Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 5899–5908
Bioorganic & Medicinal Chemistry

Synthesis and biological evaluation of novel propylamine derivatives as orally active squalene synthase inhibitors

Tsukasa Ishihara,* Hirotoshi Kakuta, Hiroshi Moritani, Tohru Ugawa and Isao Yanagisawa

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

Received 16 July 2004; revised 19 August 2004; accepted 19 August 2004
Available online 16 September 2004

Abstract—Squalene synthase inhibitors are potentially superior hypolipidemic agents. We synthesized novel propylamine derivatives, as well as evaluated their ability to inhibit squalene synthase and their lipid-lowering effects in rats. 1-Allyl-2-[3-(benzylamino)propoxy]-9*H*-carbazole (YM-75440) demonstrated potent inhibition of the enzyme derived from HepG2 cells with an IC_{50} value of 63 nM. It significantly reduced both plasma total cholesterol and plasma triglyceride levels following oral dosing to rats with a reduced tendency to elevate plasma transaminase levels.

1. Introduction

Coronary heart disease remains a major cause of death in the industrialized world despite recent advances in pharmaceutical and surgical treatments.[1] Elevated plasma cholesterol is a well established risk factor for this disease,[2] and hence research for novel antihypercholesterolemic agents has emerged as one of the most active areas of current drug discovery. Because over 70% of cholesterol in the body is derived from de novo cholesterol biosynthesis, inhibition of this pathway is a promising approach for reducing plasma cholesterol. Developments in the past decade have shown that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, the agents which inhibit cholesterol biosynthesis, are the most effective therapeutic drugs for lowering plasma cholesterol levels.[3] It has also been demonstrated that treatments with HMG-CoA reductase inhibitors improve the survival of patients with existing coronary heart disease.[4]

However, HMG-CoA reductase inhibitors interrupt the cholesterol biosynthesis pathway early on, which may also prevent the formation of biologically important isoprenoids such as dolicols, ubiquinones, and isopentenyl *t*-RNA. Moreover, their lowering effects on plasma triglyceride levels are modest.[5] Elevated plasma triglycerides has been proposed as another risk factor in the progression of coronary heart disease.[6] Thus, an agent which has the potential to reduce both plasma cholesterol and plasma triglycerides without interruption of the isoprenoid biosyntheses would provide the maximum benefit for those with coronary heart disease.

As part of the efforts to discover novel hypocholesterolemic drugs, agents that inhibit other steps of the cholesterol biosynthesis pathway have also been investigated.[7] Squalene synthase (EC 2.5.1.21), which catalyzes the reductive dimerization of two molecules of farnesyl diphosphate to form squalene, is involved in the first committed step in cholesterol biosynthesis. Inhibitors of this enzyme do not interfere with the biosyntheses of the above-mentioned isoprenoids, because the enzymatic step occurs after the branch points leading to the isoprenoids. Furthermore, squalene synthase inhibitors lower not only plasma cholesterol, but also plasma triglycerides in vivo.[8] Thus, the discovery of squalene synthase inhibitors is of great interest as treatments for hypercholesterolemia, hypertriglyceridemia, and coronary heart disease.

Several classes of squalene synthase inhibitors, such as substrate analogues,[9] transition-state analogues,[10]

Keywords: Squalene synthase; Antihypercholesterolemic effect; Antihypertriglyceridemic effect; Hepatotoxicity.
* Corresponding author. Tel.: +81 029 852 5111; fax: +81 029 852 5387; e-mail: ishihat@yamanouchi.co.jp

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.08.033

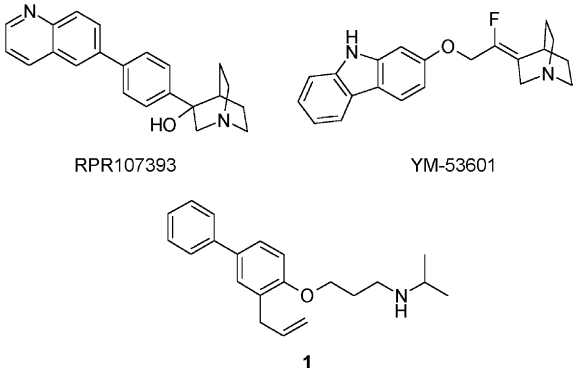


Figure 1. Structures of squalene synthase inhibitors.

2,8-dioxabicyclo[3.2.1]octane derivatives,[11] dicarboxylic acid derivatives,[12] 4,1-benzoxazepine derivatives,[13] and bicyclo[3.2.0]heptane derivatives,[14] have been reported in the literature. Quinuclidine derivatives, for example RPR107393, were also reported to inhibit the enzyme, and we discovered that introduction of a tricyclic system into the quinuclidine-based inhibitor improved its potential as a lipid-lowering agent (Fig. 1).[15] In addition to these inhibitors, Brown et al. revealed that *N*-isopropyl-biphenylyloxypropylamine derivatives, such as compound **1**, were potent inhibitors against squalene synthase.[16] Substituted quinuclidines have the potential to be other pharmacological ligands, such as muscarinic receptor agonists[17] or neurokinin-1 receptor antagonists.[18] These studies were the impetus for our work on the replacement of the ethylidenequinuclidine scaffold in the YM-53601 template with an *N*-alkyl-propylamine moiety.

In this paper, we detail the results of our studies on the synthesis, structure–activity relationships, and biological activities of novel propylamine derivatives with tricyclic systems as squalene synthase inhibitors.

2. Chemistry

Scheme 1 shows the syntheses of intermediate allyl-substituted phenol derivatives. Monoallyl intermediates **5**–**7** were prepared by the alkylation of commercially available phenol derivatives **2**–**4** with allyl bromide followed by thermal Claisen rearrangement. The monoallyl derivative **7** was resubjected to the above-mentioned two-step sequence in order to form a diallyl intermediate **9**. The formation of 9-acetyl-1-allyl-2-hydroxy-9*H*-carbazole (**8**) was accomplished in three steps: *O*-allylation, *N*-acetylation, and subsequent Claisen rearrangement, for which 2-hydroxy-9*H*-carbazole (**4**) was used as the starting material. Detailed analysis of ^{1}H NMR spectra of the rearrangement products confirmed their structures.

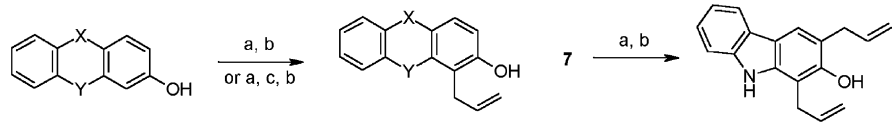
The syntheses of target aryloxypropylamines **10**–**20** are shown in Scheme 2. Compounds **10**–**19** were prepared by alkylation of the intermediates **4**–**9** with 1,3-dibromopropane and subsequent reaction with appropriate amines. The intermediate **7** was carried on to produce an *N*-phenyl derivative **20** using a three-step sequence involving alkylation with 1,3-dibromopropane, substitution with 2,2,2-trifluoro-*N*-phenylacetamide, and final deprotection of the trifluoroacetyl moiety with aqueous sodium hydroxide.[15d]

The allyl-substituted 9*H*-carbazole derivative **11** was converted into the corresponding propyl analogue **21** through catalytic hydrogenation (Scheme 3).

