Optimizing the Pharmacological Profile of New Bifunctional Antihyperlipidemic/Antioxidant Morpholine Derivatives

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Supporting Information

ABSTRACT: Among the causal risk factors directly promoting the development of coronary and peripheral atherosclerosis are reactive oxygen species and elevated low-density lipoprotein plasma levels. We hereby designed new potent squalene synthase (SQS) inhibitors that may simultaneously tackle the oxidative stress induced by lipid peroxidation. Using previously developed morpholine derivatives as a starting point, we conducted extensive structural changes by either substituting or modifying the morpholine ring, aiming at an optimal SQS-antioxidant pharmacological profile. Compounds 2, 3, and 7 emerged as the most potent bifunctional analogues, displaying IC₅₀ values for SQS inhibition of 0.014, 0.16, and 0.51 μM, respectively, and further significantly decreasing lipid peroxidation of hepatic microsomal membranes. The aforementioned activities were also confirmed *in vivo* since the most promising derivative 2 exhibited a remarkable antihyperlipidemic and antioxidant effect. In conclusion, rational drug design accompanied by structure–activity relationship studies led to compounds combining improved antioxidant and antihyperlipidemic activity that may serve as multifunctional agents against atherosclerosis.

KEYWORDS: multitarget design, synthesis, *in vitro*, *in vivo*

Accumulation of low-density lipoproteins (LDL) particles and reactive oxygen species (ROS) in the subendothelial space is one of the earliest events in atherosclerosis pathogenesis. A consequent increase in lipid peroxidation induces the formation of foam cells followed by a lipid streak and ultimately an atherosclerotic plaque.^{1,2} Despite the experimental evidence in animal models that administration of antioxidants (i.e., vitamins E and C, or probucol) can attenuate the progress of atherosclerosis, data derived from human trials are less consistent or uniform. Therefore, further studies for concluding the usefulness of antioxidants as antiatherosclerosis agents are needed.³

Currently, the first line of treatment for atherosclerosis is the administration of statins, which decrease blood lipids, especially LDL-cholesterol and total cholesterol, via inhibition of HMG-CoA reductase, the hepatic cholesterol-biosynthetic rate-limiting enzyme. However, despite their indisputable benefits, the treatment with statins are frequently associated with some very serious adverse effects, such as myotoxicity, muscle pain, hepatotoxicity, and in very rare cases rhabdomyolysis.^{4–6} In addition, when statins are administered in high doses, in high-risk patients, there is also an increased risk of diabetes mellitus type 2.^{7,8} These side effects might be caused in part by the inhibition of the biosynthesis of other isoprenoid-derived molecules, such as ubiquinone (CoQ) and dolichols, playing a vital role in a plethora of cellular

functions.^{9,10} Accordingly, an alternative antihyperlipidemic agent that does not inhibit isoprenoid biosynthesis would be desired in clinical practice. It is known that squalene synthase (SQS), an enzyme converting two molecules of farnesyl pyrophosphate (FPP) into a squalene, acts downstream of HMG-CoA reductase and catalyzes the first committed step of the cholesterol biosynthesis pathway. This implies that SQS does not interfere in the biosynthesis of several in-demand nonsteroidal isoprenoid molecules such as geranylgeranyl pyrophosphate. Therefore, SQS inhibitors could be useful as safer cholesterol-lowering agents.^{11,12}

From what has been mentioned above, it could be concluded that a monotherapy strategy against atherosclerosis might be inadequate if considering the multifactorial nature of atherosclerosis. Indeed, oxidative stress, hypercholesterolemia–hyperlipidemia, and inflammatory processes synergistically act on the arterial wall leading to a complex and quite resistant to treatment condition.^{13,14} In earlier studies, several compounds able to simultaneously address more than one pathogenic mechanisms implicated in the onset and progression of atherosclerosis were developed. In particular, several morpholine derivatives (Chart 1) have been designed

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Chart 1. Previously Designed Morpholine Derivatives with Multifunctional Activity against Atherosclerosis, and TAK-475, the First SQS Inhibitor Entered in Clinical Trials

and synthesized that are endowed with an expanded pharmacological profile (antioxidant, antihyperlipidemic, and anti-inflammatory activity as well). Accordingly, multifunctional analogues against metabolic syndrome have also been identified by our¹⁵ and other groups.¹⁶ Extended structure–activity relationships (SARs) have been explored for these molecules mainly regarding the aromatic substitution on the morpholine ring, which is essential for this multifaceted activity.^{17–20} As part of our structural optimization efforts we herein describe the design, synthesis, and pharmacological evaluation of novel (thio)morpholine derivatives (**1–14**, Chart 2) aiming to investigate the influence on antioxidant and SQS inhibitory activity of (a) the removal of the hemiketalic hydroxyl group, (b) the specific substituent of the hemiketalic hydroxyl group (with the subsequent formation of ketal structures), and (c) the replacement of the morpholine ring by its isostere thiomorpholine.

