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Design of Novel Potent Antihyperlipidemic Agents with Antioxidant/Anti-inflammatory Properties: Exploiting Phenothiazine's Strong Antioxidant Activity

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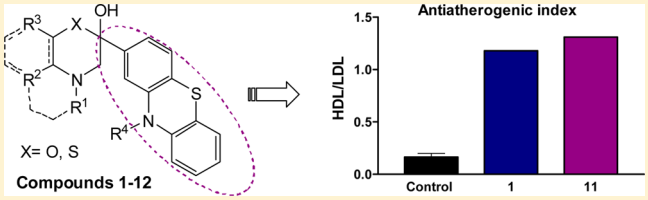
ABSTRACT: Because atherosclerosis is an inflammatory process involving a series of pathological events such as dyslipidemia, oxidative stress, and blood clotting mechanisms, we hereby report the synthesis and evaluation of novel compounds in which antioxidant, anti-inflammatory, and squalene synthase (SQS) inhibitory/hypolipidemic activities are combined in simple molecules through design. The coupling of two different pharmacophores afforded compounds **1**−**12**, whose biological profile was markedly improved compared to those of parent lead structures (i.e., the hypolipidemic 2-hydroxy-2-aryl-(benzo)oxa(or thia)zine and the antioxidant phenothiazine). Most derivatives strongly inhibited in vitro microsomal lipid and LDL peroxidation, exhibiting potent free-radical scavenging activity. They further significantly inhibited SQS activity and showed remarkable antidyslipidemic activity in vivo in animal models of acute and high-fat-induced hyperlipidemia. Finally, several compounds showed anti-inflammatory activity in vitro, inhibiting cycloxygenase (COX-1/2) activity. The multimodal properties of the new compounds and especially their combined antioxidant/SQS/COX inhibitory activity render them interesting lead compounds for further evaluation against atherosclerosis.



1. INTRODUCTION

Cardiovascular disease (CVD), currently the leading cause of death in developed countries, will soon become a predominant health problem worldwide.[1] Atherosclerosis, a progressively evolving disease characterized by the accumulation of lipids and fibrous elements in large arteries, constitutes the single most important contributor to this growing burden of CVD. Atherosclerosis involves three main processes: oxidation, inflammation, and hypercholesterolemia/hyperlipidemia.[2,3] Oxidative stress and reactive oxygen species (ROS) generation is considered a pivotal risk factor because it induces endothelial dysfunction and modification of low-density lipoprotein (LDL).[4] Moreover, a plethora of proinflammatory factors, such as cyclooxygenases (COX-1 and COX-2), lipoxygenases (LO), vascular cell adhesion molecule-1 (VCAM-1), and monocyte chemotactic protein-1 (MCP-1), interfere significantly with the onset and progress of atherosclerosis.[5,6]

The multifactorial nature of atherosclerosis renders its treatment difficult. Indeed, a plethora of studies, using antioxidants, anti-iflammatory, and cholesterol-lowering agents, have been performed with the aim of reducing atherosclerosis in both animal models and human trials. Antioxidants such as vitamin C, vitamin E, and β-carotene were successful in animal studies, although they were not conclusive in human clinical studies.[7,8] The same holds for anti-inflammatory treatment, as the overall effect and safety of various known NSAIDs (e.g., naproxen or indomethacin) on cardiovascular risk outcome may vary.[9,10] Current management of atherosclerosis focuses on reducing lipid blood levels mainly by interfering with cholesterol biosynthesis. To date, HMG-CoA reductase inhibitors (i.e., statins) are the most effective class of therapeutics in this regard. However, by inhibiting HMG-CoA, statins suppress the production of mevalonate, which is not only a precursor of cholesterol but also of other nonsterol molecules that have a vital role in diverse cellular functions. Thus, the inhibition of their synthesis has been associated with the major side effects of statins, such as hepatotoxicity and myotoxicity.[11,12] Squalene synthase (SQS), a key enzyme in the cholesterol biosynthetic pathway, occupies the first solely committed step toward the biosynthesis of the sterol nucleus of cholesterol. Because of its strategic location in this pathway, it is an attractive target for inhibition and for the development of novel, improved antihypercholesterolemic agents that may leave other nonsterol products of mevalonate metabolism unaffected.[13,14] Although the first SQS inhibitor that reached phase III clinical trials (TAK-475) was discontinued because of safety concerns at high doses, it is unclear if the toxicity of TAK-475 is a structure/metabolite- or mechanism-related effect. Furthermore, several studies on the discovery of novel SQS inhibitors have been published during the last couple of years, pointing out the intense research interest that exists in this scientific field.[15−19]

On the basis of the underlying pathophysiological processes of this disease, we have previously developed multifunctional antihyperlipidemic and antioxidant agents based on the (octahydro)benzoxazine (**A**), benzoxazine (**B**), and benzothiazine (**C**) structures (Scheme 1).[20−22] Here, aiming to enhance the antioxidant activity by increasing free-radical-scavenging

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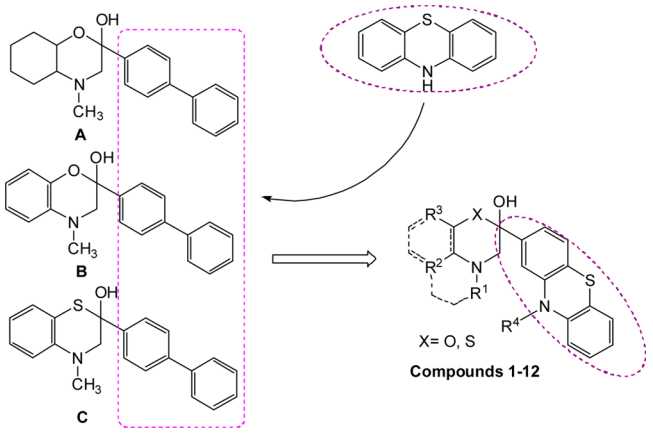
Scheme 1. Lead Structures and Design of Target Molecules

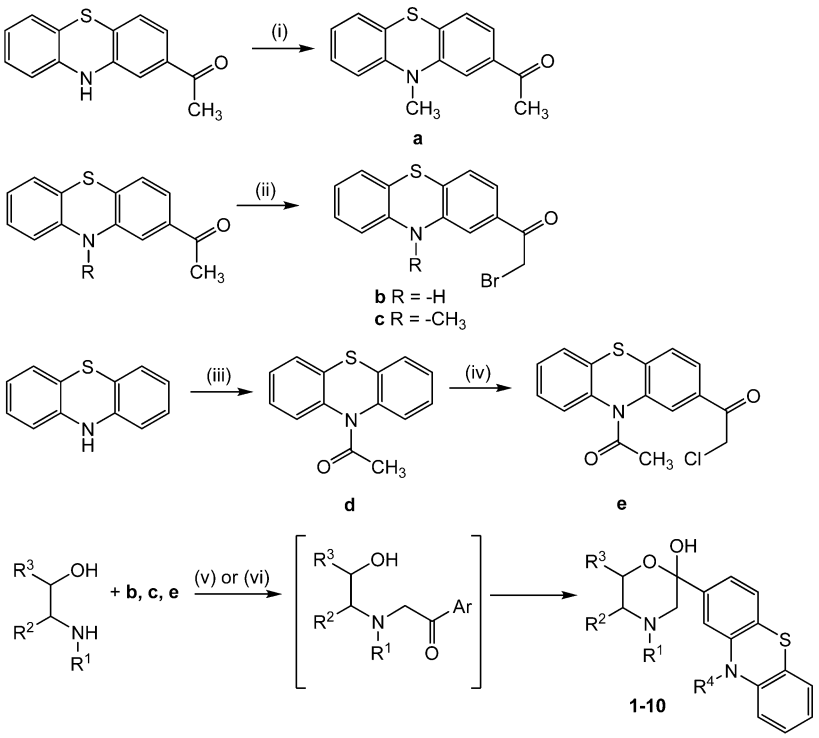


properties, the phenothiazine moiety was incorporated as the aromatic substituent into the structure of lead compounds **A**–**C**. Many phenothiazine derivatives have been described in the literature, with various activities (e.g., antihistaminic, anti-Parkinson's, or antischizophrenia) depending on the substitution of the phenothiazine ring structure.[23] Additionally, several phenothiazine derivatives have been synthesized and proven to be very potent antioxidants, free-radical scavengers, and cytoprotective agents.[24] The hereby synthesized novel phenothiazine-morpholine (compounds **1**–**9**), phenothiazine-benzoxazine (compound **10**), and phenothiazine-benzothiazine (compound **11**) derivatives were pharmacologically evaluated in vitro and in vivo for their effect on several mechanisms involved in the development of atherosclerosis, such as hyperlipidemia, oxidative stress, and inflammation.

2. RESULTS AND DISCUSSION

2.1. Chemistry. Target compounds **1**–**10** (Scheme 2) were synthesized in mostly good yields by the reaction of the appropriate aminoalcohol (2-methylaminoethanol, 2-piperidinemethanol, 2-methylaminocyclohexanol, or 2-methylaminophenol) with 2-bromoacetyl-phenothiazine **b** (for compounds **1**–**3** and **10**), 2-bromoacetyl-10-methyl-phenothiazine **c** (for compounds **4**–**6**), and 10-acetyl-2-chloroacetyl-phenothiazine **e** (for compounds **7**–**9**). The general reaction takes place via the spontaneous cyclization of the corresponding hydroxyaminoketone intermediate to a hemiketal structure (Scheme 2).

Scheme 2. Synthetic Route for Compounds 1–10[a]



1. $R^1 = CH_3$, R^2, R^3, $R^4 = H$
2. $R^1R^2 = (CH_2)_4$, R^3, $R^4 = H$
3. $R^1 = CH_3$, $R^2R^3 = (CH_2)_4$, $R^4 = H$
4. $R^1 = CH_3$, R^2, $R^3 = H$, $R^4 = CH_3$
5. $R^1R^2 = (CH_2)_4$, $R^3 = H$, $R^4 = CH_3$
6. $R^1 = CH_3$, $R^2R^3 = (CH_2)_4$, $R^4 = CH_3$
7. $R^1 = CH_3$, R^2, $R^3 = H$, $R^4 = COCH_3$
8. $R^1R^2 = (CH_2)_4$, $R^3 = H$, $R^4 = COCH_3$
9. $R^1 = CH_3$, $R^2R^3 = (CH_2)_4$, $R^4 = COCH_3$
10. $R^1 = CH_3$, $R^2R^3 =$ =CH-CH=CH-CH=, $R^4 = H$

[a]Reagents and conditions: (i) (a) *t*-BuOK, DMF, rt, (b) CH_3I, rt; (ii) phenyl trimethylammonium tribromide (PTT), THF/H_2O, rt; (iii) acetic anhydride, 120 °C; (iv) chloroacetyl chloride, $AlCl_3$, CS_2, reflux; (v) (a) acetone/ether, rt, (b) 10% HBr/ether (for compounds **1**–**9**); (vi) $NaHCO_3$, DMF, rt (for compound **10**).

