Lipid-Lowering (Hetero)Aromatic Tetrahydro-1,4-Oxazine Derivatives with Antioxidant and Squalene Synthase Inhibitory Activity

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A number of newly synthesized 2-[4-(hetero)aromatic]phenyl-2-hydroxy-tetrahydro-1,4-oxazine derivatives were found to inhibit lipid peroxidation (IC_{50} of the most potent was 20 μM) as well as rat squalene synthase (IC_{50} for most between 1−10 μM). Antidyslipidemic action was demonstrated in vivo: the most active compound decreased triglycerides, total cholesterol, and LDL-cholesterol of hyperlipidemic rats by 64, 67, and 82%, respectively, at 56 μmol/kg (ip). Most of the novel compounds are more active than the structurally related and reference biphenyl-morpholine, pointing to useful structural approaches for the design of antiatherosclerotic agents.

1. Introduction

Atherosclerosis is recognized as a major pathologic condition with serious social and economic consequences. Although at present it is a problem of the western world, the World Health Organization predicts an epidemic of atherosclerosis as developing countries adopt the western lifestyle.[1] Atherosclerosis is characterized by the accumulation of cholesterol, mainly LDL-cholesterol in macrophages, leading to lesion formation in large- and medium-size arteries.[2,3] These lesions may develop into atheromatic plaques, which are prone to rupture and cause clinical events such as heart attack and stroke.[4,5] Today, there is evidence that atherosclerosis is a condition of elevated oxidative stress in the vascular wall, expressed as lipid and protein oxidation.[6,7] Oxidation of LDL-cholesterol is an early event in atherosclerosis, and oxidized LDL-cholesterol is proatherogenic.[8] Currently, treatment of atherosclerosis aims in reducing blood cholesterol and triglyceride content. A temporary interruption of cholesterol biosynthesis can lead to decreased plasma LDL-cholesterol levels by removing LDL-cholesterol from the circulation through upregulation of hepatic LDL receptors. Inhibitors of cholesterol biosynthesis via inhibition of HMGCo-A[a] reductase are presently the most effective means for blood LDL-cholesterol reduction.[9] However, inhibition of HMGCo-A reductase may block the formation of biologically necessary isoprenoids. Squalene synthase inhibition seems to offer a preferable alternative because squalene synthase-catalyzed reductive dimerization of farnesyl pyrophosphate to squalene is the first specific step in cholesterol biosynthesis, thus isoprenoid formation is not affected. Furthermore, squalene synthase inhibitors have been found to lower triglyceride levels in addition to the decrease of plasma cholesterol.[10] It has been reported that several quinuclidine derivatives reduce cholesterol and triglyceride levels, acting as squalene synthase inhibitors.[10−13] We have previously shown that 2-biphenyl morpholine derivatives lower cholesterol and triglyceride levels in vivo. These compounds combined antidyslipidemic action with antioxidant activity[14] and nitric oxide releasing properties.[15] The two most active compounds of those studies were further pharmacologically characterized both in vitro[16,17] and in vivo.[18]

In this investigation, we synthesized and investigated further substituted 1,4-oxazine derivatives (compounds **2**−**22**), which meet the assumed structural requirements for antidyslipidemic, antioxidant, as well as squalene synthase inhibitory activity. The performed structural modifications involve mainly the 2-aryl substitution of the 1,4-oxazine ring. This substituent appears to be implicated in the antioxidant as well as the lipid lowering ability of these compounds. Compound **1**, which has been studied earlier,[14] was included as a reference.

2. Results and Discussion

2.1. Synthesis. The 2-hydroxy-2-aryl morpholine derivatives (**1**−**22**) were formed through a spontaneous cyclization of a hydroxyaminoketone intermediate to a hemiketal structure, generally in very good yields. The structures of the synthesized compounds, as well as their lipophilicity, calculated as ClogP values by the method of Leo−Hansch,[19] are shown in Table 1. As shown by previous studies[20,21] but also by theoretical studies (unpublished results), the stable conformation of the final compounds includes equatorial position of the biaryl system (and axial position of the OH) and chair or chair−chair (*trans*) conformation of the fused octahydro-oxazine ring systems. Taking the above into consideration, each final product is considered to be a racemic mixture of two enantiomers, defined at the chiral center of position 2 or 3 (for compounds **4**, **7**, **10**, **13**, **16**, **19**, and **22**), respectively.