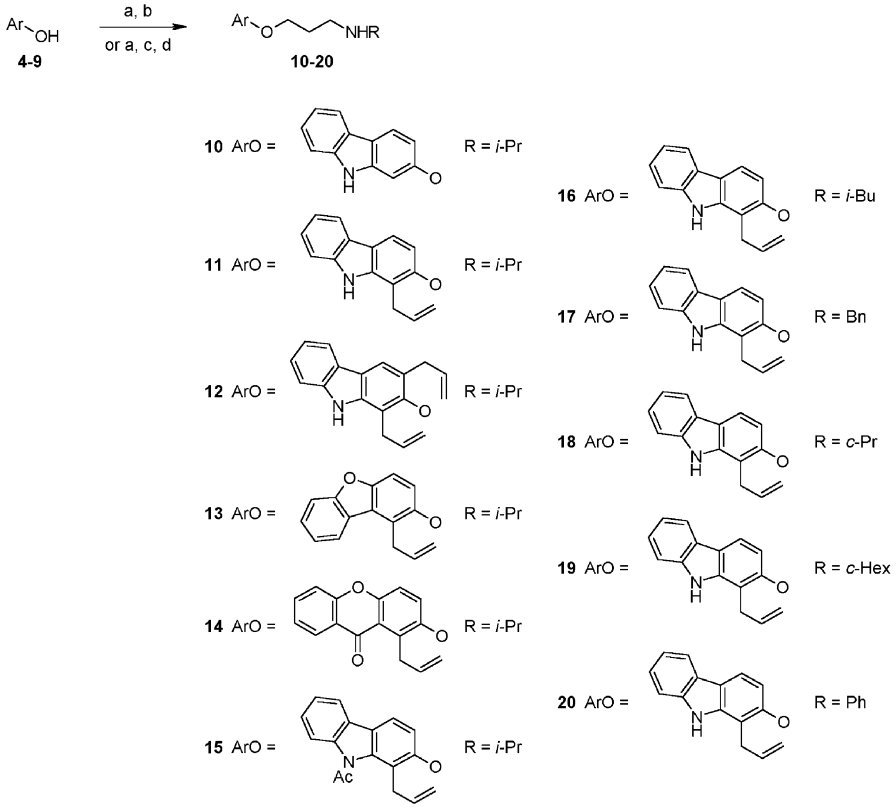
3. Results and discussion

Inhibition by the prepared compounds of the conversion of [^{3}H]farnesyl diphosphate to [^{3}H]squalene by rat liver microsomal squalene synthase was measured by the method of Amin et al.[19] with a slight modification. The selected compounds were evaluated for their inhibitory activity against squalene synthase derived from human hepatoma (HepG2) cells, and their effects on plasma total cholesterol and plasma triglycerides following oral administration to rats were also examined.

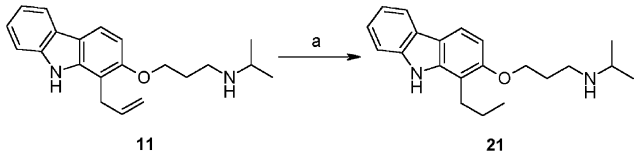
Replacement of the ethylidenequinuclidine scaffold in the YM-53601 template with an *N*-isopropyl-propylamine moiety yielded compound **10**, which showed comparable inhibitory activity to YM-53601 (Table 1). This result attracted our interest to further structural modifications of compound **10**. Brown et al. reported that an allyl substitution on the proximal phenyl ring provided an increase of inhibitory activity over the nonsubstituted parent compound in the series of propylamine-based inhibitors containing biphenylyl moieties.[16] Thus, we explored the installation of an allyl substituent into the simple 9*H*-carbazole derivative **10** in order to improve the potency, which resulted in compound **11** with slight enhancement in inhibitory activity relative to the parent. In anticipation of a further increase in activity, an additional allyl group was joined to the 9*H*-carbazole moiety of compound **11**. The resultant diallyl 9*H*-carbazole



Scheme 1. Reagents and conditions: (a) allyl bromide, K_2CO_3, DMF; (b) 200 °C; (c) Ac_2O, NaH, DMF.

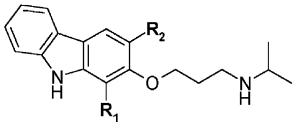


Scheme 2. Reagents: (a) 1,3-dibromopropane, K_2CO_3, DMF; (b) RNH_2, isopropanol; (c) 2,2,2-trifluoro-*N*-phenylacetamide, K_2CO_3, DMF; (d) NaOH, EtOH, H_2O.



Scheme 3. Reagents: (a) Pd–C, H_2, EtOH.

Table 1. In vitro activities of propylamine derivatives with substituted 9*H*-carbazole rings



Compd	R_1	R_2	IC_{50} (μM)[a]
YM-53601			0.090
10	H	H	0.11
11	Allyl	H	0.066
12	Allyl	Allyl	0.25
21	Et	H	0.46
1			0.093
RPR107393			0.068

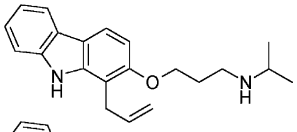
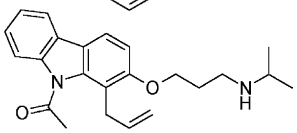
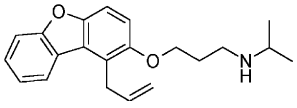
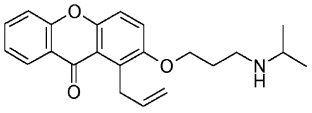
[a] Compounds were tested for their ability to inhibit the conversion of [^{3}H]farnesyl diphosphate to [^{3}H]squalene by rat liver squalene synthase. IC_{50} values were determined by a single experimental run in duplicate.

derivative **12** was, rather unexpectedly, less potent than the monoallyl inhibitor **11**. The sp^2 carbons at the side chain on the tricyclic system were found to be important for obtaining optimal activity; 1-propyl-9*H*-carbazole derivative **21** caused a significant loss of inhibitory activity compared to the corresponding 1-allyl-9*H*-carbazole derivative **11**.

We next explored alternative tricyclic systems relative to the 9*H*-carbazole moiety in compound **11**. With these chemical modifications, we focused on tricyclic systems which had no benzylic protons, such as dibenzofuran or 9*H*-xanthene-9-one, on the basis of our previous findings that inhibitors with tricyclic systems lacking benzylic protons showed improved lipid-lowering effects.[15e] The results in Table 2 show that these modifications unfortunately attenuated the ability to inhibit squalene synthase. *N*-Acetylation on the 9*H*-carbazole moiety in compound **11** yielded compound **15**, which resulted in a loss of potency compared to the parent. 9*H*-Xanthen-9-one derivative **14** was slightly less active than the 9*H*-carbazole analogue **11**, and dibenzofuran derivative **13** was a much less potent inhibitor. A combination of these results and our earlier work allowed us to conclude that 9*H*-carbazole was a suitable element for obtaining potent inhibitory activity against squalene synthase.