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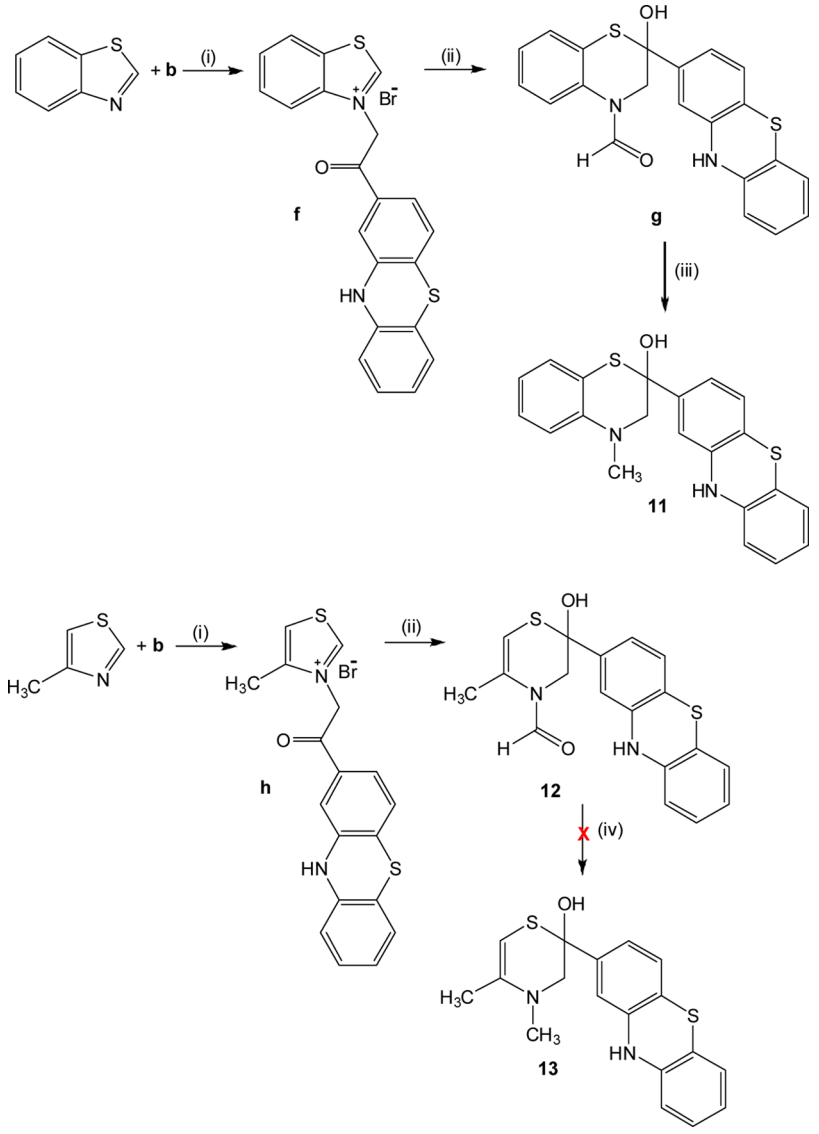
Scheme 3. Synthetic Route for Compounds 11 and 12[a]



[a]Reagents and conditions: (i) abs EtOH, 100−110 °C; (ii) 1% NaOH(aq), MeOH, Ar, rt; (iii) BH_3/THF, THF, Ar, rt; (iv) $LiAlH_4$ or BH_3/THF or catecholeborane.

As verified by spectroscopic and theoretical studies, the fused piperidino-oxazine (compounds **2**, **5**, and **8**) and octahydro-oxazine (compounds **3**, **6**, and **9**) ring systems adopt a chair−chair (trans) conformation.[21] Furthermore, the phenothiazine substituent, on the morpholine or octahydropiperidino-oxazine or octahydrobenzoxazine ring, prefers the equatorial position, as expected, because of the bulk of this substituent compared to the OH (which is at the axial position). The latter is further supported by the observed deshielding (in the ^{1}H NMR spectrum) of the axial H (compared to the equatorial H) of the CH_2 next to the ring O (compounds **1**, **2**, **4**, **5**, **7**, and **8**); this takes place only when the axial H is able to interact in space with the oxygen of the OH group that is at an axial (and not at an equatorial) position.[21]

Synthesis of benzothiazine derivative **11** was carried out by a different procedure with fewer synthetic steps and better yields than those described previously for the synthesis of lead compound **C**.[22] Specifically, the reaction of benzothiazole with 2-bromoacetyl-phenothiazine (**b**) at 110 °C gave the respective tertiary benzothiazolium bromide salt (Scheme 3, compound **f**), which, upon treatment with an aqueous solution of sodium hydroxide, is hydrolyzed and converted[25] to the 4-formyl-2-aryl-benzothiazin-2-ol derivative (Scheme 3, compound **g**). Subsequent reduction with borane/tetrahydrofuran complex (BH_3/THF) yielded target compound **11**. NMR spectra indicated that compound **11** remains in the thiohemiketal form in solution, with no traceable amounts of the open ketothiol form (Scheme 4A). This is seen by (a) the appearance in the ^{1}H NMR spectrum of two doublets with similar J coupling constants (d, 3.11 ppm J = 11.74 Hz and d, 3.41 ppm J = 11.93 Hz) for the two H atoms (axial and equatorial) of position 3 of the benzothiazine moiety (in the case of the corresponding ketothiol, these two H atoms would appear as a relatively more deshielded singlet) and (b) the appearance in the ^{13}C NMR spectrum of the 2-C of benzothiazine at 81 ppm and the lack of a corresponding carbonyl-C peak of the potential ketothiol form (Scheme 4A).

Our efforts for the synthesis of **13** were unsuccessful. Although the first and second synthetic steps were similar to those described above (Scheme 3, compounds **h** and **12**), the reduction

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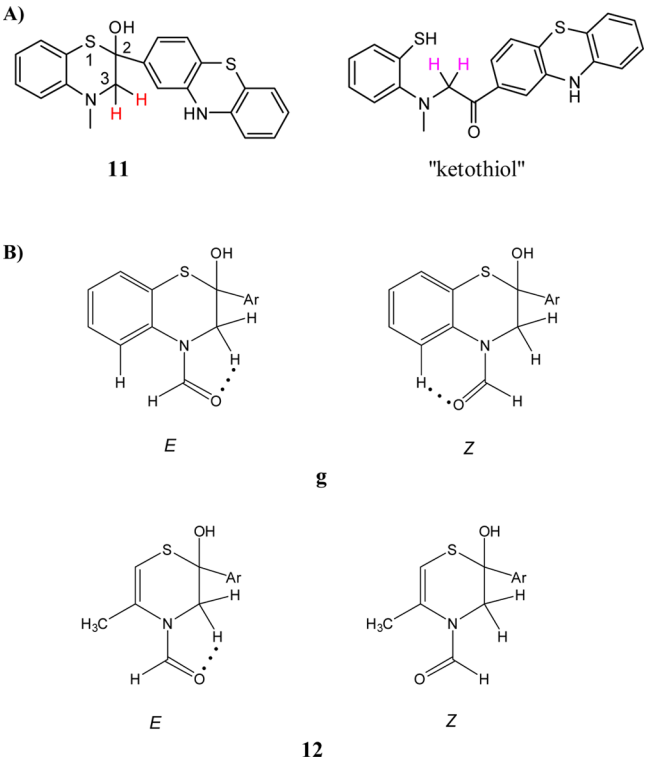
Scheme 4. Representation of (A) Compound 11 (Thiohemiketal) and Its Potential Open or Ketothiol Form and (B) the Two Rotamers (E and Z Conformations) of Compounds g and 12



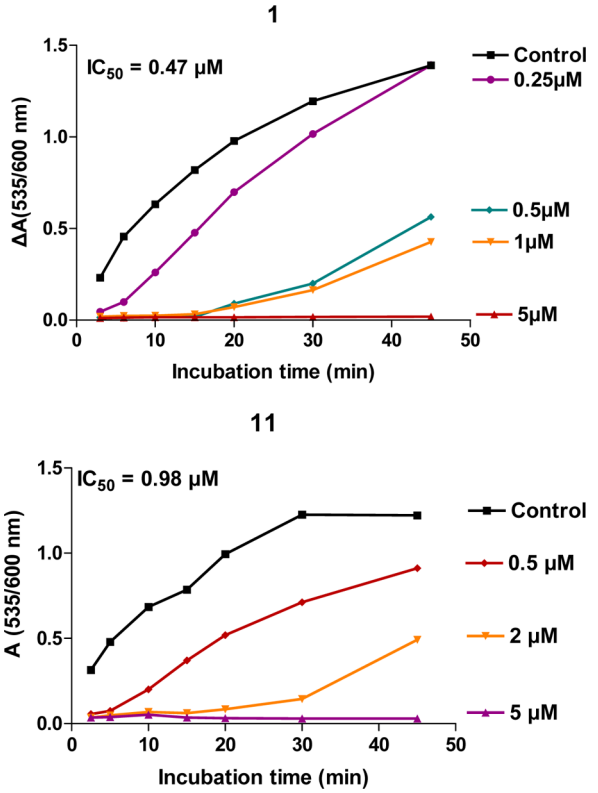


Figure 1. Time course of lipid peroxidation as affected by various concentrations of representative compounds **1** and **11**.

of the amide bond of compound **12** did not proceed as expected; the reaction of compound **12** with BH_3/THF at different temperatures led to the reduction of the ring double bond, whereas reaction with lithiumaluminumhydride ($LiAlH_4$) at different proportions and temperatures led to the opening of the thiohemiketal ring (aminoketothiol formation), and reaction with catecholborane gave compounds other than those desired.

For compound **g** and **12**, ^1H and ^{13}C NMR spectra show the presence of two rotamers; because of the hindered free rotation around the amide bond, compounds **g** and **12** exist predominantly in two conformations (E and Z), as shown in Scheme 4B. As observed by the respective ^1H NMR spectra, although E is the predominant conformation of both compounds, the ratio of E/Z is lower for compound **g** (ratio E/Z = 3:1) compared to that of compound **12** (ratio E/Z = 6:1), possibly because of more efficient intramolecular interactions of the respective Z conformation of compound **g** compared to those of the Z conformation of compound **12** (Scheme 4B).

2.2. Antioxidant Activity. *2.2.1. In Vitro Effect on Lipid Peroxidation.* All compounds were evaluated for their protective effect against lipid peroxidation of rat liver microsomal membranes. The time course of nonenzymatic lipid peroxidation, as affected by compounds **1** and **11**, is represented in Figure 1. IC_{50} values after 45 min of incubation for **1**−**12** are reported in Table 1. The new phenothiazine-morpholine derivatives **1**−**3** exhibited potent antioxidant activity, inhibiting lipid peroxidation with IC_{50} values of 0.47, 0.55, and 0.93 μM, respectively. The mechanism by which the phenothiazine moiety contributes to this potent antioxidant activity is thought to proceed through abstraction of its N−H hydrogen after a free-radical attack and subsequent formation of a radical centered on phenothiazine's nitrogen. An efficient stabilization of this radical may occur via the large conjugative system formed by the change in phenothiazine's conformation from the so-called butterfly to a planar conformation, as indicated by theoretical studies (Figure 2).[26] Compounds **10**−**12** also provoked strong inhibition of lipid peroxidation, with IC_{50} values of 1.6, 0.98, and 0.33 μM, respectively.