2.2. Antioxidant Activity. The effect of the investigated derivatives on the non enzymatic peroxidation of hepatic microsomal membrane lipids after 45 min of incubation, expressed as IC_{50} values, is shown in Table 2. Under the same

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[a] Abbreviations: SQS, squalene synthase; HMGCo-A, 3-hydroxy-3-methylglutaryl-CoA; FPP, farnesyl pyrophosphate; BSA, bovine serum albumin; NADPH, nicotinamide adenine dinucleotide phosphate; IC_{50}, inhibitory concentration (for 50% of the reaction); TC, total cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; TG, triglyceride; DMSO, dimethyl sulfoxide; trolox, (*S*)-(−)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; probucol, 4,4′-[(1-methylethylidene)bis(thio)]-bis[2,6-bis(1,1-dim ethylethyl)phenol]; TBAR, 2-thiobarbituric acid reactive material.

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Table 1. Structure of the Examined Derivatives and Their Lipophilicity (ClogP)

compd	R_1	R_2	R_3	R_4	ClogP
1	CH_3	$CH_2CH_2CH_2CH_2$		phenyl	4.72
2	CH_3	H	H	2-thienyl	2.94
3	CH_3	$CH_2CH_2CH_2CH_2$		2-thienyl	4.37
4	$CH_2CH_2CH_2CH_2$	H		2-thienyl	3.74
5	CH_3	H	H	2-bromo-5-thienyl	3.84
6	CH_3	$CH_2CH_2CH_2CH_2$		2-bromo-5-thienyl	5.27
7	$CH_2CH_2CH_2CH_2$		H	2-bromo-5-thienyl	4.64
8	CH_3	H	H	2-benzothiazolyl	3.23
9	CH_3	$CH_2CH_2CH_2CH_2$		2-benzothiazolyl	4.66
10	$CH_2CH_2CH_2CH_2$		H	2-benzothiazolyl	4.03
11	CH_3	H	H	2-fluorophenyl	3.22
12	CH_3	$CH_2CH_2CH_2CH_2$		2-fluorophenyl	4.65
13	$CH_2CH_2CH_2CH_2$		H	2-fluorophenyl	4.02
14	CH_3	H	H	4-chlorophenyl	3.79
15	CH_3	$CH_2CH_2CH_2CH_2$		4-chlorophenyl	5.23
16	$CH_2CH_2CH_2CH_2$		H	4-chlorophenyl	4.59
17	CH_3	H	H	4-bromophenyl	3.94
18	CH_3	$CH_2CH_2CH_2CH_2$		4-bromophenyl	5.37
19	$CH_2CH_2CH_2CH_2$		H	4-bromophenyl	4.74
20	CH_3	H	H	4-fluorophenyl	3.22
21	CH_3	$CH_2CH_2CH_2CH_2$		4-fluorophenyl	4.65
22	$CH_2CH_2CH_2CH_2$		H	4-fluorophenyl	4.02

Table 2. Antioxidant, SQS Inhibitory, and Antidyslipidemic Effects of the Investigated Compounds

compd	inhibition of lipid peroxidation IC_{50} (μM)	inhibition of SQS IC_{50} (μM)	% decrease compared to hyperlipidemic controls		
			TG	TC	LDL-C
1	450	36	49[a]	54[a]	51[a]
2	87	5	38[a]	48[a]	61[b]
3	20	10	47[a]	45[a]	46[a]
4	174	7	29[a]	43[a]	46[a]
5	51	13	35[a]	43[a]	26
6	160	1	26[a]	35[a]	45[a]
7	164	7	37[a]	36[a]	35[a]
8	113	2	44[a]	46[a]	53[a]
9	202	6	0	0	24
10	187	5	46[a]	55[a]	41[a]
11	182	40	64[c]	67[b]	82[c]
12	490	42	25	18	47[a]
13	390	10	15	5	11
14	111	9	70[c]	63[b]	57[b]
15	180	9	41[a]	11[a]	29[a]
16	178	15	52[a]	44[a]	35[a]
17	220	6	51[a]	47[a]	36[a]
18	200	4	7	23	53[b]
19	139	3	17	17	6
20	330	23	18[a]	23	36[a]
21	420	28	94[c]	41[a]	33[a]
22	162	10	95[c]	27	11