Other *N*-alkyl groups besides the *N*-isopropyl moiety were also explored to determine the structural requirements for gaining inhibitory activity against squalene

Table 2. In vitro activities of propylamine derivatives with tricyclic systems

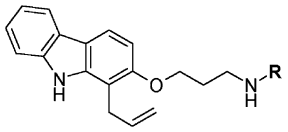
Compd	Structure	IC_{50} (μM)[a]
11		0.066
15		0.27
13		0.37
14		0.12

[a] Refer to Table 1.

synthase. The data in Table 3 summarize the structure–activity relationships of the close analogues of compound **11**. Conversion of the isopropyl group to an isobutyl chain yielded compound **16**, which led to a significant reduction in inhibitory activity. The *N*-benzyl analogue **17** was slightly less active than the *N*-isopropyl derivative **11**. Replacement with cyclic rings, such as cyclopropyl (compound **18**) or cyclohexyl (compound **19**), also reduced potency relative to the parent compound **11**, and a further loss of inhibitory activity was observed with the *N*-phenyl analogue **20**. The pK_a value of the nitrogen atom in the propylamine moiety of the *N*-cyclohexyl derivative **19** and that of the *N*-phenyl derivative **20** were 10.7 and 4.6, respectively,[20] indicating that the reduced activity of compound **20** was due to its decreased basicity.

The four most active compounds emerging from this series, compounds **10**, **11**, **14**, and **17** were evaluated in our advanced profiling assays. These compounds were tested for their ability to inhibit human-derived enzyme and their lipid-lowering effects after oral dosing in rats (Table 4). The allyl side chain on the tricyclic system markedly affected the activity against human squalene synthase in this propylamine series. Allyl-substituted 9*H*-carbazole derivative **11** was a potent inhibitor with an IC_{50} value of 32 nM, whereas nonsubstituted 9*H*-carbazole analogue **10** was a poor one with an IC_{50} value of 810 nM. The inhibitory potency of *N*-benzyl derivative **17** was also significant (IC_{50} = 63 nM) in the human enzyme assay, which was comparable to that of RPR107393.

Table 3. In vitro activities of *N*-substituted propylamine derivatives



Compd	R	IC_{50} (μM)[a]
11	*i*-Pr	0.066
16	*i*-Bu	0.40
17	Bn	0.15
18	*c*-Pr	0.23
19	*c*-Hex	0.31
20	Ph	>1

[a] Refer to Table 1.

As we reported, the 9*H*-carbazole moiety was a suitable tricyclic system for obtaining good lipid-lowering effects in vivo in the series of quinuclidine-based inhibitors. A similar boost in potency was obtained with these propylamine derivatives. 9*H*-Carbazole derivatives **11** and **17** reduced plasma total cholesterol after oral dosing in rats by 50% and 42%, respectively. However, the corresponding 9*H*-xanthen-9-one analogue **14** lowered plasma total cholesterol by only 14% despite the fact that its in vitro activity was comparable to that of compound **17** in the rat enzyme assay. Additionally, there

Table 4. In vitro and in vivo activities of propylamine derivatives

Compd	IC_{50} (μM)		TC % change[c]	TG % change[d]
	Rat[a]	Human[b]		
10	0.11	0.81	NT[e]	NT[e]
11	0.066	0.032	−50***	−25
14	0.12	0.12	−14	−8
17	0.15	0.063	−42***	−42
RPR107393	0.068	0.057	−34***	−18

[a] Refer to Table 1.

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

[c] The value represents the mean percent change from the respective control value of plasma total cholesterol after oral administration in rats (50 mg/kg, two times a day for 4 days, n = 6). The control value of plasma total cholesterol was 80 ± 3 mg/dL (mean ± SEM), and the standard error of each treated group was less than 15% of the mean.

[d] The value represents the mean percent change from the respective control value of plasma triglycerides after oral administration in rats (50 mg/kg, two times a day for 4 days, n = 6). The control value of plasma triglycerides was 12 ± 1 mg/dL (mean ± SEM), and the standard error of each treated group was less than 20% of the mean.

[e] Not tested.

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

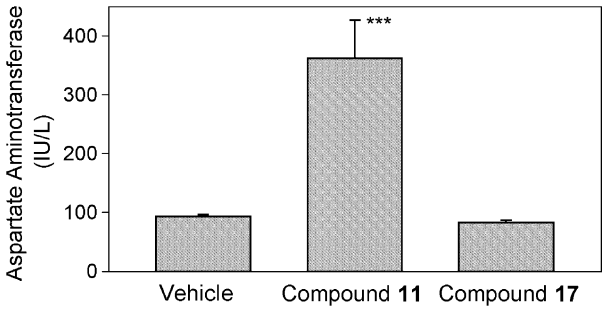


Figure 2. Effects of the oral administration of compounds **11** and **17** on plasma aspartate aminotransferase levels in rats. Compounds **11** and **17** were given at 250 mg/kg/day for 3 days ($n = 3$), respectively. ***$P < 0.001$ versus control using the Student's t-test.

was a tendency for compounds **11** and **17** to reduce plasma triglycerides following p.o. administration in rats. These results indicate that carbazole-containing squalene synthase inhibitors have the potential to be effective in the treatment of hypercholesterolemia, hypertriglyceridemia, and also coronary heart disease.

Recent studies of squalene synthase inhibitors found that the administration of some of the inhibitors resulted in the elevation of plasma transaminase levels, indicating that they might be hepatotoxic.[9f,15e] Thus, the effects of compounds **11** and **17**, the novel class of potent and orally active squalene synthase inhibitors, on plasma transaminase levels needed to be investigated. Figure 2 shows the results of this assay. Disappointingly, *N*-isopropyl derivative **11** was found to cause a 4-fold elevation of the levels of plasma transaminase in rats. However, *N*-benzyl analogue **17** had little effect on plasma transaminase levels, thereby exhibiting reduced acute hapatotoxicity.

4. Conclusion

We have synthesized a novel series of propylamine derivatives with tricyclic systems, and evaluated their ability to inhibit squalene synthase in vitro. Their effects on plasma lipid levels following oral dosing in rats were also investigated. The structure–activity relationships of the prepared compounds yielded useful information on the structural requirements for the inhibition of the enzyme. The introduction of an allyl substituent into the tricyclic system led to an increase in inhibitory activity, relative to the parent compound. 9*H*-Carbazole was a suitable tricyclic system for obtaining potent inhibition. The inhibitor with this ring system also exhibited a significant reduction of plasma lipid levels after oral administration. In this novel series, 1-allyl-2-[3-(isopropylamino)propoxy]-9*H*-carbazole (**11**) and 1-allyl-2-[3-(benzylamino)propoxy]-9*H*-carbazole (**17**) were found to be potent inhibitors of squalene synthase with IC_{50} values of 32 and 63 nM, respectively, for the HepG2 enzyme assay. These two inhibitors reduced both plasma total cholesterol and plasma triglycerides after oral administration to rats. These results indicated that they are potent and orally active squalene synthase inhibitors. Compound **11** elevated plasma transaminase levels in rats, whereas compound **17** did not affect.