Substitution on the nitrogen of the phenothiazine moiety with a methyl group (compounds **4**−**6**) or an acetyl group (compounds **7**−**9**) led to a significant decrease in antioxidant activity. Compounds **4**−**6** inhibited lipid peroxidation with IC_{50} values of 70, 68, and 57 μM, respectively, whereas the IC_{50} values of compounds **7**−**9** are 36, 12, and 14 μM, respectively. The reduced activity of compounds **4**−**9** compared to compounds **1**−**3** or **10**−**12** may be attributed to the lack of phenothiazine's N−H hydrogen, which may lead to other (than the above-mentioned) mechanisms such as the abstraction of the hemiketalic H atom or the hemiketalic OH group after free-radical attack and subsequent radical stabilization via the aromatic system of phenothiazine (Figure 2). Under the same experimental conditions, lead compounds **A**−**C** and unsubstituted phenothiazine itself exhibited IC_{50} values of 450, 9, 83, and 0.35 μM, respectively, whereas known antioxidants probucol and quercetin exhibited IC_{50} values of >1 mM and 20 μM.[22] The above results reveal that compounds **1**−**3** and **10**−**12** are among the most potent antioxidants that have been assayed against microsomal lipid peroxidation as seen in the literature. This enhanced antioxidant effect is mostly attributed to the incorporation of the phenothiazine moiety.

2.2.2. Interaction with the Stable Free Radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The ability of compounds **1**−**12** to interact with the stable free radical DPPH was evaluated

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Table 1. In Vitro Inhibitory Effect on Lipid Peroxidation (LP), DPPH, SQS, COX-1/2, and Antihyperlipidemic Activity of 1−12 and Reference Compounds

compound	inhibition of LP IC_{50} (μM)	DPPH-interaction IC_{50} (μM)	inhibition of SQS IC_{50} (μM)	inhibition of COX-1 (% at 20 μM)	inhibition of COX-2 (% at 20 μM)	percent decrease compared to hyperlipidemic controls (56 μmol/kg) TC	LDL-C	TG	ClogP
1	0.47	191	5.0	92	75	70[d]	80[d]	94[d]	2.61
2	0.55	184	2.7	55	13	36[c]	58[d]	63[c]	3.41
3	0.93	188	11.9	55	29	50[d]	68[d]	77[d]	4.04
4	70.2	>400	3.6	17	96	[a]	[a]	[a]	3.18
5	68	>400	2.3	[a]	100	55[d]	26[c]	71[d]	3.98
6	57	>400	4.7	[a]	100	26[b]	34[c]	27[c]	4.61
7	36	>400	25.3	[a]	96	63[d]	48[d]	76[d]	1.15
8	12	>400	16.4	[a]	100	[a]	[a]	[a]	1.95
9	14	>400	1.6	[a]	[a]	[a]	[a]	[a]	2.58
10	1.6	337	3.3	[a]	[a]	[a]	[a]	[a]	3.95
11	0.98	77	3.6	67	100	[a]	[a]	[a]	4.69
12	0.33	150	>50	[a]	[a]	[a]	[a]	[a]	3.17
A	450	>400	36	[a]	[a]	54[d]	51[c]	49[d]	4.24
B	9	119	12.8	36	100	62[d]	74[c]	73[d]	4.87
C	83	271	16.5	90	100	58[d]	26[b]	38[c]	5.16
simvastatin	[a]	[a]	[a]	[a]	[a]	75[d]	70[d]	0	4.48
probucol	>1000	234	[a]	[a]	[a]	18[c]	18[e]	11[e]	10.75
quercetin	20	96	[a]	[a]	[a]	[a]	[a]	[a]	1.50
nimesulide	[a]	[a]	[a]	[a]	100	[a]	[a]	[a]	3.21
diclofenac	[a]	[a]	[a]	[a]	100	[a]	[a]	[a]	4.73
indomethacin	[a]	[a]	[a]	100	80	[a]	[a]	[a]	4.18
naproxen	[a]	[a]	[a]	30	55	[a]	[a]	[a]	2.82

[a]Not tested. [b]$p < 0.05$. [c]$p < 0.01$. [d]$p < 0.005$. [e]Not significant.

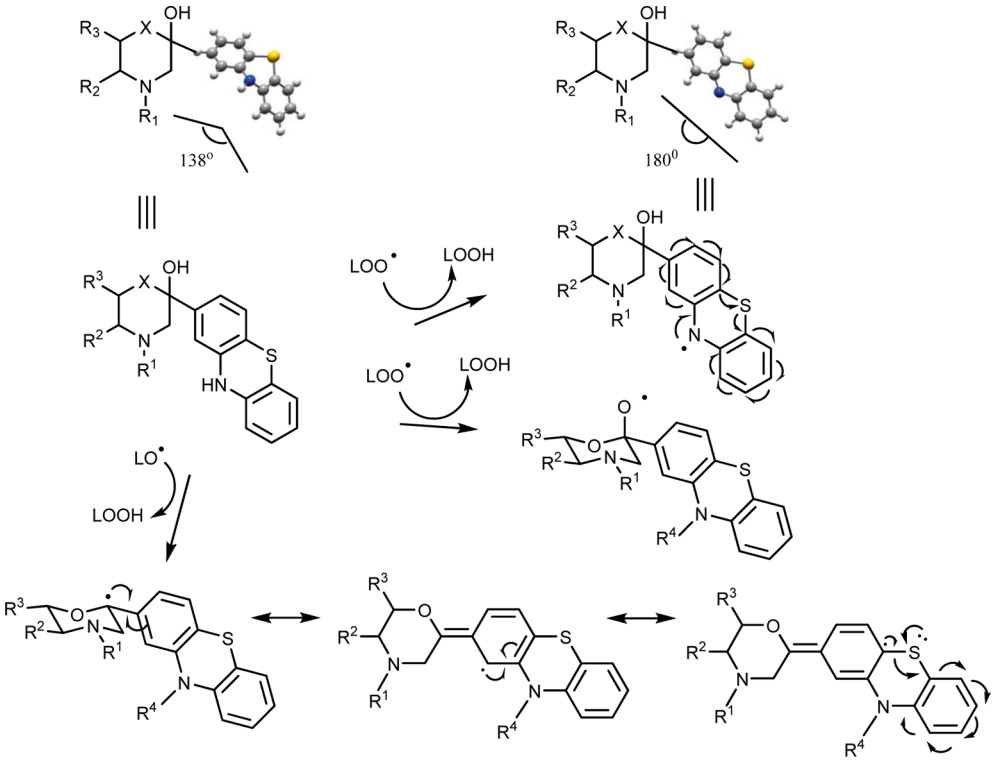

Figure 2. Representation of the butterfly conformation of the phenothiazine ring as a substituent of compounds **1−12**, and the corresponding planar conformation that is adopted when this conjugative system stabilizes a free radical: potential stabilization of an N-centered free radical of N-unsubstituted derivatives (compounds **1−3** and **10−12**) or an O- or C-centered free radical of N-substituted phenothiazine derivatives (compounds **4−9**).

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in vitro to explore the importance of some structural features in antioxidant/radical-scavenging activity. This ability to interact with DPPH is considered an indicator of reducing potential and free-radical-scavenging properties. Interaction of compounds **3** and **11** with DPPH is depicted in Figure 3, and IC_{50} values

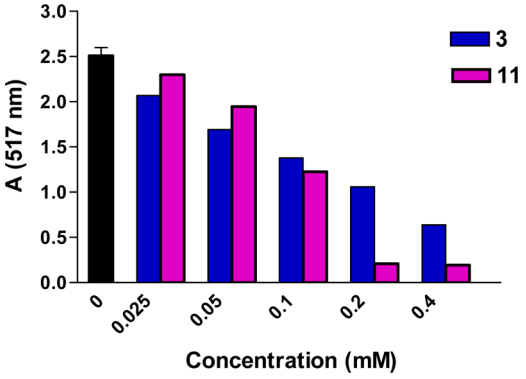

Figure 3. Interaction with DPPH (0.4 mM) of various concentrations (0.025−0.400 mM) of representative compounds **3** and **11** after 30 min of incubation.

(at 30 min of incubation) of all tested compounds are reported in Table 1. Compounds **1**−**3**, **10**, **11**, and **12** showed a very efficient and rapid (within 15 min) interaction with high-concentration DPPH (400 μM) and exhibited a very potent ability to interact, scavenge, and neutralize free radicals at relatively low concentrations (up to 50 μM). IC_{50} values (at 30 min) for **1**, **2**, **3**, **10**, **11**, and **12** are 191, 184, 188, 337, 77, and 150 μM, respectively. In contrast, activity disappears with the substitution of phenothiazine's nitrogen with methyl (compounds **4**−**6**) or acetyl (compounds **7**−**9**, Table 1). This exemplifies the decisive significance of phenothiazine's N−H for the exhibition of increased antioxidant activity, as also mentioned above in the activity against lipid peroxidation. Under the same experimental conditions, compound **A** was inactive, and compounds **B** and **C** interacted significantly with DPPH (IC_{50} values of 119 and 271 μM, respectively), whereas probucol's and quercetin's IC_{50} values are 234 and 96 μM, respectively. From these results, it is concluded that the free-radical-scavenging properties of compounds **1**−**3** are mostly attributed to the phenothiazine's structure (because they have very similar activity), whereas the activity of compounds **11** and **12** may be due to a synergistic effect between the two lead pharmacophores: the 2-hydroxy-(benzo)thiazine structure and phenothiazine.

2.2.3. Effect on LDL Oxidation. Because the oxidation of LDL renders it highly atherogenic, the effect of two active derivatives, **2** and **3**, was further investigated on Cu^{2+}-mediated oxidation of human LDL. The increase in conjugate diene formation, characteristic of LDL oxidation, was monitored spectrophotometrically at 234 nm. The antioxidant effect of compounds on LDL oxidation is expressed as the elongation of the lag time compared to control. Figure 4 depicts LDL oxidation in the presence of compounds **2** and **3**, showing a concentration-dependent effect in prolonging the control lag time. Specifically, as reported in Table 2, compound **2** prolonged the control lag time (113 min) to 152 min (35% increase, Δt = 39 min) at 0.25 μM and to 203 min (80% increase, Δt = 90 min) at 0.50 μM, whereas in the presence of 1 μM of **2**, the lag time was too prolonged to be recorded. Compound **3** prolonged the control lag time (122 min) to 200 min (64% increase, Δt = 78 min) at 0.50 μM, whereas in the presence of 1 μM of **3**, LDL oxidation

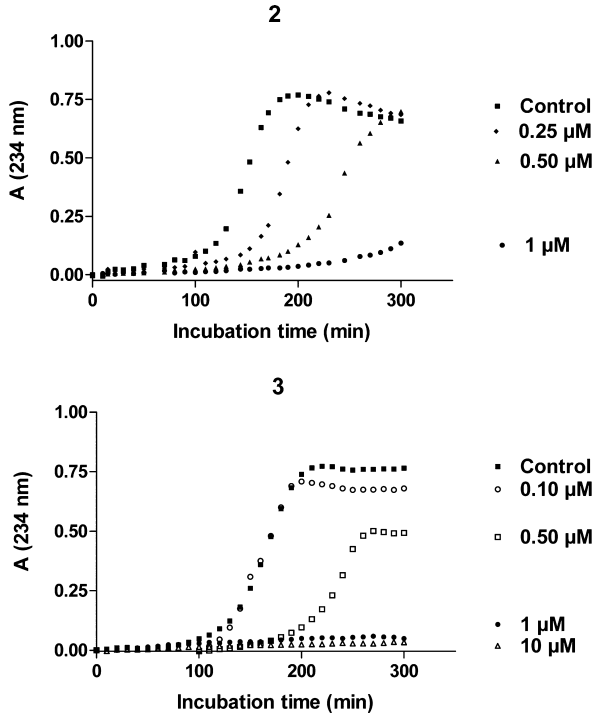

Figure 4. Effect of various concentrations of compounds **2** and **3** on the time course of in vitro Cu^{2+}-induced oxidation of LDL.

was entirely inhibited. Compounds **2** and **3** also significantly decreased the rate of oxidation (i.e., the rate of conjugate diene formation) at 0.5 μM by 20 and 41%, respectively (Table 2). Under the same experimental conditions, lead compounds **A** (at 10 μM) and **B** (at 5 μM) increased the lag time by 65 and 34% and decreased the rate of conjugate formation by 7 and 34%, respectively,[22] whereas probucol at 5 μM increased the control lag time by 22%.[27] These results identify compounds **2** and **3** as very strong inhibitors of LDL oxidation, a property that seems to be due to the additional incorporation of the phenothiazine moiety, favoring an increased interaction with LDL particles.