[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ (Student's t test)

experimental conditions, trolox and probucol, known potent antioxidants, exhibited IC_{50} values of 25 μM and >1 mM, respectively. The time course of lipid peroxidation, as affected by several concentrations of the most active compound **3**, is depicted in Figure 1. Most of the studied derivatives demonstrated significant antioxidant activity, higher than that of the reference compound **1**. Thus, the performed structural modifications, primarily the addition of a thienyl group (compounds **2**−**7**) led consistently to a considerable improvement of their antioxidant profile. This could be due to an extension of the conjugated system and/or to the presence of the sulfur atom that contributes to this action. It seems that antioxidant activity

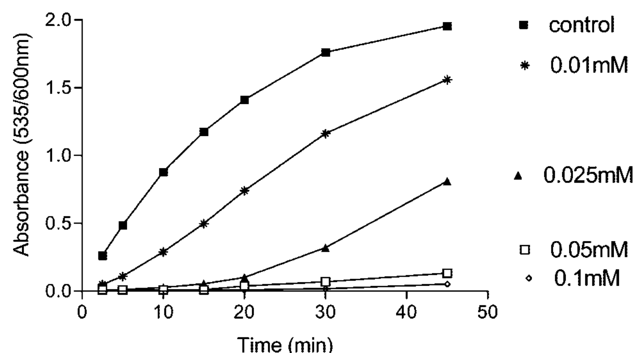

Figure 1. Representative graph of the time course of lipid peroxidation as affected by various concentrations of compound **3**.

is favored by resonance effects of the conjugated biaryl substituent because we have found that 2-hydroxy-2-phenyl-4-methyl morpholine and 2-hydroxy-2-*t*-butyl-4-methyl-morpholine had a weak (IC_{50} > 1 mM) or negligible effect on lipid peroxidation, respectively (data not shown). Although, in general, the lipophilic character of antioxidants contributes to the offered inhibition of lipid peroxidation, because lipophilic compounds may acquire an easy access to biological membranes, it seems that lipophilicity, given as ClogP values, is not a determining factor in this series of derivatives.

2.3. Inhibition of Rat Microsomal Squalene Synthase. Inhibition of the activity of squalene synthase from rat liver microsomes by the test compounds expressed as IC_{50} values is shown in Table 2. All compounds inhibited squalene synthase activity significantly and dose-dependently as shown in Figure 2, which depicts the activity of the most active compound **6** and the activity of compound **20**. Thienyl, benzothiazolyl, as well as 4-bromophenyl substitution seem to improve the activity in this series of derivatives. Closer examination of the set of compounds (**1**, **12**, **21**, **15**, **18**), as well as (**11**, **20**, **14**, **17**) and (**3**, **6**), indicates that halogen substitution in the distal aromatic ring affects activity, which goes in parallel with the lipophilic

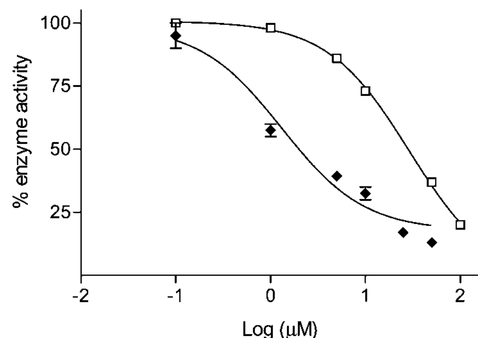

Figure 2. Representative graph showing the activity of squalene synthase as affected by various concentrations of compound **6** (◆) and **20** (□).

character and resonance effect of the halogen. This observation is in accordance with studies in a series of 3-hydroxy-3-biaryl quinuclidines, demonstrating that the best squalene synthase inhibitory activity was expressed by compounds with planar biaryl substitution and, furthermore, that there is a directional requirement for the rigid biaryl side chain.[13] As far as the mechanism of this inhibitory activity is concerned, it is considered that the biaryl substituent may act as an isoster for isoprenyl subunits of the farnesyl chain in cholesterol biosynthesis.