We propose that compound **17**, YM-75440, is a promising lipid-lowering agent with potential in the treatment of hyperlipidemia and coronary heart disease.

5. Experimental

5.1. Chemistry

^{1}H NMR spectra were measured with a JEOL EX400 spectrometer. Chemical shifts are expressed in δ units using tetramethylsilane as the standard (in the 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 using a Yanaco MP-500D melting point apparatus without correction. All reagents purchased were used without further purification.

5.1.1. 1-Allyl-2-hydroxy-9*H*-carbazole (7). To a stirred mixture of 2-hydroxy-9*H*-carbazole (**4**) (73.3 g, 400 mmol), allyl bromide (50.7 g, 420 mmol), and *N*,*N*-dimethylformamide (300 mL) was added potassium carbonate (82.8 g, 600 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h, then allowed to warm to ambient temperature and stirred for 24 h. The reaction mixture was poured into H_2O (1300 mL), and the precipitate was collected. The resulting solid in *n*-hexane/chloroform (300 mL, 85:15 by volume) was heated to reflux for 0.5 h. After cooling, the precipitate was filtered and dried in vacuo to yield 2-allyloxy-9*H*-carbazole as a gray solid (88.6 g, 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.61–4.64 (2H, m), 5.29–5.34 (1H, m), 5.43–5.49 (1H, m), 6.07–6.17 (1H, m), 6.87 (1H, dd, $J = 2.4$, 8.8 Hz), 6.92 (1H, d, $J = 2.4$ Hz), 7.18–7.22 (1H, m), 7.31–7.35 (1H, m), 7.37 (1H, d, $J = 7.6$ Hz), 7.92–7.94 (2H, m), 7.97 (1H, d, $J = 7.2$ Hz); EI-MS *m*/*z* 223 $[M]^+$.

2-Allyloxy-9*H*-carbazole (48.4 g, 217 mmol) was heated at 200 °C for 1 h. The crude product was purified by silica gel column chromatography, using *n*-hexane/ethyl acetate (4:1 by volume) as the eluant, to yield a brown solid (44.0 g). The resulting solid in *n*-hexane/chloroform (80 mL, 75:25 by volume) was heated to reflux for 0.5 h. After cooling, the precipitate was filtered and dried in vacuo to yield the title compound as a colorless solid (34.4 g, 71%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.60 (2H, d, $J = 6.0$ Hz), 4.95 (1H, dd, $J = 2.0$, 10.0 Hz), 5.09 (1H, dd, $J = 2.0$, 17.2 Hz), 5.97–6.06 (1H, m), 6.73 (1H, d, $J = 8.8$ Hz), 7.04–7.08 (1H, m), 7.22–7.29 (1H, m), 7.40 (1H, d, $J = 8.0$ Hz), 7.72 (1H, d, $J = 8.8$ Hz), 7.90 (1H, d, $J = 7.2$ Hz), 9.26 (1H, s), 10.82 (1H, s); EI-MS *m*/*z* 223 $[M]^+$.

5.1.2. 1-Allyl-2-[3-(isopropylamino)propoxy]-9*H*-carbazole (11). A mixture of 1-allyl-2-hydroxy-9*H*-carbazole (**7**) (20.1 g, 90.0 mmol), 1,3-dibromopropane (54.5 g, 270 mmol), potassium carbonate (18.6 g, 135 mmol), and *N*,*N*-dimethylformamide (90 mL) was stirred at

ambient temperature for 23 h. The reaction mixture was poured into H_2O (350 mL) and extracted with ethyl acetate (100 mL). The organic layer was washed with H_2O and then brine, dried with magnesium sulfate, and concentrated in vacuo. The residue was subjected to chromatography over silica gel eluting with *n*-hexane/ethyl acetate (90:10 by volume) to yield 1-allyl-2-(3-bromopropoxy)-9*H*-carbazole as a colorless solid (15.9 g, 51%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33–2.39 (2H, m), 3.65 (2H, t, J = 6.4 Hz), 3.69–3.72 (2H, m), 4.21 (2H, t, J = 5.6 Hz), 5.11–5.18 (2H, m), 6.00–6.10 (1H, m), 6.88 (1H, d, J = 8.4 Hz), 7.17–7.21 (1H, m), 7.31–7.35 (1H, m), 7.38 (1H, d, J = 7.6 Hz), 7.87 (1H, d, J = 8.4 Hz), 7.95–7.98 (2H, m); EI-MS *m*/*z* 343, 345 $[M]^+$.

A mixture of 1-allyl-2-(3-bromopropoxy)-9*H*-carbazole (5.68 g, 16.5 mmol), isopropylamine (3.3 mL), and isopropanol (33 mL) was heated under reflux for 15 h. The reaction mixture was concentrated in vacuo. The residue was diluted with chloroform (50 mL) and the organic layer was washed with 10% aqueous potassium carbonate. The organic layer was dried with magnesium sulfate, and concentrated in vacuo. The residue was subjected to chromatography over silica gel and eluted with chloroform/methanol/c. ammonium hydroxide (100:3:0.3 by volume). The resulting solid was crystallized from diisopropyl ether to yield the title compound as a colorless solid (1.54 g, 29%): mp 100–101 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.97 (6H, d, J = 6.0 Hz), 1.84–1.90 (2H, m), 2.68–2.72 (3H, m), 3.33 (1H, br s), 3.63 (2H, d, J = 6.4 Hz), 4.11 (2H, t, J = 6.4 Hz), 4.95 (1H, dd, J = 2.4, 10.0 Hz), 5.08 (1H, dd, J = 2.4, 16.8 Hz), 5.95–6.05 (1H, m), 6.89 (1H, d, J = 8.4 Hz), 7.07–7.11 (1H, m), 7.27–7.31 (1H, m), 7.43 (1H, d, J = 8.4 Hz), 7.88 (1H, d, J = 8.4 Hz), 7.97 (1H, d, J = 7.6 Hz), 10.93 (1H, s); FAB-MS *m*/*z* 323 $[M+H]^+$; Anal. Calcd for $C_{21}H_{26}N_2O$: C, 78.22; H, 8.13; N, 8.69. Found: C, 78.47; H, 8.29; N, 8.62.

5.1.3. 2-[3-(Isopropylamino)propoxy]-9*H*-carbazole (10). The title compound was obtained from 2-hydroxy-9*H*-carbazole (**4**) using the methods described for the synthesis of compound **11** as a colorless solid (29%): mp 170–171 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.98 (6H, d, J = 6.4 Hz), 1.66 (1H, br s), 1.84–1.91 (2H, m), 2.66–2.75 (3H, m), 4.10 (2H, t, J = 6.0 Hz), 6.75 (1H, dd, J = 2.0, 8.4 Hz), 6.95 (1H, d, J = 2.0 Hz), 7.08–7.12 (1H, m), 7.25–7.29 (1H, m), 7.40 (1H, d, J = 8.0 Hz), 7.94 (1H, d, J = 8.4 Hz), 7.97 (1H, d, J = 7.6 Hz), 11.06 (1H, br s); FAB-MS *m*/*z* 283 $[M+H]^+$; Anal. Calcd for $C_{18}H_{22}N_2O$: C, 76.56; H, 7.85; N, 9.92. Found: C, 76.52; H, 7.65; N, 9.90.