Chart 2. Design of Novel Compounds 1–14

■ DESIGN OF NOVEL COMPOUNDS-AIM

The hemiketalic morpholine derivatives **A**, **B**, and **C** were selected as starting templates for further structural modification. Compound **A** belongs to the first generation of this series of morpholine derivatives,²¹ while the choice of compounds **B** and **C** was based on the very potent antioxidant and SQS inhibitory activity they exhibited.¹⁸

Initially, the hemiketalic hydroxyl group of **A**, **B**, and **C** was substituted by the 3-thiopropoxy group (compounds **1–3**) aiming to increase antioxidant activity of the compounds, taking advantage of the potent reducing properties of the thiol group (Chart 2). Moreover, we assumed that more potent SQS inhibitors could also be achieved by this substitution due to the significant increase in lipophilicity (Table 1), which may be

Table 1. *In Vitro* Inhibitory Effect on LP and SQS, and CLogP Values of 1–14 and Reference Compounds

compd	LP, IC ₅₀ (μM)	SQS, IC ₅₀ (μM)	CLogP
1	372 ± 16	2.7 ± 0.3	4.45
2	198 ± 11	0.014 ± 0.002	5.63
3	147 ± 9	0.16 ± 0.02	5.44
4	333 ± 19	24.9 ± 1.1	3.33
5	259 ± 8	6.2 ± 0.3	4.5
6	211 ± 13	29.4 ± 1.9	4.31
7	75.8 ± 3.3	0.51 ± 0.05	3.04
8	45 ± 2	16.1 ± 1.3	2.85
9	86.8 ± 4.2	>100	3.61
10	179 ± 16	7.5 ± 0.8	4.41
11	218 ± 7	20.8 ± 0.6	5.05
12	65.1 ± 2.4	42 ± 2.9	5.56
13	299 ± 11	15 ± 1.0	4.35
14	178 ± 17	25.2 ± 1.4	3.63
A	300 ^b	33 ± 4	3.08
B	71.0 ± 1.5 ^c	1.9 ± 0.1	4.25
C	30 ± 2 ^c	6.9 ± 0.6 ^c	4.06
A1	~350 ^b	35 ± 0.4	3.88
A2	450 ^b	36 ± 2	4.51
Probucol	>500	<i>a</i>	10.75
Trolox	25 ± 1	<i>a</i>	3.09
TAK-475	<i>a</i>	0.078 ^d	3.81

^aNot tested. ^bTaken from ref 21. ^cTaken from ref 18. ^dTaken from ref 32.

favorable for interactions with SQS's active site that consists of a large lipophilic cavity.¹⁸ Compounds **4–6** were derived from the replacement of the thiol group of **1–3** with its isostere, a hydroxyl group, while compounds **7** and **8** were designed by inserting a NO-donor, i.e., a nitro-ester group, in reference compounds **B** and **C** (Chart 2). NO is known to have antioxidant, vasodilating, and antithrombotic properties well suited for antiatherosclerotic agents. The impact of the absence of the hemiketalic group on the activity of compounds **A**, **A1**, and **A2** was also investigated via the design of derivatives **9–12** (Chart 2). Finally, **13** and **14** were designed as isosteres of compound **9** (Chart 2). All derivatives were evaluated *in vitro* for their antioxidant and SQS inhibitory activity. In addition, the antihypercholesterolemic–antihyperlipidemic, antioxidant, and anti-inflammatory activity of the most promising analogue was assessed *in vivo* in order to evaluate the potential of this type of derivatives in complex and living organisms.