2.4. Antidyslipidemic Activity. The in vivo antidyslipidemic activity of these derivatives is also demonstrated in Table 2. Hyperlipidemia was successfully established 24 h after Triton WR 1339 administration as shown by the increase in plasma total cholesterol, LDL-cholesterol, and triglyceride levels that was found to be 93%, 77%, and 84%, respectively, compared to normal values. Almost all test compounds were found able to reduce the examined parameters in the plasma of hyperlipidemic rats, with the exception of some derivatives, which had a marginal or no effect on triglycerides (compounds **9**, **13**, **18**, and **19**), on total cholesterol (compounds **9**, **13**, and **19**) and on LDL-cholesterol (compounds **19**, **13**, and **22**). Under the same experimental conditions, probucol and simvastatin (used as antidyslipidemic drugs), at the same dose, reduced plasma total cholesterol by 18 and 75%, LDL-C by 11 and 70%, and triglycerides by 18 and 0%, respectively.

Most compounds had a similar or lower effect than **1** on triglyceride concentration. However, four compounds, namely **11**, **14**, **21**, and **22**, were significantly more effective than the reference compound. Compounds **11** and **14** were also more active in total cholesterol reduction compared to most of the other compounds, which presented activity similar to **1**. Furthermore, compounds **2**, **11**, and **14** were more effective than **1** in LDL-cholesterol reduction. The consistently increased effect of derivatives **11** and **14** followed by **22**, **21**, and **2**, concerning the in vivo reduction of lipid parameters, indicates useful combinations of structural features toward increased activity.

2-Bromo-substitution on the thienyl ring (compounds **5**, **6**, **7**) resulted in an overall decrease in antidyslipidemic activity, compared to **2**, **3**, and **4**. This may indicate that an increase in the bulk and/or a decrease in the electron density of the thienyl group do not favor activity.

The effect of almost all compounds on one lipid parameter seems to correlate well with its effect on the other lipid parameters measured, indicating a more or less consistent antidyslipidemic activity. This activity seems also to be somewhat related with reduced lipophilicity of the compounds of this series. Although we estimate that the basic mode of antidyslipidemic action of these compounds is via inhibition of squalene synthase, no direct correlation can be derived between in vitro and in vivo activity. Other parameters such as in vivo distribution and pharmacokinetics may disrupt the relative in vivo–in vitro order of activity of these compounds. Thus reduced lipophilicity together with increased antioxidant activity appear as more significant factors determining in vivo antihyperlipidemic action.

In conclusion, a new class of multiple acting molecules that holds promise for the treatment of atherosclerosis seems to be emerging. Together with recent studies, the observed structure–activity relationships in this series of substituted 1,4-oxazine derivatives are expected to contribute to the design of novel molecules with improved activity profile that may be useful as potential antiatherogenic agents.

3. Materials and Methods

3.1. Materials. All commercially available chemicals are of the appropriate purity and purchased from standard sources. [^{3}H] FPP (21.5 Ci/mmol), NADPH, FPP, and BSA were purchased from Sigma-Aldrich (Germany). For the in vivo experiments, Fischer-344 male rats (180–220 g) were used. Animals were kept in a controlled temperature room (22 ± 2 °C), having free access to laboratory chow and tap water, under a 12 h light/dark cycle.

3.2. Synthesis. Melting points (mp) were determined with a MEL-TEMPII (Laboratory Devices, USA) apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a BRUKER AC-200 MHz spectrometer. Elemental analyses were performed with a Perkin-Elmer 2400 CHN analyzer (analysis of C, H, and N) or by the Service Central de Microanalyse, France (analysis of C, H) and are reported in Supporting Information.