5.1.4. 1,3-Diallyl-2-hydroxy-9*H*-carbazole (9). The title compound was obtained from 1-allyl-2-hydroxy-9*H*-carbole (**7**) using the methods described for the synthesis of compound **7** as a brown solid (83%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.58 (2H, d, J = 6.4 Hz), 3.69–3.71 (2H, m) 5.15–5.27 (5H, m), 6.03–6.16 (2H, m), 7.16–7.20 (1H, m), 7.29–7.33 (1H, m), 7.37 (1H, d, J = 8.0 Hz), 7.69 (1H, s), 7.92 (1H, br s), 7.94 (1H, d, J = 8.0 Hz); FAB-MS *m*/*z* 263 $[M]^+$.

5.1.5. 1,3-Diallyl-2-[3-(isopropylamino)propoxy]-9*H*-carbazole (12). The title compound was obtained from 1,3-diallyl-2-hydroxy-9*H*-carbazole (**9**) using the methods described for the synthesis of compound **11** as a yellow solid (52%): mp 65–66 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (6H, d, J = 6.4 Hz), 2.01–2.07 (2H, m), 2.84–2.92 (3H, m), 3.57 (2H, d, J = 6.0 Hz), 3.74–3.77 (2H, m), 3.90 (2H, t, J = 6.0 Hz), 5.09–5.22 (4H, m), 6.05–6.15 (2H, m), 7.16–7.20 (1H, m), 7.32–7.36 (1H, m), 7.39 (1H, d, J = 7.6 Hz), 7.76 (1H, s), 7.97–8.01 (2H, m); FAB-MS *m*/*z* 363 $[M+H]^+$; Anal. Calcd for $C_{24}H_{30}N_2O{\cdot}H_2O$: C, 79.12; H, 8.35; N, 7.69. Found: C, 79.06; H, 8.27; N, 7.70.

5.1.6. 1-Allyl-2-hydroxydibenzofuran (5). The title compound was obtained from 2-hydroxydibenzofuran (**2**) using the methods described for the synthesis of compound **7** as a beige solid (80%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92–3.95 (2H, m), 4.81 (1H, s), 5.10–5.16 (2H, m), 6.10–6.20 (1H, m), 6.96 (1H, d, J = 8.8 Hz), 7.29–7.35 (2H, m), 7.41–7.45 (1H, m), 7.54 (1H, d, J = 8.4 Hz), 7.97 (1H, d, J = 7.6 Hz); EI-MS *m*/*z* 224 $[M]^+$.

5.1.7. 1-Allyl-2-[3-(isopropylamino)propoxy]dibenzofuran hydrochloride (13). In a procedure similar to that described above, 1-allyl-2-(3-bromopropoxy)dibenzofuran was prepared from 1-allyl-2-hydroxydibenzofuran (**5**) as a yellow solid (89%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33–2.39 (2H, m), 3.66 (2H, t, J = 6.4 Hz), 3.90–3.94 (2H, m), 4.16 (2H, t, J = 6.0 Hz), 4.99–5.06 (2H, m), 6.05–6.15 (1H, m), 7.05 (1H, d, J = 9.2 Hz), 7.27–7.33 (1H, m), 7.38 (1H, d, J = 9.2 Hz), 7.41–7.45 (1H, m), 7.54 (1H, d, J = 8.0 Hz), 7.98 (1H, d, J = 7.6 Hz); FAB-MS *m*/*z* 344, 346 $[M]^+$.

A mixture of 1-allyl-2-(3-bromopropoxy)dibenzofuran (2.42 g, 7.01 mmol), isopropylamine (6.0 mL), isopropanol (7.0 mL), and tetrahydrofuran (2.0 mL) was heated under reflux for 3 h. The reaction mixture was concentrated in vacuo. The residue was subjected to chromatography over silica gel eluting with chloroform/methanol/c. ammonium hydroxide (90:1:0.1 by volume). The resulting material was dissolved in chloroform. The organic layer was washed with 25% aqueous potassium carbonate, dried with magnesium sulfate, and concentrated in vacuo to yield a yellow oil (1.40 g). The resulting oil (1.40 g) was dissolved in diethyl ether (10 mL). To the mixture was added hydrogen chloride in ethyl acetate (4 M, 1.2 mL) at ambient temperature and the mixture was stirred for 15 min. The resulting precipitate was filtered to yield the title compound as a colorless solid (1.37 g, 55%): mp 165–167 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.28 (6H, d, J = 6.8 Hz), 2.14–2.22 (2H, m), 3.08 (2H, t, J = 8.0 Hz), 3.26–3.36 (1H, m), 3.90 (2H, d, J = 6.0 Hz), 4.16 (2H, t, J = 6.0 Hz), 4.99–5.05 (2H, m), 6.02–6.12 (1H, m), 7.24 (1H, d, J = 8.8 Hz), 7.36–7.40 (1H, m), 7.50–7.54 (1H, m), 7.55 (1H, d, J = 8.8 Hz), 7.67 (1H, d, J = 8.0 Hz), 8.07 (1H, d, J = 8.0 Hz), 9.04 (2H, br s); FAB-MS *m*/*z* 324 $[M+H]^+$; Anal. Calcd for $C_{21}H_{25}NO_2{\cdot}HCl{\cdot}0.2H_2O$: C, 69.39; H, 7.32; N, 3.85; Cl, 9.75. Found: C, 69.48; H, 7.36; N, 3.83; Cl, 9.68.

5.1.8. 1-Allyl-2-hydroxy-9*H*-xanthen-9-one (6). The title compound was obtained from 2-hydroxy-9*H*-xanthen-9-one (**3**) using the methods described for the synthesis of compound **7** as a colorless solid (51%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.33–4.36 (2H, m), 5.10–5.13 (1H, m), 5.15–5.16 (1H, m), 5.28 (1H, s), 6.12–6.22 (1H, m), 7.29 (1H, d, J = 9.2 Hz), 7.31–7.36 (2H, m), 7.43 (1H, d, J = 8.0 Hz), 7.66–7.70 (1H, m), 8.28 (1H, dd, J = 2.0, 8.0 Hz); FAB-MS m/z 253 $[M+H]^+$.