2.3. In Vitro Effect on Squalene Synthase Activity. Inhibition of the activity of squalene synthase (SQS), from rat liver microsomes, by the test compounds, expressed as IC_{50} values, is shown in Table 1. Compounds **1**−**11** decreased SQS activity significantly and dose-dependently, as representatively shown in Figure 5, which depicts the activity of compounds **5** and **11**. It seems that replacement of the biphenyl moiety of reference compounds **A**−**C** with that of phenothiazine leads to a 3- to 11-fold higher SQS inhibitory activity. The IC_{50} values, for example, of **3**, **10**, and **11**, are 11.9, 3.3, and 3.6 μM, respectively, whereas those of corresponding lead compounds **A**−**C** are 36, 12.8, and 16.5 μM under the same experimental conditions.[21,22] Methylation of phenothiazine's nitrogen (compounds **4**−**6**) preserves or marginally increases the inhibitory activity on SQS, whereas acetylation (compounds **7**−**9**) leads (with the exception of **9**) to relatively decreased activity that is comparable to that of the reference compounds (Table 1).

2.4. Anti-Inflammatory Activity. *2.4.1. In Vitro Cyclooxygenase (COX-1 and COX-2) and Lipoxygenase Inhibitory Activity.* Upregulation of cycloxygenases is considered, among others, to be responsible for the inflammatory processes that take place during atheroma formation, whereas lipoxygenases can oxidize LDL particles, rendering them noxious.[5,6] Furthermore, several structurally related morpholine derivatives have been

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Table 2. Effect of 2 and 3 on the Lag Time and Rate of Conjugate Diene Formation in Cu^{2+}-Induced Lipid Peroxidation of LDL

compound	concentration (μM)	lag time (min)	Δt (min)	percent increase in lag time	rate of conjugate diene formation (nmol/min)/mg protein	percent decrease in oxidation or rate of conjugate diene formation
2	0	113			6.96	
	0.25	152	39	35	7.32	0
	0.50	203	90	80	5.54	20
	1	[a]		100		100
	0	122			6.10	
3	0.10	109	−13	0	6.25	0
	0.50	200	78	64	3.57	41
	1	[a]		100		100

[a] 100% inhibition of conjugate diene formation.

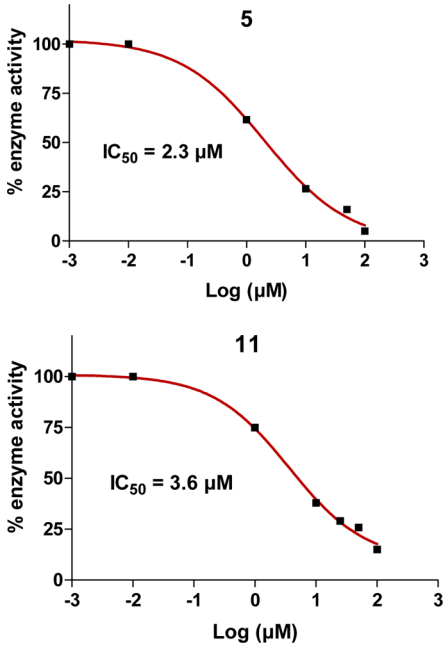

Figure 5. Representative graphs showing the in vitro activity of squalene synthase as affected by various concentrations of compounds **5** and **11**.

shown to have anti-inflammatory activity.[21] Thus, we screened our compounds (at 20 μM) for inhibitory activity against COX-1, COX-2, and soybean lipoxygenase. Compounds **1** and **11** showed very good inhibition of both COX isoforms, whereas other compounds inhibited COX-1 or COX-2 more selectively (Table 1). In the presence of 20 μM compound **1**, COX-1 and COX-2 activities were inhibited by 92 and 75%, respectively, and by 67 and 100% in the presence of 20 μM **11**. Methylation or acetylation of phenothiazine's nitrogen led to a significant increase in COX-2 inhibitory activity (compounds **4**−**8**) in relation to compounds **1**−**3**; compounds **4**−**8** inhibited COX-2 by 96−100%, whereas compounds **1**−**3** inhibited COX-2 by 75, 13, and 29%, respectively. Under the same experimental conditions and concentration (20 μM), indomethacin and naproxen, used as reference compounds, inhibited COX-1 by 100 and 30% and COX-2 by 80 and 55%, respectively, whereas nimesulide and diclofenac inhibited COX-2 by 100%. The phenothiazine moiety seems to contribute to the significant COX-1 and/or COX-2 inhibitory activity of these compounds, as this has been observed also with several other phenothiazine derivatives reported in the literature.[29]

Soybean lipoxygenase is inhibited by nonsteroidal anti-inflammatory drugs in a similar manner to that of the rat mast cell lipoxygenase and is often used as a reliable means for identifying lipoxygenase inhibitors.[30] Thus, the effect of compounds **1**−**9** on lipoxygenase activity was also investigated. However, none of the compounds that we evaluated showed any significant activity up to a concentration of 100 μM. Under the same experimental conditions, naproxen inhibited soybean lipoxygenase, with an IC_{50} value of 24 μM.

2.4.2. In Vivo Anti-Inflammatory Activity. Compound **3**, one of the most active agents of this series, was further evaluated for its in vivo anti-inflammatory activity by the method of carrageenan-induced paw edema, a nonspecific inflammation maintained by the release of histamine and serotonin and later by prostaglandins.[31] The examined compound was administered i.p. at a dose of 300 μmol/kg immediately after the injection of carrageenan. Compound **3** showed a good anti-inflammatory activity, decreasing edema by 41%. Despite the significant COX-1 inhibition offered by compound **3**, its potent antioxidant activity may also contribute to its anti-inflammatory effect because it is known that free radicals act synergistically in vivo in the catalyzed COX-mediated formation of prostaglandins from arachidonic acid. This activity was comparable to that of naproxen and indomethacin, which, under the same experimental conditions and dose, decreased edema formation by 51 and 54%, respectively.

2.5. In Vivo Antidyslipidemic Activity. The antidyslipidemic activity of selected compounds was evaluated after their administration at a single i.p. dose of 56 μmol/kg (to allow comparison with related compounds of previous studies) to the experimentally induced hyperlipidemic rat model (after Triton WR1339 administration). Results, presented in Table 1, show a remarkable in vivo antidyslipidemic activity of compounds **1**−**3**, reducing total cholesterol (TC), LDL, and triglyceride (TG) levels of hyperlipidemic rats by 36−94%. Compound **1** displayed the best activity, decreasing the above lipidemic parameters by 70, 80, and 94%, respectively. Methylation of phenothiazine's nitrogen preserved (compound **5**) or fairly decreased (compound **6**) the antidyslipidemic activity (as compared to their structural analogues **2** and **3**), whereas acetylation (compound **7**) offered a significant reduction of lipidemic parameters, although this reduction was less compared to compound **1**. Under the same experimental conditions and at the same dose, reference compounds **A**−**C** reduced plasma TC by 54, 62, and 58%, LDL by 51, 74, and 26%, and TG by 49, 73, and 38%, respectively. In addition, probucol and simvastatin offered a reduction of TC levels by 18 and 75%, LDL levels by 18 and 70%, and TG levels by 11 and 0%, respectively.[21,22] Overall, compounds **1**−**3** and **7** had a remarkable effect on all lipidemic indices, which was comparable or even greater than that of lead compounds **A**−**C**, probucol, and simvastatin.

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It has been shown that SQS inhibitors, such as the compounds of this study, decrease circulating cholesterol (and LDL) by an increased expression of hepatic LDL receptors in a manner similar to statins.[32] However, unlike statins, they reduce plasma TG through a LDL receptor-independent mechanism. Specifically, SQS inhibitors interfere with TG biosynthesis, suppressing it through the farnesol pathway.[33] Thus, SQS inhibitors (such as **1**−**3**, **5**−**7**, and **11**) most probably exhibit their antihypercholesterolemic/antihyperlipidemic effects through the above-mentioned mechanisms. Furthermore, long-term administration of statins has been shown to be associated with the escape phenomenon in which plasma cholesterol levels of human subjects are initially reduced but subsequently return to pretreatment levels. The mechanism by which statins induce the escape phenomenon has been a subject of considerable research and is believed to involve the inhibition not only of cholesterol but also of other mevalonate derivatives, such as farnesol.[34,35] In contrast, it has been observed that SQS inhibitors do not provoke such an escape phenomenon.[35]

2.6. Antidyslipidemic Effect of Compounds 1, 3, and 11 on Mice Fed a High-Fat Diet (HFD). *2.6.1. Effect of Compound 3 on Mice Fed a HFD (35.5% w/w fat).* The antidyslipidemic effect of compound **3** was evaluated in male SKH-2 mice after high-fat-diet (HFD) administration (35.5% w/w fat) for 28 days. The consumption of HFD increased total cholesterol (TC) and LDL levels by ca. 140 (111 mg/dL) and 120% (57 mg/dL), respectively, at the end of the experiment, whereas HDL and triglyceride (TG) levels were relatively unchanged. Long-term administration of compound **3** (twice daily at a dose of 56 μmol/kg for 28 days) significantly decreased TC by 54% and LDL by 76%, leaving HDL and TG unaffected (Figure 6).

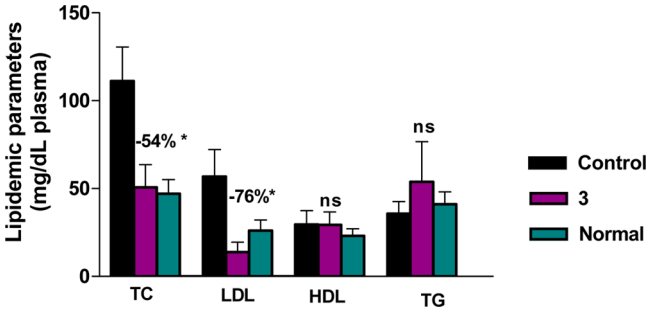

Figure 6. Effect of compound **3** on lipidemic parameters of mice fed with a high-fat diet (35.5% w/w fat). Significant difference compared to the respective control value is indicated by *, $p < 0.05$. ns, not significant.

Interestingly, the HDL/LDL ratio (considered an antiatherogenic index), was significantly higher in the group treated with compound **3** (1.7) compared to control group (0.5). Furthermore, compound **3** indicated a very good antioxidant activity in vivo, reducing plasma malonodialdehyde (MDA) levels by 38%, as determined after 28 days (Figure 7). Body weight, food intake, and glucose levels (data not shown) were not found to differ significantly among the HFD groups, and no macroscopic toxicity was observed after the long-term administration of **3**.