RESULTS AND DISCUSSION

Chemistry. The synthesis of the ketalic morpholine derivatives 1–8 is depicted in Scheme 1. The substituted

Scheme 1. Synthesis of Morpholine Ketalic Derivatives 1–8

hemiketalic morpholines **A**, **B**, and **C** were formed according to the procedures previously described.^{18,21,22} For the synthesis of the ketalic morpholine derivatives 1–3, reference compounds **A–C** initially reacted with 3-bromopropanol under acidic conditions to give the respective 2-(3-bromopropoxy) derivatives 15–17, which subsequently reacted with thioacetic acid to produce the 2-(3-acetylthiopropoxy) analogues 18–20. Hydrolysis of the thioester group of 18–20 with an aqueous KOH solution afforded the final products 1–3. The acid-catalyzed reaction of the hemiketals **A–C** with 1,3-propanediol directly afforded the corresponding 2-(3-hydroxypropoxy) derivatives 4–6, while nitric acid esters 7 and 8 were synthesized through the reaction of 16 and 17 and silver nitrate in acetonitrile according to the literature.²³

The synthesis of morpholine derivatives 9–12 is displayed in Scheme 2a. Starting from the hemiketalic morpholine derivative **A** and its analogues **A1** and **A2**, a reduction with sodium borohydride took place to afford mixtures of diastereomeric diols 21–23. The substituted morpholine ring (9–11) can then be formed by cyclization using sulfuric acid in dichloromethane. The absence of the hemiketalic hydroxyl group in these compounds was confirmed by the peak shift in ^{13}C NMR spectra of the initial quaternary hemiketalic-C of **A**, **A1**, and **A2** (~ 95 ppm) at higher shield range (~ 75 ppm), attributed to the tertiary-C of compounds 9–11. Compound 12 was formed by the dehydration of compound **A2** at high temperature (90°C). The formation of the double bond was confirmed by IR.

Finally, 13 and 14 were synthesized following the synthetic procedure depicted in Scheme 2b: the synthesis of thiomorpholine derivatives 28 and 29 was carried out by reaction of 2-aminoethanethiol hydrochloride with α -bromoarylacetate derivatives 26 and 27. Reduction of the lactams 28 and 29 with sodium borohydride/acetic acid in dioxane was employed to obtain the aminoborane complexes, which were subsequently hydrolyzed to thiomorpholine derivatives 30 and 31 with 10% HCl/MeOH . Methylation of 30 and 31 with either CH_3I or $\text{CH}_2\text{O}/\text{NaBH}_4$ gave the final products 13 and 14.²⁴ Compound 13 was isolated as a hydrobromide salt and 14 as a free base due to the high hygroscopicity that was noticed for its hydrobromide salt.

Scheme 2. Synthesis of Compounds (a) 9–12 and (b) 13–14

Biological Studies. Antioxidant Activity. In Vitro Lipid Peroxidation Inhibition. All final products (1–14) were assayed *in vitro* as inhibitors of nonenzymatic ferrous salt/ascorbate-induced lipid peroxidation of membrane lipids of rat hepatic microsomes. Reference compounds **A–C**, **A1**, **A2**, probucol, and trolox were also considered for reasons of comparison. The antioxidant effect of the investigated compounds, expressed as IC_{50} values after 45 min of incubation, is shown in Table 1. Furthermore, the time course of lipid peroxidation as affected by several concentrations of two of the most active compounds, 8 and 9, is presented in Figure A (Supporting Information). All examined derivatives showed fairly better antioxidant activity than known reference antioxidants including probucol ($\text{IC}_{50} > 500 \mu\text{M}$), while the antioxidant effect of 8 and 12 was comparable to that of the known antioxidant trolox ($\text{IC}_{50} = 25 \mu\text{M}$). It is observed, from the results shown in Table 1, that the substitution of **A–C** with a 3-thiopropoxy (1–3) or a 3-hydroxypropoxy (4–6) group led to a relative decrease in antioxidant activity, rendering the hemiketalic hydroxyl group on the morpholine ring as an important structural element for increased antioxidant activity and not particularly favorable to substitution. We propose that this may be due to the abstraction of the hemiketalic hydroxyl group after a radical attack, thus forming an intermediate free radical on the prehemiketalic carbon of the morpholine ring.^{19,20} This radical could subsequently be stabilized via resonance with the adjacent aromatic system (Figure B, Supporting Information). As a result, it may be that the substitution on the hemiketalic hydroxyl group may retard or even impede the abstraction of the ketalic moiety and subsequently the formation of the intermediate radical. The slight increase of the antioxidant activity showed by compounds 2 and 3 compared to that of 5 and 6 may be attributed to the better reducing properties of the thiol ($-\text{SH}$) in relation to the hydroxyl ($-\text{OH}$) group. However, the substitution of **B** and **C** with 3-nitroxypropoxy group (compounds 7 and 8) preserved their antioxidant activity,