5.1.9. 1-Allyl-2-[3-(isopropylamino)propoxy]-9*H*-xanthen-9-one (14). The title compound was obtained from 1-allyl-2-hydroxy-9*H*-xanthen-9-one (**6**) using the methods described for the synthesis of compound **11** as a yellow solid (51%): mp 82–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.10 (6H, d, J = 6.4 Hz), 2.01–2.07 (2H, m), 2.83–2.89 (3H, m), 4.10 (2H, t, J = 6.0 Hz), 4.24–4.27 (2H, m), 4.94–5.05 (2H, m), 6.08–6.18 (1H, m), 7.30–7.41 (4H, m), 7.64–7.68 (1H, m), 8.28 (1H, dd, J = 1.6, 8.0 Hz); FAB-MS m/z 352 $[M+H]^+$; Anal. Calcd for $C_{22}H_{25}NO_3 \cdot 0.3H_2O$: C, 74.42; H, 7.21; N, 3.95. Found: C, 74.47; H, 7.22; N, 4.02.

5.1.10. 9-Acetyl-1-allyl-2-hydroxy-9*H*-carbazole (8). To a stirred mixture of 2-allyloxy-9*H*-carbazole (3.35 g, 15.1 mmol) and tetrahydrofuran (30 mL) was added sodium hydride (664 mg, 16.6 mmol, 60% dispersion in mineral oil) at ambient temperature and the mixture was stirred for 1 h. To the mixture was added acetic anhydride (10 mL) at ambient temperature and the mixture was stirred at 50 °C for 70 h. After the addition of H_2O (6.0 mL) at ambient temperature, 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 subjected to chromatography over silica gel eluting with *n*-hexane/ethyl acetate (91:9 by volume) to yield 9-acetyl-2-allyloxy-9*H*-carbazole as a colorless solid (2.29 g, 57%): ^{1}H NMR (400 MHz, DMSO-d_6) 2.87 (3H, s), 4.68–4.70 (2H, m), 5.29–5.34 (1H, m), 5.44–5.50 (1H, m), 6.06–6.16 (1H, m), 7.07 (1H, dd, J = 2.4, 8.4 Hz), 7.36–7.45 (2H, m), 7.85 (1H, d, J = 2.4 Hz), 8.05–8.09 (2H, m), 8.17 (1H, d, J = 8.0 Hz); FAB-MS m/z 266 $[M+H]^+$.

9-Acetyl-2-allyloxy-9*H*-carbazole (2.26 g, 8.52 mmol) was heated at 200 °C for 4 h. The crude product was purified by silica gel column chromatography eluting with *n*-hexane/ethyl acetate (89:11 by volume) to yield the title compound as a beige solid (1.13 g, 50%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.70 (3H, s), 3.60 (2H, d, J = 6.4 Hz), 4.86–4.92 (1H, m), 4.93–4.97 (1H, m), 5.69–5.79 (1H, m), 6.99 (1H, d, J = 8.4 Hz), 7.29–7.38 (2H, m), 7.83 (1H, d, J = 8.4 Hz), 7.84 (1H, d, J = 8.0 Hz), 7.96–7.99 (1H, m), 9.79 (1H, s); FAB-MS m/z 266 $[M+H]^+$.

5.1.11. 9-Acetyl-1-allyl-2-[3-(isopropylamino)propoxy]-9*H*-carbazole hydrochloride (15). The title compound was obtained from 9-acetyl-1-allyl-2-hydroxy-9*H*-carbazole (**8**) using the methods described for the synthesis of compound **13** as a colorless solid (55%): mp 148–150 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.27 (6H, d, J = 6.4 Hz), 2.14–2.22 (2H, m), 2.73 (3H, s), 3.05–3.13 (2H, m), 3.27–3.36 (1H, m), 3.63 (2H, d, J = 6.0 Hz), 4.20 (2H, t, J = 6.0 Hz), 4.91–5.00 (2H, m), 5.73–5.83 (1H, m), 7.18 (1H, d, J = 8.4 Hz), 7.33–7.43 (2H, m), 7.88 (1H, d, J = 8.0 Hz), 8.02 (1H, d, J = 8.4 Hz), 8.07 (1H, d, J = 7.6 Hz), 8.95 (2H, br s); FAB-MS m/z 365 $[M+H]^+$; Anal. Calcd for $C_{23}H_{28}N_2O_2 \cdot HCl$: C, 68.90; H, 7.29; N, 6.99; Cl, 8.84. Found: C, 68.50; H, 7.29; N, 6.82; Cl, 8.62.

5.1.12. 1-Allyl-2-[3-(isobutylamino)propoxy]-9*H*-carbazole (16). Methods similar to those described for the synthesis of compound **11**, but using isobutylamine, yielded the title compound as a yellow solid (85%): mp 106–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (6H, d, J = 6.4 Hz), 1.71–1.81 (1H, m), 1.99–2.06 (2H, m), 2.46 (2H, d, J = 6.8 Hz), 2.84 (2H, t, J = 6.8 Hz), 3.71 (2H, d, J = 6.0 Hz), 4.15 (2H, t, J = 6.4 Hz), 5.10–5.19 (2H, m), 6.00–6.10 (1H, m), 6.88 (1H, d, J = 8.4 Hz), 7.16–7.20 (1H, m), 7.31–7.34 (1H, m), 7.38 (1H, d, J = 8.0 Hz), 7.86 (1H, d, J = 8.4 Hz), 7.95–7.99 (2H, m); FAB-MS m/z 336 $[M+H]^+$; Anal. Calcd for $C_{22}H_{28}N_2O$: C, 78.53; H, 8.39; N, 8.33. Found: C, 78.19; H, 8.45; N, 8.32.

5.1.13. 1-Allyl-2-[3-(benzylamino)propoxy]-9*H*-carbazole hydrochloride (17). Methods similar to those described for the synthesis of compound **13**, but using 1-allyl-2-hydroxy-9*H*-carbazole (**7**) and benzylamine, yielded the title compound as a colorless solid (50%): mp 190–192 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.16–2.23 (2H, m), 3.07–3.14 (2H, m), 3.60 (2H, d, J = 6.0 Hz), 4.15 (2H, t, J = 6.0 Hz), 4.17–4.21 (2H, m), 4.90–4.94 (1H, m), 5.03–5.09 (1H, m), 5.88–5.96 (1H, m), 6.89 (1H, d, J = 8.8 Hz), 7.08–7.12 (1H, m), 7.28–7.31 (1H, m), 7.40–7.47 (4H, m), 7.57–7.60 (2H, m), 7.90 (1H, d, J = 8.8 Hz), 7.99 (1H, d, J = 8.0 Hz), 9.40 (2H, br s), 11.01 (1H, s); FAB-MS m/z 371 $[M+H]^+$; Anal. Calcd for $C_{25}H_{26}N_2O \cdot HCl$: C, 73.79; H, 6.99; N, 6.88; Cl, 8.71. Found: C, 73.74; H, 6.77; N, 6.79; Cl, 8.67.