3.2.1. General Procedure for the Preparation of the Final Products. The final products (Table 1) were obtained by the reaction of 3 mmol of 2-methylaminoethanol, *trans*-2-methylamino-cyclohexanol,[22] or 2-piperidinemethanol[23] with 1.2 mmol of 2-(4-bromoacetylphenyl)thiophene (compounds **2**, **3**, **4**) or 2-bromo-5-(4-bromoacetylphenyl)thiophene (compounds **5**, **6**, **7**) or 2-(4-bromoacetylphenyl)benzothiazole (compound **8**, **9**, **10**) or 4-(2′-fluorophenyl)-bromoacetophenone (compound **11**, **12**, **13**) or 4-(4′-chlorophenyl)-bromoacetophenone (compound **14**, **15**, **16**) or 4-(4′-bromophenyl)-bromoacetophenone (compound **17**, **18**, **19**) or 4-(4′-fluorophenyl)-bromoacetophenone (compound **20**, **21**, **22**) in anhydrous acetone (40 mL) at room temperature with stirring for 15 h. Acetone was then distilled off, ether was added to the residue, the mixture was washed with saturated sodium chloride solution, dried (K_2CO_3), and the products were isolated as hydrobromides.

2-[4-(2-Thienyl)phenyl]-4-methylmorpholin-2-ol hydrobromide (2). Yield 30%, mp 175–177 °C. ^{1}H NMR ($CDCl_3$) δ 2.65 (s, 3H), 2.84–3.27 (m, 5H), 3.75 (dd, J_1 = 12.9 Hz, J_2 = 3.5 Hz, 1H), 4.30 (dt, J_1 = 12.9 Hz, J_2 = 3.3 Hz, 1H), 6.80–7.40 (m, 7H), 10.0 (bs, 1H). Anal. ($C_{15}H_{18}BrNO_2S \cdot 0.5\ H_2O$) C, H, N.

2-[4-(2-Thienyl)phenyl]-4-methyl-octahydro-1,4-benzoxazin-2-ol hydrobromide (3). Yield 87%, mp 76–78 °C. ^{1}H NMR ($CDCl_3$) δ 1.29–2.1 (m, 9H), 2.74 (d, J = 5.0 Hz, 1H), 2.81 (d, J = 5.0 Hz, 3H), 2.96–3.04 (t, J = 14.0 Hz, 1H), 3.61–3.66 (dd, J_1 = 15.0 Hz, J_2 = 2.0 Hz, 1H), 4.50 (m, 1H), 7.05–7.30 (m, 5H), 7.65 (d, J = 8.0 Hz, 2H), 11.2 (bs, 1H). Anal. ($C_{19}H_{24}BrNO_2S \cdot 2.2H_2O$) C, H, N.

3-[4-(2-Thienyl)phenyl]-octahydro-1,4-pyrido[2,1-*c*]oxazin-3-ol hydrobromide (4). Yield 99%, mp 189–190 °C. Anal. ($C_{18}H_{22}BrNO_2S \cdot H_2O$) C, H.

2-[4-(2-Bromo-5-thienyl)phenyl]-4-methylmorpholin-2-ol hydrobromide (5). Yield 57%, mp 168–170 °C. Anal. ($C_{15}H_{17}Br_2NO_2S \cdot H_2O$) C, H, N.

2-[4-(2-Bromo-5-thienyl)phenyl]-4-methyl-octahydro-1,4-benzoxazin-2-ol hydrobromide (6). Yield 90%, mp 113–115 °C. ^{1}H NMR ($CDCl_3$) δ 1.20–2.28 (m, 8H), 2.75 (m, 2H), 2.81 (d, J = 5.0 Hz, 3H), 3.00 (d, J = 14.0 Hz, 1H), 3.67 (d, J = 14.0 Hz, 1H), 4.46–4.58 (m, 1H), 7.00–7.06 (m, 2H), 7.49–7.52 (d, J = 8.0

Hz, 2H), 7.64−7.67 (d, J = 8.0 Hz, 2H), 11.5 (bs, 1H). Anal. ($C_{19}H_{23}Br_2NO_2S \cdot H_2O$) C, H, N.