while significantly increasing it when compared to the other ketalic derivatives 1–6. We presume that compounds 7 and 8 could act as NO-donors. This assumption is also supported by previous studies of structurally related 2-(3-nitroxypoxy) morpholine derivatives in which the *in situ* liberated NO and not the nitric ester group, under the same experimental conditions, was responsible for their antioxidant activity.²³ Consequently, 7 and 8 possibly undergo nonenzymatic hydrolysis in the biological medium of the experiment (hepatic microsomal membranes) giving NO and the corresponding 2-(3-hydroxypropoxy) derivatives 5 and 6, respectively. Thus, their increased antioxidant activity compared to the respective ketalic derivatives 1–6 could be attributed to the additive effect of the *in situ* liberated NO, which reinforces the antioxidant potential of 5 and 6. As anticipated, the positive resonance effect (+R) of the methoxy group contributes to the exhibition of a better antioxidant effect (compare 3, 6, and 8 with 2, 5, and 7).

Regarding the implication of the hemiketalic hydroxyl group of morpholine compounds A, A1, and A2 in the antioxidant activity, we observe that the absence of this group led to derivatives with 2- to 7-fold increased antioxidant activity (compounds 9–12). This difference was also observed in related 1,4-benzoxazine and 1,4-benzothiazine derivatives.²⁵ In this case, the hydrogen of the tertiary carbon next to oxygen of the morpholine ring, reminiscent of an allylic hydrogen, could be abstracted this time after radical attack. Further, this increase in activity might be also attributed to the higher lipophilicity (Table 1) achieved through this simple structural modification since the lipophilic character of antioxidant molecules is an important factor for protection against lipid peroxidation due to their optimum partition into lipid bilayers.²⁶ The replacement of the morpholine ring of one of the most active antioxidant molecules (compound 9, IC₅₀ = 86.8 μ M) with its isostere thiomorpholine ring (compound 13) decreased significantly the antioxidant activity by approximately 3.5 times (IC₅₀ value of 299 μ M for compound 13) consistent with previous results obtained for 1,4-benzoxazine and 1,4-benzothiazine derivatives.²⁵ Furthermore, replacement of the biphenyl group of 13 with the more condensed and planar moiety of naphthalene (compound 14) led to a relative increase of antioxidant activity (IC₅₀ = 178 μ M).

Finally, a study of 2/3D descriptors of these molecules (calculated by MOE software)²⁷ indicated a positive, linear relationship (r^2 = 0.89, RMSE = 0.05) between IC₅₀ values (expressed as pIC₅₀) of compounds 1–6 and their atomic polarizability (*apol* descriptor) values (Table A, Supporting Information). Polarizability is a parameter describing the charge distribution in molecules²⁸ and their solvation properties (which depend on solute/solvent interactions) and may also serve as an index of chemical reactivity. Altogether, these properties may explain further the antioxidant profile exhibited by these compounds.

Squalene Synthase Inhibitory Activity. All compounds were evaluated based on the inhibition offered against rat microsomal squalene synthase activity (Table 1). The inhibitory effect on enzyme activity was measured according to the method of Amin et al.,²⁹ while the inhibitory activity for two of the most active compounds, 2 and 7, is depicted in Figure C (Supporting Information). From the results presented in Table 1 it can be concluded that the substitution of the hemiketalic hydroxyl group of reference compounds A–

C with a 3-thiolpropoxy group led up to 136-fold higher squalene synthase inhibitory activity (IC₅₀ values of 1, 2, and 3 are 2.7, 0.014, and 0.16 μ M compared to 33, 1.9, and 6.9 μ M for compounds A, B, and C, respectively). This outstanding difference in activity may be attributed to the increase in lipophilicity offered by this substitution, favoring further interactions of 1–3 with the active site of SQS that consists of a very large lipophilic cavity.¹⁸ In fact, pIC₅₀ values (of 1–3, A–C, Table A, Supporting Information) correlate extensively (r^2 = 0.92) with ClogP values (Table 1). Replacement of the thiol group of 1–3 with its isostere, a hydroxyl group, notably decreased the inhibitory activity against SQS (IC₅₀ values of 4, 5, and 6 are 24.9, 6.2, and 29.4 μ M, respectively). Although pIC₅₀ values of all nine compounds (1–6 and A–C, Table A, Supporting Information) still correlate well (r^2 = 0.79) with ClogP values (Table 1), this striking difference in activity could emanate from the potential interaction between the thiol group of 1–3 and the two magnesium ions used by the enzyme as a cofactor. Aliphatic sulfhydryl groups are known to bind as thiolates to metal ions in the active site of enzymes,³⁰ while complexes with thiolates bridging two magnesium ions have been described previously.³¹ The respective interaction with the hydroxyl group of compounds 4–6 is anticipated to be much weaker.