2.6.2. Effect of Compounds 1 and 11 on Mice Fed a HFD (2.5% w/w Cholesterol, 0.5% w/w Cholic Acid, and 10% w/w Peanut Oil). The antidyslipidemic effect of compounds **1** and **11** was evaluated in male SKH-2 mice administered HFD, which consisted of 2.5% w/w cholesterol, 0.5% w/w cholic acid, and 10% w/w peanut oil, for 30 days. This modification in HFD has been shown to induce a larger increase in lipidemic parameters[36]

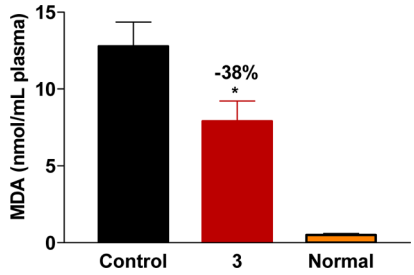

Figure 7. Effect of compound **3** on plasma MDA levels of mice fed with a high-fat diet (35.5% w/w fat). Significant difference compared to the respective control value is indicated by *, $p < 0.05$.

as compared to the above-mentioned type of HFD (35.5% w/w fat) that did not produce severe hypercholesterolemia/hyperlipidemia in mice after 28 days. The administration of this cholesterol-rich HFD to mice for 30 days significantly increased plasma TC (up to 270%) and LDL levels (up to 770%) relative to their initial levels. The initial and final TC, LDL, and HDL levels of control groups and groups treated with compounds **1** and **11** are presented in Figure 8. Long-term administration of compound **1** led to a 45% decrease in TC levels and a 58% decrease in LDL levels after 21 days of administration, reaching a decrease of 53 and 76%, respectively, at the end of the experiment (30 days) compared to the control group. Compound **1** also provoked a very significant increase of 120% in HDL levels at the end of the experiment (Figure 8A), whereas no effect was observed on TG levels (data not shown). Regarding the activity of compound **11**, TC and LDL levels were remarkably decreased after its prolonged administration (by 48 and 80%, respectively), whereas a significant increase (32%) of HDL levels compared to control group (Figure 8B) was also observed. Furthermore, after 30 days of administration, compound **11** provoked an increase of HDL levels by 100% compared to initial levels (Figure 8B). Compounds **1** and **11** did not affect the feeding pattern of the animals, and no significant differences in body weight and glucose levels (data not shown) were observed between control and treatment groups through the end of the experiment. Additionally, in both groups treated with **1** or **11**, the HDL/LDL ratio was significantly higher in relation to the control groups (1.2 for group treated with compound **1** and 1.3 for the compound **11**-treated group against 0.2 for control group).

The results from the long-term experiments confirm the potent antihyperlipidemic activity of compounds **1**, **3**, and **11**. Compounds **1** and **3** also showed potent antihyperlipidemic activity in the hyperlipidemic rat experimental model after Triton WR 1339 administration (Table 1). The mechanism of their antihyperlipidemic effect is considered to proceed via squalene synthase inhibition.[13,14,21,28] Furthermore, compounds **1** and **11** elevated HDL levels significantly. This trend has not been observed in morpholine and benzothiazine derivatives with other aromatic substituents (unpublished data). HDL leads cholesterol out of the tissue through a reverse cholesterol pathway and thus protects against atherosclerosis. However, the mechanism by which compounds **1** and **11** increase plasma HDL deserves further investigation. It has been observed that ApoA-I is decreased in patients with treatment-resistant schizophrenia, whereas it is significantly increased in rats treated with chloropromazine (an antipsychotic phenothiazine derivative); elevation of HDL levels has been considered as a possible alternative therapeutic target for schizophrenia treatment.[37]

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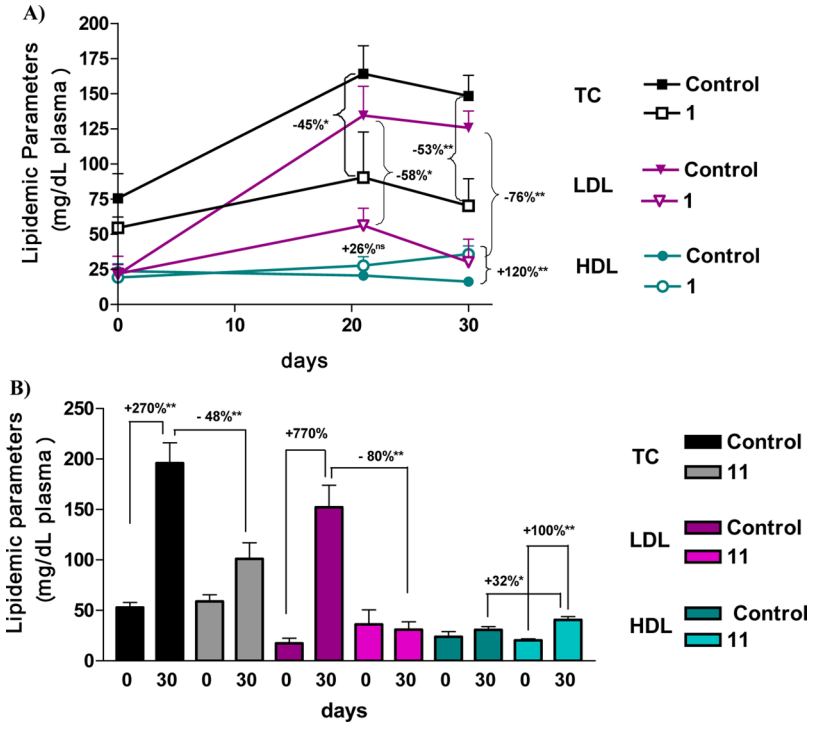

Figure 8. Decrease of lipidemic parameters by compound **1** (**A**) and **11** (**B**) (at the end of the experiment, 30 days, compared to the initial levels, 0 days) in mice fed a high-fat diet (standard chow enriched with 2.5% w/w cholesterol, 0.5% w/w cholic acid, and 10% w/w peanut oil). Significant difference compared to the respective control value is indicated by *, $p < 0.05$ and **, $p < 0.005$. ns, not significant.

ApoA-I is synthesized in the liver and small intestine and participates, among others, in the reverse transport of cholesterol incorporated in HDL particles. Moreover, ApoA-I acts as a cofactor for the lecithin cholesterol acyltransferase (LCAT), an enzyme that participates in the reverse cholesterol transport pathway.[38] Thus, the phenothiazine moiety of **1** and **11** may be postulated to be responsible for interaction with other targets that are implicated in HDL metabolism.

In conclusion, novel multitargeted compounds able to act against the multifactorial nature of atherosclerosis are presented. Most compounds showed very promising in vitro results against oxidative stress (lipid peroxidation, LDL oxidation, and free-radical scavenging), inflammation (COX-1 and/or COX-2 inhibition), and hypercholesterolemia/hyperlipidemia (SQS inhibition). These activities were confirmed in vivo as well. It seems that the incorporation of phenothiazine and its strong antioxidant/free-radical-scavenging properties into lead compounds **A**–**C** significantly enhanced and broadened their pharmacological profile. Furthermore, it is worth mentioning that multitarget compound **1** has been evaluated for its anti-atherosclerotic activity after long-term administration (3.5 months) in apoE$^{-/-}$ transgenic mice, showing a significant decrease of primary atherosclerotic lesions while at the same time not showing any toxicity, as reflected by the measurement of several liver enzymes (unpublished data). To the best of our knowledge, this is the first time that simple molecules with combined SQS/COX inhibitory activities also display at the same time such a multifaceted pharmacological profile against atherosclerosis.

3. EXPERIMENTAL SECTION

3.1. Materials. All commercially available chemicals were of the appropriate purity and purchased from standard sources. For the in vivo experiments, Wistar male rats (200–250 g) and SKH-2 male mice (25–30 g) were used. Animals were kept in a temperature-controlled 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 digital Electrothermal IA 9000 series apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with Bruker Avance DRX 400 (400 MHz) and DPX 200 (200 MHz) spectrometers, respectively. Elemental analysis was used to determine the purity of new compounds, confirming ≥95% purity. Elemental analyses were performed by the Service Central de Microanalyse, France (analysis of C and H).

*2-Acetyl-10-methyl-phenothiazine (**a**).* To a solution of 2-acetyl-phenothiazine (4.97 mmol) in anhydrous DMF (12 mL) was added 7.46 mmol of *t*-BuOK in portions, and the mixture was stirred for 3 h at room temperature. Then, 14.91 mmol of CH_3I was added dropwise, and the mixture was stirred overnight. After the addition of water, the mixture was extracted with ethyl acetate, and the organic phase was washed with a saturated aqueous solution of NaCl (brine), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:6) to give **a** (60%) as a yellow solid. mp 70–71 °C (68.5 °C).[39] ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.50 (s, 3H, $COCH_3$), 3.33 (s, 3H, N-CH_3), 6.75 (d, J = 8.22 Hz, 1H, 9-H phenothiazine), 6.88 (dt, J_1 = 1.87 Hz, J_2 = 7.63 Hz, 1H, 8-H phenothiazine), 7.04 (dd, J_1 = 1.36 Hz, J_2 = 7.63 Hz, 1H, 6-H phenothiazine), 7.09–7.14 (m, 2H, 4,7-H phenothiazine), 7.29 (s, 1H, 1-H phenothiazine), 7.41 (dd, J_1 = 1.67 Hz, J_2 = 7.93 Hz, 1H, 3-H phenothiazine).

*General Procedure for the Preparation of Bromoacetyl Derivatives **b** and **c**.* To a solution of the acetyl derivative 2-acetyl-phenothiazine or 2-acetyl-10-methyl-phenothiazine (**a**) (5 mmol) in 30 mL of anhydrous THF was added phenyltrimethylammonium tribromide (PTT) (5 mmol). The mixture was stirred at room temperature for 1.5–24 h, and then cold water was added. The crystalline precipitate was filtered, washed with water, dried, and subjected to flash column chromatography on silica gel to give the desired product.

*2-Bromoacetyl-phenothiazine (**b**).* Purified by flash column chromatography on silica gel (dichloromethane/petroleum ether 5:2) to give **b** (55%) as a red solid. mp 189–192 °C (188–190 °C).[40] ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.72 (s, 2H, -CH_2), 6.58 (d, J = 7.33 Hz, 1H, 8-H phenothiazine), 6.84 (d, J = 6.31 Hz, 1H, 5-H phenothiazine),

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6.93 (t, J = 7.47 Hz, 1H, 6-H phenothiazine), 6.99 (t, J = 7.51 Hz, 1H, 7-H phenothiazine), 7.07−7.09 (m, 2H, 1,4-H phenothiazine), 7.33 (d, J = 7.94 Hz, 1H, 3-H phenothiazine), 8.74 (brs, 1H, -NH-).

*2-Bromoacetyl-10-methyl-phenothiazine (**c**).* Purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:6) to give **c** (56%) as a yellow semisolid.[41] ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.32 (s, 3H, N-CH_3), 4.33 (s, 2H, -CH_2-), 6.75 (d, J = 8.22 Hz, 1H, 9-H phenothiazine), 6.88 (dt, J_1 = 1.18 Hz, J_2 = 7.63 Hz, 1H, 8-H phenothiazine), 7.04 (dd, J_1 = 1.56 Hz, J_2 = 7.63 Hz, 1H, 6-H phenothiazine), 7.09−7.15 (m, 2H, 4,7-H phenothiazine), 7.28 (ds, J = 1.76 Hz, 1H, 1-H phenothiazine), 7.40 (dd, J_1 = 1.67 Hz, J_2 = 7.83 Hz, 1H, 3-H phenothiazine).

