, 50%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.79–2.01 (4H, m), 2.55–2.69 (1H, m), 2.88–3.19 (6H, m), 3.81 (2H, s), 4.35 (2H, d, $J=5.9$ Hz), 4.57 (2H, d, $J=6.3$ Hz), 5.61–5.80 (1H, m), 6.61–6.76 (2H, m), 7.29–7.80 (3H, m), 7.92–8.04 (2H, m); EI–MS *m*/*z* 375 (M^+).

(*Z*)-9-(2-Aminoethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole hydrochloride (25). The title compound was obtained from (*Z*)-9-(2-aminoethyl)-2-[2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole-*N*-borane **17** using the methods described for the synthesis of compound **8** as a colorless crystalline solid (23%): mp 246–248 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.80–1.88 (2H, m), 1.92–2.00 (2H, m), 2.68–2.71 (1H, m), 3.16–3.22 (2H, m), 3.26–3.33 (4H, m), 4.16 (2H, s), 4.69–4.73 (4H, m), 5.76–5.78 (1H, m), 6.85 (1H, dd, $J=2.0$ Hz, 8.5 Hz), 7.18–7.21 (1H, m), 7.37–7.40 (1H, m), 7.48 (1H, d, $J=2.0$ Hz), 7.64 (1H, d, $J=8.0$ Hz), 8.01–8.06 (2H, m), 8.49 (3H, br s), 10.82 (1H, br s); EI–MS *m*/*z* 361 (M^+). Anal. calcd for $C_{23}H_{27}N_3O{\cdot}2HCl{\cdot}1.5H_2O$: C, 59.87; H, 6.99; N, 9.11; Cl, 15.37. Found: C, 60.08; H, 6.83; N, 9.08; Cl, 15.53.

(*E*)-3-(1-Fluoro-2-hydroxyethylidene)quinuclidine-*N*-borane (27). To a stirred solution of triethyl 2-fluoro-2-phosphonoacetate (17.8 g, 73.5 mmol) in tetrahydrofuran (140 mL) was added sodium hydride (2.94

g, 73.5 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 (8.76 g, 70.0 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 (30 mL), the reaction mixture was extracted with ethyl acetate. The extract was washed with brine, dried over magnesium sulfate and concentrated in vacuo to give a colorless oil. The resulting oil was dissolved in tetrahydrofuran (75 mL) and cooled to −78 °C. To this solution was added a solution of borane-tetrahydrofuran complex in tetrahydrofuran (75.0 mL, 1.0 M, 75.0 mmol) was added 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 and the extract was washed with brine, dried over magnesium sulfate and concentrated in vacuo to give a colorless solid. The resulting solid was dissolved in ethanol (200 mL). To this solution was added sodium hydride (0.300 g, 7.50 mmol, 60% dispersion in mineral oil) at 0 °C and stirred for 0.5 h. The reaction mixture was stirred at 50 °C for a further 28 h. After cooling down, acetic acid (0.43 mL) was added at ambient temperature. The reaction mixture was concentrated in vacuo and the residue was diluted with ethyl acetate. The organic layer was washed with H_2O and brine, dried over magnesium sulfate and concentrated in vacuo to give a colorless solid. The resulting solid was dissolved in toluene (80 mL) and cooled to 0 °C. To this solution was added a solution of diisobutylaluminum hydride in toluene (144 mL, 1.02 M, 144 mmol) and stirred for 1 h. H_2O (20 mL) was added at 0 °C, allowed to warm to ambient temperature and stirred for 10 h. The reaction mixture was extracted with ethyl acetate and the extract was washed with brine, dried over magnesium sulfate and concentrated in vacuo to give a colorless solid. The resulting residue was chromatographed over silica gel eluting with *n*-hexane–ethyl acetate (1:1 by volume) to give the title compound as a colorless oil (3.61 g, 27%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.80–1.88 (4H, m), 3.00–3.15 (5H, m), 3.67 (2H, s), 4.14 (2H, d, J = 20.0 Hz); FAB-MS m/z 184 ($M-H^+$).