Insertion of a NO-donor in compounds B and C either increased (compound 7, IC₅₀ = 0.51 μ M) or decreased (compound 8, IC₅₀ = 16.1 μ M) the inhibitory effect on SQS. Furthermore, compounds 7 and 8 were less active inhibitors compared to the 2-(3-thiolpropoxy)-compounds (2, 3) but more active compared to their respective 2-(3-hydroxypropoxy)-counterparts (5, 6).

Comparing the hemiketalic morpholine derivatives A, A1, and A2 with those lacking the hydroxyl group (compounds 9–11), we observe (Table 1) that this structural modification (the OH removal) led to a 2- and 5-fold higher SQS inhibitory activity (compounds 11 and 10, respectively), although compound 9 exhibited an IC₅₀ value of over 100 μ M. Furthermore, the inhibitory activity of compound 12 was decreased 2-fold compared to 11 and marginally (1.2-fold) compared to reference compound A2, indicating that a conformational alteration of the morpholine ring due to the presence of the double bond in compound 12 does not allow favorable interactions with the active site of SQS. An unexpected increase of the inhibitory effect against SQS activity was observed by replacing the morpholine ring of compound 9 with its isostere thiomorpholine (compound 13). Specifically, the very weak SQS inhibitor 9 (IC₅₀ > 100 μ M) was converted to a derivative with a significant inhibitory activity (compound 13, IC₅₀ = 15 μ M). Furthermore, the replacement of the biphenyl moiety in 13 by naphthalene (compound 14) more or less preserved this activity (IC₅₀ = 25.2 μ M).

Thus, most of the new compounds showed higher SQS inhibitory activity compared to reference compounds (A–C, A1, and A2), while three compounds (2, 3, and 7) exhibited a value comparable to the activity of TAK-475 (IC₅₀ = 0.078 μ M; Table 1, Chart 1), the first SQS inhibitor that entered in advanced clinical trials.³²

In Vivo Antihypercholesterolemic–Antihyperlipidemic, Antioxidant, and Anti-inflammatory Effect of Compound 2. The very promising *in vitro* pharmacological profile of compound 2 prompted us to evaluate further its pharmacological activity *in vivo*. Besides the antihypercholesterolemic–

antihyperlipidemic and antioxidant activity, its anti-inflammatory activity was also assessed in order to study the possible interference of compound **2** in inflammatory mechanisms taking place during atherosclerosis onset and development. With regard to compound **2**'s antihypercholesterolemic–antihyperlipidemic and antioxidant activity, severe acute experimental hyperlipidemia was induced in female C57BL/6 mice after the *ip* administration of tyloxapol (Triton WR 1339). Tyloxapol is a nonionic detergent of polymeric structure exhibiting a potent inhibitory activity against lipoprotein lipase³³ playing a critical role in breaking down TGs. It has been demonstrated that tyloxapol induces the liberation of cholesterol from the liver to the circulation, whereby the hepatic synthesis of cholesterol is triggered,³⁴ and therefore, it has been successfully used in several studies to induce hypercholesterolemia.^{19,23,25,35,36} The effect of compound **2** against the increase in TC and TG plasma levels induced by tyloxapol is displayed in Figure 1. The

Figure 1. Effect of compound **2** (84 μ mol/kg) on total cholesterol, triglyceride, and total antioxidant capacity (%TAC) levels of experimentally induced hyperlipidemic C57BL/6 mice, and % paw edema decrease (300 μ mol/kg). Statistically significant difference from control group: * $P < 0.05$, *** $P < 0.0001$.