*10-Acetyl-phenothiazine (**d**).* A mixture of 15.05 mmol of phenothiazine and 63.47 mmol acetic anhydride was heated at 120 °C for 4 h. The mixture was filtered at room temperature, washed with acetic acid, and dried to give compound **d** (94%) as a gray-green solid. mp 201−203 °C (198−200 °C).[42] ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.22 (s, 3H, -CH_3), 7.22−7.26 (m, 2H, 2,8-H phenothiazine), 7.34 (t, J = 7.45 Hz, 2H, 3,7-H phenothiazine), 7.45 (d, J = 7.67 Hz, 2H, 4,6-H phenothiazine), 7.51 (brd, J = 6.94 Hz, 2H, 1,9-H phenothiazine).

*10-Acetyl-2-chloroacetyl-phenothiazine (**e**).* A stirred solution of 12.43 mmol of 10-acetyl-phenothiazine (**d**) and 12.43 mmol of chloroacetylchloride in 50 mL of anhydrous CS_2 was treated at room temperature with 37.29 mmol of anhydrous $AlCl_3$. The mixture was stirred at room temperature for 2.5 h and at reflux for 3 h. The insoluble oily complex formed was separated and decomposed with acidified ice water. The precipitated solid was filtered and diluted in acetone, and the organic phase was dried over Na_2SO_4 and concentrated. The gray solid was recrystallized from EtOH to give **e** (85%) as a yellow solid. mp 168−169.5 °C (171−172.5 °C).[42] ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (s, 3H, -CH_3), 4.70 (s, 2H, -CH_2), 7.30 (dd, J_1= 1.28 Hz, J_2= 7.57 Hz, 1H, 9-H phenothiazine), 7.39 (dt, J_1= 1.46 Hz, J_2= 7.45 Hz, 1H, 7-H phenothiazine), 7.45−7.50 (m, 2H, 6,8-H phenothiazine), 7.55 (d, J = 8.18 Hz, 1H, 4-H phenothiazine), 7.83 (dd, J_1= 1.87 Hz, J_2= 8.23 Hz, 1H, 3-H phenothiazine), 8.12 (s, 1H, 1-H phenothiazine).

*General Procedure for the Preparation of (Benzo)thiazolium Salts **f** and **h**.* A mixture of benzothiazole or 4-methyl-thiazole (7.5 mmol), 2-bromoacetyl-phenothiazine (**b**) (5 mmol), and absolute EtOH (4 mL) was heated in an oil bath at 100−110 °C for 5−9 h. On completion of the reaction, the reaction mixture turned into a solid. Ethyl ether (50 mL) was then added, and the mixture was sonicated for 30 min. The solid was filtered, washed with ethyl ether, and dried.[43]

*3-(2-Phenothiazinyl-acyl)benzothiazolium Bromide (**f**).* Red solid (83%). mp 209−210 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.61 (s, 2H, -CH_2-), 6.67 (d, J = 7.83 Hz, 1H, 6-H phenothiazine), 6.77 (t, J = 7.24 Hz, 1H, 7-H phenothiazine), 6.91−7.05 (m, 2H, 8,9-H phenothiazine), 7.18−7.22 (m, 2H, 1,4-H phenothiazine), 7.51 (d, J = 8.22 Hz, 1H, 3-H phenothiazine), 7.82−7.93 (m, 2H, 5,6-H benzothiazole), 8.35 (d, J = 7.43 Hz, 1H, 7-H benzothiazole), 8.56 (d, J = 8.02 Hz, 1H, 4-H benzothiazole), 8.94 (s, 1H, -NH), 10.56 (s, 1H, 2-H benzothiazole). Anal. Calcd for $C_{21}H_{15}BrN_2OS_2$: C, 55.39; H, 3.32. Found: C, 55.70; H, 3.13.

*3-(2-Phenothiazinyl-acyl)-4-methylthiazolium Bromide (**h**).* Red solid (72%). mp 167−169 °C: ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.32 (s, 3H, -CH_3), 6.19 (s, 2H, -CH_2-), 6.52−6.56 (m, 1H, 9-H phenothiazine), 6.69 (t, J = 7.15 Hz, 1H, 7-H phenothiazine), 6.84 (d, J = 7.24 Hz, 1H, 6-H phenothiazine), 6.93 (t, J = 7.05 Hz, 1H, 8-H phenothiazine), 7.05−7.13 (m, 2H, 1,4-H phenothiazine), 7.37 (d, J = 7.04 Hz, 1H, 3-H phenothiazine), 8.00 (s, 1H, 5-H thiazole), 8.87 (s, 1H, -NH), 9.99 (s, 1H, 2-H thiazole). Anal. Calcd for $C_{21}H_{15}BrN_2OS_2$: C, 51.55; H, 3.61. Found: C, 51.80; H, 3.81.

*General Procedure for the Preparation of Compounds **g** and **12**.* To a solution of quaternary salts **f** and **h** (2 mmol) in MeOH (150 mL) was added an aqueous solution of 1% NaOH (2.2 mmol) under argon. The reaction mixture was then stirred at room temperature and under argon for 2 h. Cold water was added, and the formed solid was separated, washed with cold acetonitrile, and dried.[44]

*4-Formyl-2-(2-phenothiazinyl)-3,4-dihydro-2H-1,4-benzothiazin-2-ol (**g**).* Brown semisolid (87%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.30 (d, J = 13.30 Hz, 3/4 × 1H, 3-H banzothiazine), 3.47 (d, J = 13.31 Hz, 1/4 × 1H, 3-H benzothiazine)*, 3.87 (d, J = 13.31 Hz, 1/4 × 1H, 3-H benzothiazine)*, 4.44 (d, J = 12.91 Hz, 3/4 × 1H, 3-H benzothiazine), 6.50−6.69 (m, 2H, 1,3-H phenothiazine), 6.82−6.97 (m, 4H, 6,7,8,9-H phenothiazine), 7.04−7.14 (m, 4H, 4-H phenothiazine and 6,7,8-H benzothiazine), 7.31−7.35 (m, 1H, 5-H benzothiazine), 8.12 (s, 1/4 × 1H, -CHO)*, 8.63 (s, 3/4 × 1H, -NH), 8.70 (s, 3/4 × 1H, −CHO), 8.77 (s, 1/4 × 1H, -NH). Anal. Calcd for $C_{21}H_{16}BrN_2O_2S_2{\cdot}H_2O$: C, 61.44; H, 4.43. Found: C, 61.50; H, 4.42.

*4-Formyl-5-methyl-2-(2-phenothiazinyl)-2,3-dihydro-4H-1,4-thiazin-2-ol (**12**).* Orange solid (90%). mp 160−163 °C: ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 1.99 (s, 1/7 × 1H, -OH)*, 2.07 (s, 6/7 × 3H, -CH_3), 2.19 (s, 1/7 × 3H, -CH_3)*, 2.42 (s, 6/7 × 1H, -OH), 2.94 (d, J = 12.96 Hz, 6/7 × 1H, 3-H), 3.14 (d, J = 12.96 Hz, 1/7 × 1H, 3-H)*, 3.45 (d, J = 13.20 Hz, 1/7 × 1H, 3-H)*, 4.61 (d, J = 13.21 Hz, 6/7 × 1H, 3-H), 5.16 (s, 1/7 × 1H, 6-H)*, 5.25 (s, 6/7 × 1H, 6-H), 6.33−6.47 (m, 1H, 7-H phenothiazine), 6.61 (t, J = 7.58 Hz, 1H, 8-H phenothiazine), 6.71−6.86 (m, 5H, 1,3,4,6,9-H phenothiazine), 7.05 (s, 6/7 × 1H, -NH), 7.38 (s, 1/7 × 1H, -NH)*, 7.79 (s, 1/7 × 1H, -CHO)*, 8.53 (s, 6/7 × 1H, -CHO). ^{13}C NMR (400 MHz, DMSO-d_6): δ 25.64 (-CH_3), 26.31 (-CH_3)*, 54.58 (3-C thiazine), 60.61 (3-C thiazine)*, 83.66 (2-C thiazine), 85.45 (2-C thiazine)*, 105.00 (6-C thiazine), 111.30 (6-C thiazine)*, 117.22−147.99 (5-C thiazine, 5-C thiazine*, phenothiazine's carbons, phenothiazine's carbons*), 166.00 (-CHO), 168.71 (-CHO)*. Anal. Calcd for $C_{18}H_{16}N_2O_2S_2{\cdot}2H_2O$: C, 55.08; H, 5.15. Found: C, 55.50; H, 5.27.

*Because of the existence of two rotamers, two signals were observed in the NMR spectra of **g** and **12**.

*General Procedure for the Preparation of the Final Compounds **1**−**9**.* Final products **1**−**9** (Scheme 2) were obtained by the reaction of 2.2 mmol of 2-methylaminophenol or 2-piperidinemethanol or *trans*-2-methylaminocyclohexanol with 1.0 mmol of either 2-bromoacetyl-phenothiazine **b** (compounds **1**−**3**) or 2-bromoacetyl-10-methyl-phenothiazine **c** (compounds **4**−**6**) or 10-acetyl-2-chloroacetyl-phenothiazine **e** (compounds **7**−**9**) in anhydrous acetone (50 mL) at room temperature with stirring for 24 h. Acetone was then distilled off, ethyl ether was added to the residue, and the mixture was washed with water and saturated NaCl solution (brine), and dried over K_2CO_3. The products, compounds **1**−**9**, were isolated as hydrobomide salts and are racemic mixtures.[20,21]

*4-Methyl-2-(2-phenothiazinyl)-morpholin-2-ol Hydrobromide (**1**).* Red solid. Yield 44%. mp 136−140 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.42 (s, 3H, N-CH_3), 2.65 (s, 2H, 2 × 3-H), 2.77−3.00 (m, 2H, 5-H_{ax} and -OH), 3.54−3.68 (m, 1H, 5-H_{eq}), 3.86 (d, J = 11.94 Hz, 1H, 6-H_{eq}), 4.14 (t, J = 11.94 Hz, 1H, 6-H_{ax}), 6.61−6.69 (m, 3H, 1,3,7-H phenothiazine), 6.82−6.93 (m, 3H, 4,6,9-H phenothiazine), 7.02−7.25 (m, 1H, 8-H phenothiazine), 8.67 (s, 1H, -NH phenothiazine), 9.69 (brs, 1H, NH). ^{13}C NMR (200 MHz, DMSO-d_6): δ 43.55 (N−CH_3), 52.11 (5-C oxazine), 57.32 (6-C oxazine), 59.96 (3-C oxazine), 93.90 (2-C oxazine), 112.25 (9-C phenothiazine), 114.97 (4a-C phenothiazine), 119.60 (5a-C phenothiazine), 122.44 (3-C phenothiazine), 126.40 (1-C phenothiazine), 126.76 (6,8-C phenothiazine), 128.16 (7-C phenothiazine), 128.62 (4-C phenothiazine), 141.35 (2-C phenothiazine), 142.30 (10a-C phenothiazine), 142.40 (9a-C phenothiazine). Anal. Calcd for $C_{17}H_{19}BrN_2O_2S{\cdot}2.5H_2O$: C, 46.40; H, 4.26. Found: C, 46.36; H, 4.31.