(*E*)-2-[2-Fluoro-2-(quinuclidin-3-ylidene)ethoxy]-9*H*-carbazole hydrochloride (28). To a stirred solution of (*E*)-3-(1-fluoro-2-hydroxyethylidene)quinuclidine-*N*-borane **27** (1.22 g, 6.59 mmol), lithium chloride (1.40 g, 33.0 mmol) and triethylamine (3.77 g, 37.3 mmol) in dichloromethane (18 mL) was added methanesulfonyl chloride (1.13 g, 9.89 mmol) at 0 °C. The reaction mixture was stirred for 1 h, allowed to warm to ambient temperature and 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 brine, dried over magnesium sulfate and concentrated in vacuo to give a colorless solid. A mixture of the resulting solid, 2-hydroxy-9H-carbazole (1.12 g, 6.09 mmol), potassium carbonate (4.21 g, 30.5 mmol) and *N*,*N*-dimethylformamide (18 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 brine, dried over magnesium sulfate and concentrated in vacuo to give a colorless solid. The resulting solid in ethanol–acetone (10 mL, 1:2 by volume) was treated with hydrogen chloride in ethanol (5 M, 5.0 mL) at ambient temperature and stirred for 0.5 h. Ether (15 mL) was added and the resulting precipitate was filtered to give the title compound as a colorless crystalline solid (1.00 g, 44%): mp 241–243 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.80–1.86 (2H, m), 1.95–2.02 (2H, m), 3.13–3.16 (1H, m), 3.20–3.33 (4H, m), 4.10 (2H, s), 4.76 (2H, d, J = 20.0 Hz), 6.82 (1H, dd, J = 2.0 Hz, 8.5 Hz), 7.03 (1H, d, J = 2.0 Hz), 7.10–7.13 (1H, m), 7.28–7.32 (1H, m), 7.45 (1H, d, J = 8.5 Hz), 7.79–8.01 (2H, m), 10.85 (1H, br s), 11.25 (1H, br s); EI–MS m/z 336 (M^+). Anal. calcd for $C_{21}H_{21}N_2OF\cdot HCl\cdot 0.3H_2O$: C, 66.68; H, 6.02; N, 7.41; Cl, 9.37; F, 5.02. Found: C, 66.33; H, 5.98; N, 7.40; Cl, 9.64; F, 5.02.

(*Z*)-3-(1-Hydroxyprop-2-ylidene)quinuclidine-*N*-borane (29). The title compound was obtained from triethyl 2-phosphonopropionate using the methods described for the synthesis of compound **28** as a colorless oil (28%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.75–1.93 (7H, m), 2.85–2.90 (1H, m), 2.94–3.13 (4H, m), 3.71 (2H, s), 4.04 (2H, s); FAB-MS m/z 180 ($M-H^+$).

(*Z*)-2-[2-(Quinuclidin-3-ylidene)propoxy]-9*H*-carbazole hydrochloride (30). The title compound was obtained from (*Z*)-3-(1-hydroxyprop-2-ylidene)quinuclidine-*N*-borane **29** using the methods described for the synthesis of compound **28** as a colorless crystalline solid (40%): mp 260–262 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.74–1.82 (5H, m), 1.92–2.00 (2H, m), 3.03–3.04 (1H, m), 3.21–3.29 (4H, m), 4.08 (2H, s), 4.51 (2H, s), 6.79 (1H, dd, J = 2.5 Hz, 8.5 Hz), 6.99 (1H, d, J = 2.5 Hz), 7.09–7.12 (1H, m), 7.27–7.30 (1H, m), 7.43 (1H, d, J = 8.0 Hz), 7.96–8.00 (2H, m), 10.73 (1H, br s), 11.22 (1H, br s); FAB-MS m/z 333 ($M+H^+$). Anal. calcd for $C_{22}H_{24}N_2O\cdot HCl$: C, 71.63; H, 6.83; N, 7.59; Cl, 9.61. Found: C, 71.45; H, 6.84; N, 7.54; Cl, 9.67.

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.[17] 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.[18]

Assay of squalene synthase inhibitory activity

Squalene synthase activities of these microsomes were assayed using the technique of Amin with a 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), farnesyl diphosphate (5 μM), [^{3}H]farnesyl diphosphate (0.017 μM, 15 Ci/mmol), NB-598 (10 μM) and sodium pyrophosphate decahydrate (1 mM). After pre-incubation 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 h 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).

Plasma lipid lowering effect in guinea pigs

Four-week-old male Hartley guinea pigs (from Charles River, Kanagawa, Japan) were fed GC-4 diet (CLEA Japan Inc., Tokyo, Japan), and water ad libitum. Guinea pigs were orally given compound **8** at dose of 50 mg/kg of body weight, or pravastatin 25 mg/kg of body weight once a day for 14 days. Compound **8** and pravastatin were suspended in a 0.5% methylcellulose vehicle solution. The no-trearment control group was given an equal volume of the 0.5% methylcellulose vehicle solution. In all experimants blood specimens were obtained 2 h after the last compound dose from animals which had fasted 18 h. All plasma samples were analysed for non-HDL cholesterol which was subtracted HDL cholesterol from total cholesterol, for HDL cholesterol and for 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 GC-4 diet (CLEA Japan Inc., Tokyo, Japan), and water ad libitum. Rats were orally given test compounds at dose of 250 mg/kg of body weight once a day for 3 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. In all experiments blood specimens were obtained 24 h after the last compound dose from animals. All plasma samples were analysed for aspartate aminotransferase and for alanine aminotransferase using a Hitachi 7250 Automatic analyzer (Tokyo, Japan).

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