5.1.14. 1-Allyl-2-[3-(cyclopropylamino)propoxy]-9*H*-carbazole (18). Methods similar to those described for the synthesis of compound **11**, but using cyclopropylamine, yielded the title compound as a yellow solid (86%): mp 104–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.32–0.36 (2H, m), 0.42–0.46 (2H, m), 1.98–2.05 (2H, m), 2.13–2.18 (1H, m), 2.94 (2H, t, J = 6.8 Hz), 3.71 (2H, d, J = 6.4 Hz), 4.13 (2H, t, J = 6.0 Hz), 5.03–5.08 (1H, m), 5.10–5.16 (1H, m), 6.01–6.11 (1H, m), 6.86 (1H, d, J = 8.4 Hz), 7.14–7.17 (1H, m), 7.29–7.33 (1H, m), 7.41 (1H, d, J = 8.0 Hz), 7.84 (1H, d, J = 8.4 Hz), 7.94 (1H, d, J = 7.2 Hz), 9.03 (1H, br s); FAB-MS m/z 321 $[M+H]^+$; Anal. Calcd for $C_{21}H_{24}N_2O$: C, 78.72; H, 7.55; N, 8.74. Found: C, 78.55; H, 7.63; N, 8.71.

5.1.15. 1-Allyl-2-[3-(cyclohexylamino)propoxy]-9*H*-carbazole (19). Methods similar to those described for the synthesis of compound **11**, but using cyclohexylamine, yielded the title compound as a yellow solid (86%): mp 105–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03–1.32

(5H, m), 1.58–1.65 (1H, m), 1.70–1.77 (2H, m), 1.87–1.94 (2H, m), 1.98–2.04 (2H, m), 2.42–2.49 (1H, m), 2.88 (2H, t $J = 6.8$ Hz), 3.72 (2H, d, $J = 5.6$ Hz), 4.14 (2H, t, $J = 5.6$ Hz), 5.10–5.20 (2H, m), 6.00–6.10 (1H, m), 6.88 (1H, d, $J = 8.4$ Hz), 7.16–7.20 (1H, m), 7.31–7.35 (1H, m), 7.38 (1H, d, $J = 8.0$ Hz), 7.86 (1H, d, $J = 8.4$ Hz), 7.95–7.99 (2H, m); FAB-MS *m/z* 363 $[M+H]^+$; Anal. Calcd for $C_{24}H_{30}N_2O$: C, 79.52; H, 8.34; N, 7.73. Found: C, 79.68; H, 8.56; N, 7.74.

5.1.16. 1-Allyl-2-(3-anilinopropoxy)-9*H*-carbazole (20). A mixture of 1-allyl-2-(3-bromopropoxy)-9*H*-carbazole (1.38 g, 4.01 mmol), 2,2,2-trifluoro-*N*-phenylacetamide (908 mg, 4.80 mmol), potassium carbonate (1.11 g, 8.02 mmol), and *N*,*N*-dimethylformamide (6.0 mL) was stirred at 70 °C for 20 h. The reaction mixture was poured into H_2O (100 mL) and extracted with ethyl acetate (100 mL). The organic layer was washed with H_2O and then brine, dried with magnesium sulfate, and concentrated in vacuo to yield a colorless solid (2.17 g). The resulting solid (2.16 g) was dissolved in EtOH (30 mL). To this solution was added aqueous sodium hydroxide (3 N, 6.0 mL) at ambient temperature. The mixture was stirred for 18 h, and then concentrated in vacuo. The residue was diluted with ethyl acetate (100 mL). The organic layer was washed with H_2O then brine, dried with magnesium sulfate, and concentrated in vacuo. The residue was subjected to chromatography over silica gel eluting with *n*-hexane/ethyl acetate (90:10 by volume) to yield the title compound as a colorless solid (915 mg, 64%): mp 114–115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.12–2.19 (2H, m), 3.04 (2H, d, $J = 6.8$ Hz), 3.73–3.76 (2H, m), 3.89 (1H, br s), 4.21 (2H, t, $J = 6.0$ Hz), 5.12–5.20 (2H, m), 6.03–6.13 (1H, m), 6.63–6.66 (2H, m), 6.68–6.72 (1H, m), 6.88 (1H, d, $J = 8.4$ Hz), 7.15–7.21 (3H, m), 7.32–7.36 (1H, m), 7.39 (1H, d, $J = 8.0$ Hz), 7.87 (1H, d, $J = 8.4$ Hz), 7.95–7.98 (2H, m); FAB-MS *m/z* 357 $[M+H]^+$; Anal. Calcd for $C_{24}H_{24}N_2O$: C, 80.87; H, 6.79; N, 7.86. Found: C, 80.91; H, 6.85; N, 7.80.

5.1.17. 2-[3-(Isopropylamino)propoxy]-1-propyl-9*H*-carbazole (21). To a solution of 1-allyl-2-[3-(isopropylamino)propoxy]-9*H*-carbazole (**11**) (3.20 g, 9.92 mmol) in ethanol (40 mL) was added palladium on carbon (10 wt%, 500 mg), and the mixture was stirred under an atmosphere of hydrogen at ambient temperature for 5 h. The reaction mixture was filtered through a pad of Celite and concentrated in vacuo. The residue was subjected to chromatography over silica gel, eluted with chloroform/methanol/c. ammonium hydroxide (90:10:1 by volume). The resulting solid was crystallized from *n*-hexane to yield the title compound as a yellow solid (3.08 g, 96%): mp 106–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (3H, t, $J = 7.2$ Hz), 1.09 (6H, d, $J = 6.0$ Hz), 1.67–1.76 (2H, m), 1.99–2.08 (2H, m), 2.80–2.90 (5H, m), 4.14 (2H, t, $J = 6.0$ Hz), 6.86 (1H, d, $J = 8.8$ Hz), 7.16–7.20 (1H, m), 7.31–7.35 (1H, m), 7.40 (1H, d, $J = 7.2$ Hz), 7.82 (1H, d, $J = 8.8$ Hz), 7.86 (1H, br s), 7.96 (1H, d, $J = 8.0$ Hz); FAB-MS *m/z* 325 $[M+H]^+$; Anal. Calcd for $C_{21}H_{28}N_2O$: C, 77.74; H, 8.70; N, 8.63. Found: C, 77.83; H, 8.92; N, 8.59.

5.2. Preparation of microsomes from rat liver and HepG2 cells

Microsomes were prepared from the livers of rats and from HepG2 cells, a human hepatoma cell line described previously.[21] 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 Lowry method.[22]

5.3. Assay of squalene synthase inhibitory activity

The squalene synthase activities of these microsomes were assayed using the Amin technique with a modification. The test compounds were dissolved in DMSO and the assay was carried out in HEPES buffer (50 mM, pH 7.5) containing: NaF (11 mM), $MgCl_2$ (5.5 mM), dithiothreitol (3 mM), NADPH (1 mM), 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 initiated by the addition of microsomes (10 μg protein). The reaction was carried out at 30 °C for 20 min and then terminated by the addition of 40% KOH–ethanol solution (100 μL, 1:1 by volume). Synthesized [^{3}H]-squalene was extracted in petroleum ether after saponification at 60 °C for 30 min and counted in Aquasol-2 using a Beckman liquid scintillation counter.