*3-(2-Phenothiazinyl)-octahydro-1,4-pyrido[2,1-c]oxazin-3-ol Hydrobromide (**2**).* Yellow solid. Yield 47%. mp 128−132 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.30−1.44 (m, 3H, 8,9,7-H_{ax}), 1.55−1.78 (m, 5H, 7,8,9-H_{eq}, -OH, 6-H_{ax}), 2.98 (t, J = 10.76 Hz, 1H, 4-H_{ax}), 3.48−3.71 (m, 2H, 4-H_{eq} and 9a-H_{ax}), 3.82 (dd, J_1 = 2.92 Hz, J_2 = 12.22 Hz, 1H, 1-H_{eq}), 3.91 (m, 1H, 1-H_{ax}), 6.53−6.70 (m, 2H, 1,3-H phenothiazine), 6.83−6.91 (m, 3H, 4,6,7-H phenothiazine), 7.06−7.08 (m, 1H, 8-H phenothiazine), 7.32 (s, 1H, 9-H phenothiazine), 8.69 (s, 1H, -NH phenothiazine), 9.66 (brs, 1H, NH). Anal. Calcd for $C_{20}H_{23}BrN_2O_2S{\cdot}H_2O$: C, 52.98; H, 5.12. Found: C, 52.65; H, 5.00.

*4-Methyl-2-(2-phenothiazinyl)-octahydro-1,4-benzoxazin-2-ol Hydrobromide (**3**).* Yellow solid. Yield 68%. mp 169−171 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.23−1.38 (m, 4H, 5,8-H_{ax} *και* 2 × 7-H), 1.53−1.73 (m, 3H, 2 × 6-H and 8-H_{eq}), 1.81−1.90 (m, 1H, 5-H_{eq}), 2.14 (d, J = 11.00 Hz, 1H, 4a-H_{ax}), 2.65 (s, 3H, N-CH_3), 2.90 (brs, 1H, -OH), 3.03 (q, J = 21.16 Hz, 1H, 3-H_{ax}), 3.39 (d, J = 12.22 Hz, 1H, 3-H_{eq}),

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3.93−4.06 (m, 1H, 8a-H_{ax}), 6.61 (d, J = 7.83 Hz, 1H, 4-H phenothiazine), 6.67 (t, J = 7.34 Hz, 1H, 3-H phenothiazine), 6.84−6.92 (m, 4H, 6,7,8,9-H phenothiazine), 7.35 (s, 1H, 1-H phenothiazine), 8.71 (s, 1H, -NH phenothiazine), 9.84 (brs, 1H, NH). Anal. Calcd for $C_{21}H_{25}BrN_2O_2S·3H_2O$: C, 50.10; H, 5.00. Found: C, 50.16; H, 4.52.

*4-Methyl-2-(10-methyl-2-phenothiazinyl)-morpholin-2-ol Hydrobromide (**4**).* Green-yellow solid. Yield 51%. mp 183−185 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.73 (ds, J = 4.69 Hz, 3H, N-CH_3), 2.86−2.91 (m, 1H, 5-H_{ax}), 3.01−3.13 (m, 1H, 5-H_{eq}), 3.28 (s, 3H, N-CH_3 phenothiazine), 3.38−3.50 (m, 2H, 3-H_{ax} and -OH), 3.69−4.20 (m, 2H, 3-H_{eq} *και* 6-H_{eq}), 4.55−4.73 (m, 1H, 6-H_{ax}), 6.65−6.76 (m, 1H, 4-H phenothiazine), 6.80−6.89 (m, 1H, 3-H phenothiazine), 6.94−7.09 (m, 4H, 6,7,8,9-H phenothiazine), 7.20 (s, 1H, 1-H phenothiazine), 9.97 (brs, 1H, NH). Anal. Calcd for $C_{18}H_{21}BrN_2O_2S$: C, 52.81; H, 5.17. Found: C, 53.20; H, 5.26.

*3-(10-Methyl-2-phenothiazinyl)-octahydro-1,4-pyrido[2,1-c]-oxazin-3-ol Hydrobromide (**5**).* White solid. Yield 96%. mp 218.5−219.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.56−1.91 (m, 4H, 8,9-H_{ax} and 2 × 7-H), 1.94−2.07 (m, 2H, 8-H_{eq} and -OH), 2.32−2.43 (m, 1H, 9-H_{eq}), 2.60−2.71 (m, 1H, 6-H_{ax}), 2.79−2.85 (m, 1H, 6-H_{eq}), 3.16−3.24 (m, 1H, 9a-H_{ax}), 3.33 (s, 3H, N-CH_3), 3.40−3.43 (m, 1H, 4-H_{ax}), 3.82 (dd, J_1 = 3.13 Hz, J_2 = 13.30 Hz, 1H, 1-H_{eq}), 4.16 (d, J = 8.81 Hz, 1H, 4-H_{eq}), 4.44 (m, 1H, 1-H_{ax}), 6.77 (d, J = 8.02 Hz, 4-H, 1-H phenothiazine), 6.87 (dd, J_1 = 1.50 Hz, J_2 = 7.24 Hz, 1H, 3-H phenothiazine), 7.04−7.13 (m, 5H, 1,6,7,8,9-H phenothiazine), 11.41 (brs, 1H, NH). ^{13}C NMR (200 MHz, DMSO-d_6): δ 21.69 (8-C oxazine), 22.06 (7-C oxazine), 23.85 (9-C oxazine), 35.72 (N−CH_3), 53.79 (6-C oxazine), 59.61 (4-C oxazine), 61.20 (9a-C oxazine), 61.85 (1-C oxazine), 94.50 (3-C oxazine), 112.51 (9-C phenothiazine), 115.18 (4a,5a-C phenothiazine), 120.29 (3-C phenothiazine), 122.23 (1-C phenothiazine), 123.15 (7-C phenothiazine), 123.55 (6-C phenothiazine), 126.85 (8-C phenothiazine), 127.30 (4-C phenothiazine), 128.38 (2-C phenothiazine), 141.27 (10a-C phenothiazine), 145.58 (9a-C phenothiazine). Anal. Calcd for $C_{21}H_{25}BrN_2O_2S·0.25H_2O$: C, 55.56; H, 5.62. Found: C, 55.28; H, 5.73.

*4-Methyl-2-(10-methyl-2-phenothiazinyl)-octahydro-1,4-benzoxazin-2-ol Hydrobromide (**6**).* Yellow solid. Yield 74%. mp 149−150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28−1.46 (m, 4H, 5,8-H_{ax} and 2 × 7-H), 1.77−1.84 (m, 1H, 6-H_{ax}), 1.90−1.96 (m, 2H, 6-H_{eq} and -OH), 2.02−2.11 (m, 1H, 8-H_{eq}), 2.16−2.22 (m, 1H, 5-H_{eq}), 2.72 (s, 3H, N-CH_3), 2.87−2.96 (m, 1H, 4a-H_{ax}), 3.32 (s, 3H, N-CH_3 phenothiazine), 3.38−3.44 (m, 1H, 3-H_{ax}), 3.53 (d, J = 12.13 Hz, 1H, 3-H_{eq}), 4.41 (dt, J_1 = 4.31 Hz, J_2 = 9.59 Hz, 1H, 8a-H_{ax}), 6.77 (d, J = 8.22 Hz, 1-H phenothiazine), 6.84−6.89 (m, 1H, 3-H phenothiazine), 7.01−7.13 (m, 5H, 2,6,7,8,9-H phenothiazine), 11.48 (brs, 1H, NH). ^{13}C NMR (200 MHz, DMSO-d_6): δ 23.80 (6,7-C oxazine), 24.37 (5-C oxazine), 24.66 (8-C oxazine), 31.02 (N−CH_3), 35.69 (N−CH_3 phenothiazine), 61.21 (3-C oxazine), 66.17 (4a-C oxazine), 70.38 (8a-C oxazine), 94.12 (2-C oxazine), 112.51 (9-C phenothiazine), 115.21 (4a,5a-C phenothiazine), 120.27 (3-C phenothiazine), 122.26 (1-C phenothiazine), 123.16 (7-C phenothiazine), 123.61 (6-C phenothiazine), 126.85 (8-C phenothiazine), 127.31 (4-C phenothiazine), 128.38 (2-C phenothiazine), 141.27 (10a-C phenothiazine), 145.61 (9a-C phenothiazine). Anal. Calcd for $C_{22}H_{27}BrN_2O_2S·0.75H_2O$: C, 55.40; H, 5.71. Found: C, 55.25; H, 5.62.

*2-(10-Acetyl-2-phenothiazinyl)-4-methylmorpholin-2-ol Hydrobromide (**7**).* White solid. Yield 20%. mp 140−142 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.06 (s, 3H, CO-CH_3), 2.69 (s, 3H, N-CH_3), 2.87 (s, 1H, -OH), 3.03 (t, J = 10.47 Hz, 1H, 5-H_{ax}), 3.10−3.23 (m, 1H, 5-H_{eq}), 3.30 (d, J = 7.04 Hz, 2H, 2 × 3-H), 3.94 (dd, J_1 = 3.13 Hz, J_2 = 12.71 Hz, 1H, 6-H_{eq}), 4.19 (dt, J_1 = 2.36 Hz, J_2 = 11.74 Hz, 1H, 6-H_{ax}), 7.23−7.30 (m, 1H, 1-H phenothiazine), 7.34 (t, J = 8.02 Hz, 1H, 7-H phenothiazine), 7.39 (d, J = 8.60 Hz, 1H, 3-H phenothiazine), 7.50 (d, J = 7.63 Hz, 1H, 4-H phenothiazine), 7.55−7.62 (m, 2H, 6,8-H phenothiazine), 7.68 (s, 1H, 9-H phenothiazine), 9.84 (brs, 1H, NH). ^{13}C NMR (200 MHz, DMSO-d_6): δ 23.04 (CO**C**H_3), 43.42 (N−CH_3), 51.92 (5-C oxazine), 57.46 (6-C oxazine), 59.44 (3-C oxazine), 93.86 (2-C oxazine), 124.79 (1-C phenothiazine), 125.37 (3-C phenothiazine), 127.63 (7-C phenothiazine), 127.92 (6,9-C phenothiazine), 128.13 (8-C phenothiazine), 128.42 (4-C phenothiazine), 132.28 (10a-C phenothiazine), 133.28 (9a-C phenothiazine), 138.90 (2-C phenothiazine), 138.98 (4a-C phenothiazine), 140.97 (5a-C phenothiazine), 168.91 (**C**O−CH_3). Anal. Calcd for $C_{19}H_{21}BrN_2O_3S·0.25H_2O$: C, 51.64; H, 4.91. Found: C, 51.52; H, 4.65.

*3-(10-Acetyl-2-phenothiazinyl)-octahydro-1,4-pyrido[2,1-c]-oxazin-3-ol Hydrobromide (**8**).* White solid. Yield 40%. mp 136−139 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.30−1.51 (m, 3H, 7,8,9-H_{ax}), 1.57 (s, 1H, −OH), 1.64−1.95 (m, 5H, 7,8,9-H_{eq} and 2 × 6-H), 2.06 (s, 3H, CO-CH_3), 2.86−2.89 (m, 1H, 9a-H_{ax}), 3.11−3.20 (m, 1H, 4-H_{ax}), 3.46−3.73 (m, 2H, 4-H_{eq} and 1-H_{eq}), 3.84−3.97 (m, 1H, 1-H_{ax}), 7.25 (t, J = 7.53 Hz, 1H, 7-H phenothiazine), 7.34 (t, J = 7.44 Hz, 1H, 8-H phenothiazine), 7.41 (d, J = 8.03 Hz, 1H, 3-H phenothiazine), 7.50 (d, J = 6.82 Hz, 1H, 4-H phenothiazine), 7.56−7.59 (m, 2H, 6,9-H phenothiazine), 7.69 (s, 1H, 1-H phenothiazine), 9.74 (brs, 1H, NH). Anal. Calcd for $C_{22}H_{25}BrN_2O_3S·H_2O$: C, 53.33; H, 5.49. Found: C, 53.41; H, 5.61.