3-[4-(2-Bromo-5-thienyl)phenyl]-octahydro-1,4-pyrido[2,1-*c*]oxazin-3-ol hydrobromide (7). Yield 99%, mp 166−167 °C. ^1H NMR ($CDCl_3$ with drops of DMSO-d_6) δ 1.21−1.82 (m, 7H), 2.40−3.00 (m, 3H), 3.20−3.40 (m, 2H), 3.70−3.77 (dd, J_1 = 13.0 Hz, J_2 = 3.0 Hz, 1H), 4.26 (t, J = 11.0 Hz, 1H), 6.88−6.96 (m, 2H), 7.38 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 8.0 Hz, 2H), 11.05 (bs, 1H). Anal. ($C_{18}H_{21}Br_2NO_2S$) C, H.

2-[4-(2-Benzothiazolyl)phenyl]-4-methylmorpholin-2-ol hydrobromide (8). Yield 68%, mp 110−115 °C. ^1H NMR ($CDCl_3$) δ 2.55 (s, 3H), 3.10−3.55 (m, 5H), 4.05 (dd, J_1 = 11.6 Hz, J_2 = 3.2 Hz, 1H), 4.36 (dt, J_1 = 12.0 Hz, J_2 = 3.1 Hz, 1H), 7.40−8.20 (m, 8H), 9.8 (bs, 1H). Anal. ($C_{18}H_{19}BrN_2O_2S$) C, H.

2-[4-(2-Benzothiazolyl)phenyl]-4-methyl-octahydro-1,4-benzoxazin-2-ol hydrobromide (9). Yield 90%, mp 90−91 °C. Anal. ($C_{22}H_{25}BrN_2O_2S \cdot 2H_2O$) C, H, N.

2-[4-(2-Benzothiazolyl)phenyl]-octahydro-1,4-pyrido[2,1-*c*]oxazin-3-ol hydrobromide (10). Yield 23%, mp 215−217 °C. ^1H NMR ($CDCl_3$) δ 1.20−2.20 (m, 7H), 2.40−3.00 (m, 2H), 3.30−3.60 (m, 2H), 3.90 (d, J = 12.0, 1H), 4.21 (d, J = 12.1, 1H), 4.60 (t, J = 12.4, 1H), 7.10−8.20 (m, 8H), 11.4 (bs, 1H). Anal. ($C_{21}H_{23}BrN_2O_2S \cdot 0.6CH_2Cl_2$) C, H, N.

2-[4-(2-Fluorophenyl)phenyl]-4-methyl-morphonyl-2-ol hydrobromide (11). Yield 40%, mp 135−136 °C. Anal. ($C_{17}H_{19}BrFNO_2$) C, H, N.

2-[4-(2-Fluorophenyl)phenyl]-4-methyl-octahydro-1,4-benzoxazine-2-ol hydrobromide (12). Yield 89%, mp 105−106 °C. Anal. ($C_{21}H_{25}BrFNO_2 \cdot 0.5H_2O$) C, H, N.

3-[4-(2-Fluorophenyl)phenyl]-octahydro-1,4-pyrido[2,1-*c*]oxazine-3-ol hydrobromide (13). Yield 99%, mp 150−151 °C. Anal. ($C_{20}H_{23}BrFNO_2 \cdot 3H_2O$) C, H.

2-[4-(4-Chlorophenyl)phenyl]-4-methyl-morphonyl-2-ol hydrobromide (14). Yield 31%, mp 168−169 °C. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 2.28 (s, 3H), 2.50 (m, 1H), 2.84 (m, 4H), 3.45 (dd, J_1 = 12.6 Hz, J_2 = 3.3 Hz, 1H), 3.90 (dt, J_1 = 12.1 Hz, J_2 = 2.9 Hz, 1H), 6.82−7.14 (m, 9H). Anal. ($C_{17}H_{19}BrClNO_2 \cdot 0.3H_2O$) C, H, N.