administration of 300 mg/kg tyloxapol (Control group) led to a 2-fold increase in plasma TC levels and 18-fold increase in TG levels compared to normal (untreated) mice (normal group), 24 h after its administration. Of note, the *ip* administration of compound **2** (84 μ mol/kg) decreased significantly both lipidemic parameters by 67 and 25%, respectively, therefore confirming its involvement in lipid metabolism. Furthermore, **2** exhibited a similar antihypercholesterolemic and a better antihyperlipidemic activity than simvastatin, which in the same experimental animal protocol decreased TC blood levels of tyloxapol-induced hyperlipidemic rats by 70% without, however, affecting TG levels (0%).^{17–19,25} The outstanding *in vivo* antihyperlipidemic effect of **2** could be attributed to its potent inhibitory activity on SQS ($IC_{50} = 14$ nM). Additionally, since compound **2** exhibited a significant antioxidant activity against lipid peroxidation (Table 1) *in vitro*, its ability to increase the total antioxidant capacity (TAC) of blood was estimated in the same experimental hyperlipidemia animal model. TAC is a term used to describe the ability of all antioxidant species found in blood able to interact with (harmful) free radicals. As depicted in Figure 1,

the administration of tyloxapol (control group) did not affect the TAC of normal mice after 24 h. Notably, the %TAC of the group treated with **2** was significantly increased by 20% (Figure 1), thus highlighting the positive effect of **2** in increasing the antioxidant status of plasma after a single dose and only after 24 h of its administration. Specifically, this points out its capability to contribute to the increase of the reducing properties of antioxidants or electron-donating components circulating in blood. Accordingly, a higher increase of %TAC blood levels is anticipated by a more prolonged administration of **2**.

The *in vivo* anti-inflammatory activity of **2** was evaluated based on the % paw edema decrease offered after paw *id* administration of carrageenan. Carrageenan is an inflammatory agent inducing a nonspecific inflammation maintained initially by the release of histamine and serotonin and later by prostaglandins.³⁷ Figure 1 shows that a successfully established inflammation was achieved 3.5 h postadministration of carrageenan. However, **2** decreased the formed edema by 40% ($\pm 4\%$) reflecting the potential of compound **2** to interfere with inflammatory processes. This activity is similar to that of the known NSAID naproxen, which under the same experimental conditions decreased edema by 51% (results not shown). Since it has been demonstrated that inflammatory processes actively participate in atherosclerosis' onset and progression,¹⁴ this preliminary result of anti-inflammatory activity of **2** (its mechanism of action needs to be elucidated) adds to an interesting activity profile for **2** designed as a potential antiatherosclerosis agent.

Finally, these preliminary *in vivo* experiments did not clarify whether the *in vivo* activity of **2** is due to the thiol or any other (active) metabolite (such as disulfide, sulfonic acid, or sulfone) after its potential respective oxidation or metabolism. Although this remains to be further investigated in the future, the results of this *in vivo* study serve as a successful means of proof-of-principle as far as the potential use and therapeutic value of (at least one) of these derivatives.

CONCLUSION

As part of our optimization efforts to determine the appropriate structural features for optimal dual function/activity (antioxidant and SQS inhibitory activity) toward the multifactorial disease of atherosclerosis, we herein investigate novel morpholine and thiomorpholine analogues derived from structural modifications of previously described counterparts (A–C). Several very potent compounds were developed, and structure–activity relationships, regarding the inhibitory effect of substituted morpholine derivatives against SQS activity as well as antioxidant activity, were elucidated. Several compounds showed a very potent SQS inhibitory activity (at a low nanomolar range) maintaining at the same time significant antioxidant activity. Furthermore, the replacement of the hydroxyl group of **4–6** with its respective thiol (**1–3**) favors higher inhibitory activity against SQS, a fact that could possibly be ascribed to the efficiency of the thiol group to interact with the Mg^{2+} ions of the active site of SQS. One of the most active SQS inhibitors/antioxidants of this series, compound **2**, also exhibited a remarkable antihypercholesterolemic–antihyperlipidemic and antioxidant effect *in vivo*, substantiating the concept for multifunctional agents to combat multifactorial diseases such as atherosclerosis. The very important dual pharmacological profile of the novel derivatives renders them interesting multifunctional lead compounds for atherosclerosis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.8b00469.

Detailed experimental procedures, Table, and Figures (PDF)

NMR spectra of key compounds (PDF)

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The manuscript was written through contributions of both authors. Both authors have given approval to the final version of the manuscript. Both authors contributed equally in the experimental part.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

SQS, squalene synthase; HMGCo-A, 3-hydroxy-3-methylglutaryl-CoA; FPP, farnesyl pyrophosphate; PSPP, presqualene pyrophosphate; ROS, reactive oxygen species; rt, room temperature; BSA, bovine serum albumin; NADPH, nicotinamide adenine dinucleotide phosphate; IC₅₀, inhibitory concentration for 50% of the reaction; TC, total cholesterol; LDL, low-density lipoprotein cholesterol; TG, triglycerides; TAC, total antioxidant capacity

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