*2-(10-Acetyl-2-phenothiazinyl)-4-methyl-octahydro-1,4-benzoxazin-2-ol Hydrobromide (**9**).* White solid. Yield 40%. mp 118−121 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.24−1.39 (m, 4H, 5,8-H_{ax} and 2 × 7-H), 1.55−1.71 (m, 4H, 2 × 6-H and 5,8-H_{eq}), 1.85 (t, J = 12.91 Hz, 1H, 4a-H_{ax}), 2.06 (s, 3H, CO-CH_3), 2.50 (s, 1H, -OH), 2.67 (ds, J = 4.11 Hz, 3H, N-CH_3), 3.00−3.14 (m, 1H, 3-H_{ax}), 3.51−3.64 (m, 1H, 3-H_{eq}), 3.98−4.04 (m, 1H, 8a-H_{ax}), 7.24 (t, J = 7.54 Hz, 1H, 7-H phenothiazine), 7.33 (t, J = 7.63 Hz, 1H, 8-H phenothiazine), 7.41 (d, J = 8.02 Hz, 1H, 3-H phenothiazine), 7.50 (d, J = 6.46 Hz, 1H, 4-H phenothiazine), 7.55−7.60 (m, 2H, 6,9-H phenothiazine), 7.68 (s, 1H, 1-H phenothiazine), 9.92 (brs, 1H, NH). Anal. Calcd for $C_{23}H_{27}BrN_2O_3S$: C, 56.21; H, 5.54. Found: C, 56.55; H, 5.51.

*4-Methyl-2-(2-phenothiazinyl)-3,4-dihydro-2H-1,4-benzoxazin-2-ol (**10**).* To a solution of 2-methylaminophenol (2.03 mmol)[22] in dry DMF were added $NaHCO_3$ (2.13 mmol) and 2-bromoacetyl-phenothiazine **b** (2.23 mmol). After stirring at room temperature overnight, dichloromethane was added, and the mixture was washed with water and brine, dried, and concentrated in vacuum. The residue was purified by flash column chromatography on silica gel (ethyl acetate/petroleum ether 1:10) to give **10** (31%) as a deep red semisolid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.13 (s, 3H, N-CH_3), 3.46 (d, J = 11.74 Hz, 1H, 3-H benzoxazine), 3.65 (d, J = 11.93 Hz, 1H, 3-H benzoxazine), 4.11 (s, 1H, -OH), 5.61 (s, 1H, -NH), 6.66 (d, J = 7.63 Hz, 1H, 5-H benzoxazine), 6.84−6.90 (m, 2H, 1,3-H phenothiazine), 6.93 (d, J = 8.02 Hz, 1H, 4-H phenothiazine), 6.98−7.04 (m, 3H, 6,7,8-H benzoxazine), 6.08−7.16 (m, 4H, 6,7,8,9-H phenothiazine). Anal. Calcd for $C_{21}H_{18}N_2O_2S·H_2O$: C, 66.29; H, 5.31. Found: C, 66.36; H, 5.33.

*4-Methyl-2-(2-phenothiazinyl)-3,4-dihydro-2H-1,4-benzothiazin-2-ol (**11**).* Benzothiazine derivative **g** (1 mmol) was dissolved in anhydrous THF under argon, and BH_3/THF (1M, 7 mL) was added dropwise. After stirring at room temperature for 1 h, water was added, and the mixture was extracted with dichloromethane, washed with water and saturated $NaHCO_3$ solution, and dried, and the solvent was distilled off. The residue was purified by flash column chromatography on silica gel (dichloromethane/petroleum ether 1:4) to give **11** (63%) as a brown solid. mp 128.5−129.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.00 (s, 3H, N−CH_3), 3.11 (d, J = 11.74 Hz, 1H, 3-H benzothiazine), 3.41 (d, J = 11.93 Hz, 1H, 3-H benzothiazine), 3.87 (s, 1H, -OH), 5.81 (s, 1H, -NH), 6.46 (d, J = 7.63 Hz, 1H, 5-H benzothiazine), 6.74−6.80 (m, 2H, 1,3-H phenothiazine), 6.83 (d, J = 8.02 Hz, 1H, 4-H phenothiazine), 6.88−6.94 (m, 3H, 6,7,8-H benzothiazine), 6.98−7.06 (m, 4H, 6,7,8,9-H phenothiazine). ^{13}C NMR (200 MHz, $CDCl_3$): δ 40.74 (N-CH_3), 64.03 (3-C benzothiazine), 81.75 (2-C benzothiazine), 112.11 (5-C benzothiazine), 112.27 (4a-C phenothiazine), 113.39 (9-C phenothiazine), 114.53 (3-C phenothiazine), 115.58 (5a-C phenothiazine), 119.94 (1-C phenothiazine), 120.10 (7-C benzothiazine), 120.43 (8a-C benzothiazine), 122.74 (7-C phenothiazine), 125.43 (6-C benzothiazine), 126.63 (6-C phenothiazine), 126.72 (8-C phenothiazine), 126.82 (4-C phenothiazine), 127.46 (8-C benzothiazine), 129.02 (2-C phenothiazine), 130.08 (9a-C phenothiazine), 140.16 (10a-C phenothiazine), 143.18 (4a-C benzothiazine). Anal. Calcd for $C_{21}H_{18}N_2OS_2·CH_2Cl_2$: C, 56.35; H, 4.31. Found: C, 56.36; H, 4.08.

3.3. In Vitro Microsomal Lipid Peroxidation. Heat-inactivated hepatic microsomes from untreated rats were prepared as described

previously. The inhibitory effect of compounds on lipid peroxidation was assessed spectrophotometrically (535 against 600 nm) as TBAR material.[20−22]

3.4. In Vitro Interaction with the Stable Radical DPPH. Compounds, dissolved in absolute ethanol at concentrations of 10−400 μM, were added to an equal volume of an ethanolic solution of DPPH (final concentration 400 μM) at room temperature. Absorbance (517 nm) was recorded at different time intervals for 90 min.[45]

3.5. Isolation and in Vitro LDL Oxidation. Blood was collected from a normolipidemic human volunteer, and LDL was isolated by discontinuous density gradient (using KBr) ultracentrifugation according to previous studies.[22,46] LDL (56 μg of protein/mL) in PBS was incubated at 37 °C in the absence or presence of various concentrations of **2** and **3** (in 10% DMSO in H_2O). Oxidation was initiated by 10 μM $CuSO_4{\cdot}5H_2O$. Conjugated dienes were determined every 10 min for 5 h as the increase in absorbance at 234 nm and calculated using the extinction coefficient of 29 500.[22,27]

3.6. In Vitro Squalene Synthase Activity Assay. SQS activity was evaluated by determining the amount of [^{3}H]FPP converted to squalene by rat hepatic SQS as previously described.[21,22]

3.7. In Vitro Evaluation of Cycloxygenase Activity (COX-1 and COX-2). The effect of compounds on COX-1 and COX-2 activity was measured using bovine COX-1 and human recombinant COX-2 enzymes included in the COX inhibitor screening assay kit by Cayman Chemical Co. (Ann Arbor, MI, USA). The assay directly measures PGF_{2a} produced by $SnCl_2$ reduction of COX-derived PGH_2. The prostanoid product is quantified via enzyme immunoassay using a broadly specific antibody that binds to all major prostaglandin compounds.

The inhibitory activity of the test compounds (at a final concentration of 20 μM) was measured in the presence of 100 μM arachidonic acid (substrate) and 10^{-9} IU/mL of the respective COX isoform.

3.8. In Vitro Evaluation of Lipoxygenase Activity. Lipoxygenase activity was determined using soybean lipoxygenase (250 U/mL) and sodium linoleate (100 μM) as substrate in Tris-HCl buffer, pH 9.0.[30] The test compounds dissolved in 60% ethanol were added, and the reaction was monitored for 6 min at 28 °C, recording absorbance at 234 nm.

3.9. In Vivo Antidyslipidemic Activity.[20−22,47] An aqueous solution of Triton WR 1339 was given i.p. to rats (200 mg/kg), and 1 h later, the test compounds (56 μmol/kg), finely suspended or dissolved in saline with a few drops of Tween 80 (1−2%), or saline only were administered i.p. After 24 h, blood was collected 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. Levels of plasma lipids were determined in duplicate, and values presented are the mean from six to eight rats (per compound). All standard errors are within 10% of the respective reported values.

3.10. In Vivo Anti-Inflammatory Activity of Compound 3. An aqueous solution of carrageenan was prepared (2% w/v in physiological saline), and 0.05 mL of this was injected into the right hind paw of male mice, with the left paw serving as control. Compound **3** (dissolved in saline with a few drops of Tween 80) was given i.p. (0.30 mmol/kg) immediately after the carrageenan injection. After 3.5 h, the hind paws were excised, and the produced edema was estimated as the paw weight increase. This value is the mean of six rats, and the standard error is 8% of the respective reported value.[31]

3.11. Evaluation of Compound 3 in Mice Fed a High-Fat Diet (35.5% w/w fat). Twelve week old SKH-2 male mice were acclimated for 1 week and allowed water and normal rodent chow ad libitum. Subsequently, they were divided into three groups:

control group:	HFD
treatment group:	HFD + compound **3**
normal group:	normal diet

From day 1 to 28, mice in all groups were allowed access to their respective a high-fat diet (HFD) 35.5% w/w or a normal chow diet ad libitum. Compound **3** (or drug vehicle alone) was administered i.p. at a dose of 56 μmol/kg, twice a day, during the whole experimental period (28 days).

For lipid analysis, blood was collected on day 28 of the experiment. Total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride plasma levels were determined using commercially available kits. Plasma MDA content was estimated by measuring the complex of MDA with *N*-methyl-2-phenyl indole (586 nm).[48]

3.12. Chronic Cholesterol-Fed Mouse Model. The evaluation of the hypocholesterolemic/hypolipidemic activity of compounds **1** and **11** took place in two distinct experiments as follows: 12 week old SKH-2 male mice were acclimated for 1 week and allowed water and normal rodent chow ad libitum. Subsequently, they were divided into 2 groups:

control group:	HFD
treatment group:	HFD + compound **1** or **11**

From day 1 to day 30, mice in both groups received ad libitum a chow diet supplemented with cholesterol (2.5% w/w), cholic acid (0.5% w/w), and peanut oil (10% w/w). Compounds **1** or **11** (or drug vehicle alone) were administered i.p. at a dose of 56 μmol/kg, twice a day, during the whole experimental period (30 days) to the treatment groups.

For lipid analysis, blood was taken on day 30 of the experiment. Total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride plasma levels were determined using commercially available kits.[36]

3.13. Protein Determination. The protein content of microsomal and LDL fractions was determined according to Lowry's method.[49]

3.14. Statistical Analysis. Data are expressed as the mean ± SD. Where indicated, statistical comparisons were made using Student's *t* test, and a statistically significant difference was inferred if $p < 0.05$.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

CVD, cardiovascular disease; SQS, squalene synthase; HMGCo-A, 3-hydroxy-3-methylglutaryl-CoA; FPP, farnesyl pyrophosphate; PSPP, presqualene pyrophosphate; COX-1, cycloxygenase-1; COX-2, cycloxygenase-2; rt, room temperature; BSA, bovine serum albumin; NADPH, nicotinamide adenine dinucleotide phosphate; IC_{50}, inhibitory concentration for 50% of the reaction; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglyceride; MDA, malonodihaldehyde

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