2-[4-(4-Chlorophenyl)phenyl]-4-methyl-octahydro-1,4-benzoxazine-2-ol hydrobromide (15). Yield 79%, mp 141−142 °C. ^1HNMR ($CDCl_3$ + DMSO-d_6) δ 1.20−1.50 (m, 4H), 1.70−1.85 (m, 3H), 2.05−2.10 (m, 1H), 2.60 (m, 1H), 2.75 (s, 3H), 2.87−3.08 (m, 2H), 3.45, (d, J = 12.3 Hz, 1H), 4.26 (dt, J_1 = 11.0 Hz, J_2 = 3.9 Hz, 1H),7.34−7.60 (m, 8H), 10.7 (bs, 1H). Anal. ($C_{21}H_{25}BrClNO_2 \cdot 0.8H_2O$) C, H, N.

3-[4-(4-Chlorophenyl)phenyl]-octahydro-1,4-pyrido[2,1-*c*]oxazine-3-ol hydrobromide (16). Yield 73%, mp 202−204 °C. Anal. ($C_{20}H_{23}BrClNO_2 \cdot H_2O$) C, H, N.

2-[4-(4-Bromophenyl)phenyl]-4-methyl-morphonyl-2-ol hydrobromide (17). Yield 61%, mp 178.4−179 °C. Anal. ($C_{17}H_{19}Br_2NO_2 \cdot 0.5H_2O$) C, H.

2-[4-(4-Bromophenyl)phenyl]-4-methyl-octahydro-1,4-benzoxazine-2-ol hydrobromide (18). Yield 46%, mp 227−229 °C. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 1.20−2.00 (m, 8H), 2.80 (s, 3H), 3.49 (m, 1H), 3.67 (m, 2H), 3.90, (m, 1H), 4.51 (m, 1H), 7.41−7.73 (m, 8H), 11.62 (bs, 1H). Anal. ($C_{21}H_{25}Br_2NO_2$) C, H.

3-[4-(4-Bromophenyl)phenyl]-octahydro-1,4-pyrido[2,1-*c*]oxazine-3-ol hydrobromide (19). Yield 93%, mp 196−198 °C (decom 169 °C). ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 1.20−2.02 (m, 7H), 3.06−3.33 (m, 4H), 3.65 (m, 1H), 3.83 (d, J = 12.3 Hz, 1H), 4.05 (t, J = 12.5 Hz, 1H), 7.03−7.52 (m, 8H), 10.55 (bs, 1H). Anal. ($C_{20}H_{23}Br_2NO_2 \cdot 0.5H_2O$) C, H.

2-[4-(4-Fluorophenyl)phenyl]-4-methyl-morphonyl-2-ol hydrobromide (20). Yield 40%, mp 120−122 °C. Anal. ($C_{17}H_{19}BrFNO_2$) C, H, N.

2-[4-(4-Fluorophenyl)phenyl]-4-methyl-octahydro-1,4-benzoxazine-2-ol hydrobromide (21). Yield 41%, mp 140−142 °C. Anal. ($C_{21}H_{25}BrFNO_2 \cdot 0.5H_2O$) C, H, N.

3-[4-(4-Fluorophenyl)phenyl]-octahydro-1,4-pyrido[2,1-*c*]oxazine-3-ol hydrobromide (22). Yield 98%, mp 157−157.5 °C. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 1.40−2.27 (m, 8H), 3.20−3.55 (m, 3H), 3.70 (m, 1H), 3.85−4.05 (m, 1H), 4.25 (t, J = 11.6 Hz, 1H), 7.10−7.70 (m, 8H), 10.7 (bs, 1H). Anal. ($C_{20}H_{23}BrFNO_2$) C, H.