5.4. Plasma lipid-lowering effect in rats

Five-week-old male SD rats, which were purchased from SLC (Shizuoka, Japan), were fed CE-2 diet (CLEA Japan, Inc., Tokyo, Japan) and water ad libitum. Rats received test compounds orally at doses of 50 mg/kg of body weight two times a day for 4 days. The test compounds were suspended in a 0.5% methylcellulose vehicle solution. The no-treatment control groups were administered equal volumes of the 0.5% methylcellulose vehicle solution. In all experiments, blood specimens were obtained from the animals 24 h after the last dose. All plasma samples were analyzed for total cholesterol and triglycerides using a Hitachi 7250 Automatic Analyzer (Tokyo, Japan).

5.5. Acute toxic study in rats

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

analyzed for aspartate aminotransferase using a Hitachi 7250 Automatic Analyzer (Tokyo, Japan).

Acknowledgements

The authors wish to acknowledge and thank Drs. Koyo Matsuda and Masahiko Isaka for their important input on the study. We would like to express our gratitude to Drs. Minoru Okada and Fukushi Hirayama for helpful support in the preparation of this manuscript, and we are also grateful to the staff of the Division of Analytical Science Laboratories for the elemental analysis and spectral measurements.

References and notes

1. (a) Rosamond, W. D.; Chambless, L. E.; Folsom, A. R.; Cooper, L. S.; Conwill, D. E.; Clegg, L.; Wang, C.-H.; Heiss, G. *N. Engl. J. Med.* **1998**, *339*, 861; (b) Murray, C. J. L.; Lopez, A. D. *Lancet* **1997**, *349*, 1269.
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. Ooi, T. C.; Heinonen, T.; Alaupovic, P.; Davignon, J.; Leiter, L.; Lupien, P. J.; Sniderman, A. D.; Tan, M. H.; Tremblay, G.; Sorisky, A.; Shurzinske, L.; Black, D. M. *Arterioscler. Thromb. Vasc. Biol.* **1997**, *17*, 1793.
6. Study Group *Eur. Heart J.* **1998**, *9*, 571.
7. McCarthy, P. A. *Med. Res. Rev.* **1993**, *13*, 139.
8. (a) Bergstrom, J. D.; Dufresne, C.; Bills, G. F.; Nallin-Omstead, M.; Byrne, K. *Annu. Rev. Microbiol.* **1995**, *49*, 607; (b) Amin, D.; Rutledge, R. Z.; Needle, S. J.; Hele, D. J.; Neuenschwander, K.; Bush, R. C.; Bilder, G. E.; Perrone, M. H. *Naunyn Schmiedebergs Arch. Pharmacol.* **1996**, *353*, 233; (c) Amin, D.; Rutledge, R. Z.; Needle, S. N.; Galczenski, H. F.; Neuenschwander, K.; Scotese, A. C.; Maguire, M. P.; Bush, R. C.; Hele, D. J.; Bilder, G. E.; Perrone, M. H. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 746.
9. (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.
10. (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.
11. 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.* **1996**, *35*, 1622.
12. (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.
13. (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.
14. Fairlamb, I. J. S.; Dickinson, J. M.; O'connor, R.; Higson, S.; Grieveson, L.; Marin, V. *Bioorg. Med. Chem.* **2002**, *10*, 2641.
15. (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; (c) Amin, D.; Rutledge, R. Z.; Needle, S. N.; Galczenski, H. F.; Neuenschwander, K.; Scotese, A. C.; Maguire, M. P.; Bush, R. C.; Hele, D. J.; Bilder, G. E.; Perrone, M. H. *J. Pharmacol. Exp. Ther.* **1997**, *281*, 746; (d) Ishihara, T.; Kakuta, H.; Moritani, H.; Ugawa, T.; Sakamoto, S.; Tsukamoto, S.; Yanagisawa, I. *Bioorg. Med. Chem.* **2003**, *11*, 2403; (e) Ishihara, T.; Kakuta, H.; Moritani, H.; Ugawa, T.; Sakamoto, S.;

Tsukamoto, H. J., Jr.; Yanagisawa, I. *Bioorg. Med. Chem.* **2003**, *11*, 3735.

16. Brown, G. R.; Butlin, R. J.; Chapman, S.; Eakin, M. A.; Foubister, A. J.; Freeman, S.; Griffiths, D.; Harrison, P. J.; Johnson, M. C.; Mallion, K. B.; McTaggart, F.; Reid, A. C.; Smith, G. J.; Taylor, M. J.; Walker, R. P.; Whittamore, P. R. O. *J. Med. Chem.* **1995**, *38*, 4157.
17. (a) Saunders, J.; Cassidy, M.; Freedman, S. B.; Harley, E. A.; Iversen, L. L.; Kneen, C.; MacLeod, A. M.; Merchant, K. J.; Snow, R. J.; Baker, R. *J. Med. Chem.* **1990**, *33*, 1128; (b) Street, L. J.; Baker, R.; Book, T.; Reeve, A. J.; Saunders, J.; Willson, T.; Marwood, R. S.; Patel, S.; Freedman, S. B. *J. Med. Chem.* **1992**, *35*, 295; (c) Bromidge, S. M.; Brown, F.; Cassidy, F.; Clark, M. S. G.; Dabbs, S.; Hadley, M. S.; Loudon, J. M.; Orlek, B. S.; Riley, G. J. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 787; (d) Orlek, B. S.; Cassidy, F.; Clark, M. S. G.; Faulkner, R. E.; Collings, E. J.; Hawkins, J.; Riley, G. J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1411; (e) Nordvall, G.; Sundquist, S.; Ilvebrant, L. N.; Hacksell, U. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2837; (f) Ward, J. S.; Merritt, L.; Calligaro, D. O.; Bymaster, F. P.; Shannon, H. E.; Sawyer, B. D.; Mitch, C. H.; Deeter, J. B.; Peters, S. C.; Sheardown, M. J.; Olesen, P. H.; Swedberg, M. D. B.; Sauerberg, P. *J. Med. Chem.* **1995**, *38*, 3469; (g) Somanadhan, B.; Loke, W.-K.; Sim, M.-K.; Go, M.-L. *Bioorg. Med. Chem.* **2001**, *10*, 207.
18. (a) Swain, C. J.; Seward, E. M.; Sabin, V.; Owen, S.; Baker, R.; Cascieri, M. A.; Sadowski, S.; Strader, C.; Ball, R. G. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1703; (b) Swain, C. J.; Fong, T. M.; Haworth, K.; Owen, S. N.; Seward, E. M.; Strader, C. D. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1261; (c) Swain, C. J.; Seward, E. M.; Cascieri, M. A.; Fong, T. M.; Herbert, R.; MacIntyre, D. E.; Merchant, K. J.; Owen, S. N.; Owens, A. P.; Sabin, V.; Teall, M.; VanNiel, M. B.; Williams, B. J.; Sadowski, S.; Strader, C.; Ball, G.; Baker, R. *J. Med. Chem.* **1995**, *38*, 4793.
19. 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.
20. The pK_a values were calculated using the ACD/pK_a data base (Advanced Chemistry Development Inc.).
21. Lindsey, S.; Harwood, H. J., Jr. *J. Biol. Chem.* **1995**, *270*, 9083.
22. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265.