3.2.2. Preparation of Intermediate and Starting Materials. Synthesis and characterization of the following starting materials and intermediate bromoacetylphenyl derivatives, *trans*-2-methylamino-cyclohexanol,[22] 2-(4-acetylphenyl)thiophene (**23**),[24,25] 2-(4-bromoacetylphenyl)thiophene (**24**), 2-bromo-5-(4-bromoacetylphenyl)thiophene (**25**), 2-(acetylphenyl)benzothiazole (**26**),[26] 2-(4-bromoacetylphenyl)benzothiazole (**27**),[27] 4-(2′-fluorophenyl)acetophenone (**28**),[28] 4-(4′-fluorophenyl)acetophenone (**29**),[29] 4-(4′-chlorophenyl)acetophenone (**30**),[30] 4-(2′-fluorophenyl)-bromoacetophenone (**31**),[28] 4-(4′-fluorophenyl)bromoacetophenone (**32**), 4-(4′-chlorophenyl)-bromoacetophenone (**33**),[31] and 4-(4′-bromophenyl)-bromoacetophenone (**34**)[32] are reported in Supporting Information.

3.3. In Vitro Lipid Peroxidation. Heat-inactivated hepatic microsomes from untreated rats were prepared as described.[14] The incubation mixture contained microsomal fraction (corresponding to 2.5 mg of hepatic protein per mL or 4 mM fatty acid residues), ascorbic acid (0.2 mM) in Tris-HCl/KCl buffer (50 mM/150 mM, pH 7.4), and the studied compounds (10 μM to 1 mM) dissolved in DMSO. The reaction was initiated by addition of a freshly prepared $FeSO_4$ solution (10 μM), and the mixture was incubated at 37 °C for 45 min. Lipid peroxidation of aliquots was assessed spectrophotometrically (535 against 600 nm) as TBAR.[14] All compounds and solvents were found not to interfere with the assay. Each assay was performed in duplicate, and IC_{50} values represent the mean concentration of compounds that inhibits the peroxidation of control microsomes by 50% after 45 min of incubation. All standard errors are within 10% of the respective reported values.

3.4. In Vitro Squalene Synthase Activity Assay. SQS activity was evaluated by determining the amount of [^{3}H] FPP converted to squalene as previously described.[33,16] The assay was performed in 1 mL of 50 mM phosphate buffer, pH 7.4, containing 10 mM $MgCl_2$, 0.5 mM NADPH, rat liver microsomes (18 μg protein/mL), various concentrations of the test compounds dissolved in ethanol, and [^{3}H] FPP (0.5 μM, 0.27 Ci/mmol) in a glass screw-cap tube. All components except [^{3}H] FPP were preincubated for 10 min (37 °C). The reaction was initiated by the addition of [^{3}H] FPP. After 10 min (37 °C), the reaction was terminated by the addition of 1 mL 15% KOH in ethanol. The mixture was incubated at 65 °C for 30 min, extracted with 5 mL of petroleum ether, which was subsequently washed with 2 mL distilled water. Then 1.5 mL of the upper organic phase was counted with 3 mL of scintillation liquid using a Beckman scintillation counter. Each assay was performed in triplicate, and IC_{50} values represent the mean concentration of compounds that inhibits the activity of the enzyme by 50%. All standard errors are within 10% of the respective reported values.

3.5. In Vivo Evaluation of Antihypercholesterolemic and Antihyperlipidemic Activity. An aqueous solution of Triton WR 1339 was given ip to rats (200 mg/kg), and 1 h later, the test compounds (56 μmol/kg), dissolved in saline or saline only were administered ip. After 24 h, blood was taken from the aorta and used for the determination of plasma total cholesterol (TC), LDL-cholesterol (LDL-C), and triglyceride (TG) levels, using commercially available kits.[15] Levels of plasma lipids were determined in duplicate, while values presented are the mean from 8−10 rats (per compound). All standard errors are within 10% of the respective reported values.

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Supporting Information Available: Methods for preparation of intermediate and starting materials, ^{1}HNMR data for compounds **4**, **5**, **9**, **11**, **12**, **13**, **16**, **17**, **19**, **20**, and **21**, as well as elemental analysis data of all final and some intermediate compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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