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Design of more potent squalene synthase inhibitors with multiple activities

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Lipoxygenase

ABSTRACT

With the increasing realization that modulating a multiplicity of targets can be an asset in the treatment of multifactorial disorders, we hereby report the synthesis and evaluation of the first compounds in which antioxidant, anti-inflammatory as well as squalene synthase (SQS) inhibitory activities are combined by design, in a series of simple molecules, extending their potential range of activities against the multifactorial disease of atherosclerosis. The activity of the initially synthesized antihyperlipidemic morpholine derivatives (**1**–**6**), in which we combined several pharmacophore moieties, was evaluated in vitro (antioxidant, inhibition of SQS and lipoxygenase) and in vivo (anti-dyslipidemic and anti-inflammatory effect). We further compared the in vitro SQS inhibitory action of these derivatives with theoretically derived molecular interactions by performing an in silico docking study using the X-ray crystal structure of human SQS. Based on low energy preferred binding modes, we designed potentially more potent SQS ligands. We proceeded with synthesizing and evaluating these new structures (**7**–**12**) in vitro and in vivo, to show that the new derivatives were significantly more active than formerly developed congeners, both as SQS inhibitors (20–70-fold increase in activity) and antioxidants (4–30-fold increase in activity). A significant correlation between experimental activity [Log($1/IC_{50}$)] and the corresponding binding free energy (ΔG_b) of the docked compounds was shown. These results, taken together, show a promising alternative and novel approach for the design and development of multifunctional antiatherosclerosis agents.

1. Introduction

Atherosclerosis, the underlying cause of coronary artery disease that is the leading cause of death in most parts of the World,[1] is a multifactorial disease. Several mechanisms participate in its initial manifestation and progression, mainly involving the triad: hyperlipidemia, oxidative stress, and inflammation.[2] It is generally accepted that elevated levels of cholesterol, that is, mainly LDL-cholesterol, provide a higher substrate concentration for lipid peroxidation by reactive oxygen species. The oxidatively altered LDL is taken up in large amounts by macrophages which are transformed into foam cells, which are deposited in the vasculature and excrete several proinflammatory factors. Thus, lesion formation in the vascular tissue progresses into atheromatic plaques and the subsequent complications of the disease.

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 respect. However, by inhibiting HMG-CoA, statins suppress the production of mevalonate which is not only a precursor of cholesterol but also of nonsterol molecules which have a vital role in diverse cellular functions. Thus, the inhibition of their synthesis has been associated with statins' major side-effects, hepatotoxicity, and myotoxicity.

Squalene synthase, a key enzyme in the cholesterol biosynthetic pathway, occupies the first and solely committed step towards the biosynthesis of the sterol nucleus of cholesterol. Because of its unique and strategic location in this pathway, it is an attractive target for inhibition and the development of novel and improved antihypercholesterolemic agents. These agents are considered not to interfere with the biosynthesis of other biologically important molecules and thus may offer a better side-effect profile than the extensively used inhibitors of (upstream) HMG-CoA reductase.

Several squalene synthase inhibitors have been studied[3,4] including zaragozic acids, quinuclidine as well as 4,1-benzoxazepine-3-acetic acid derivatives, such as CP-320473 and TAK-475. TAK-475 (or else 'Lapaquistat') reached clinical phase III trials but was eventually discontinued based on safety concerns at higher doses.[5] Using a very different pharmacophore structure, we have

Abbreviations: SQS, squalene synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; FPP, farnesyl pyrophosphate; BSA, bovine serum albumin; NADPH, nicotinamide adenine dinucleotide phosphate; IC_{50}, inhibitory concentration (for 50% of the reaction); TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride.

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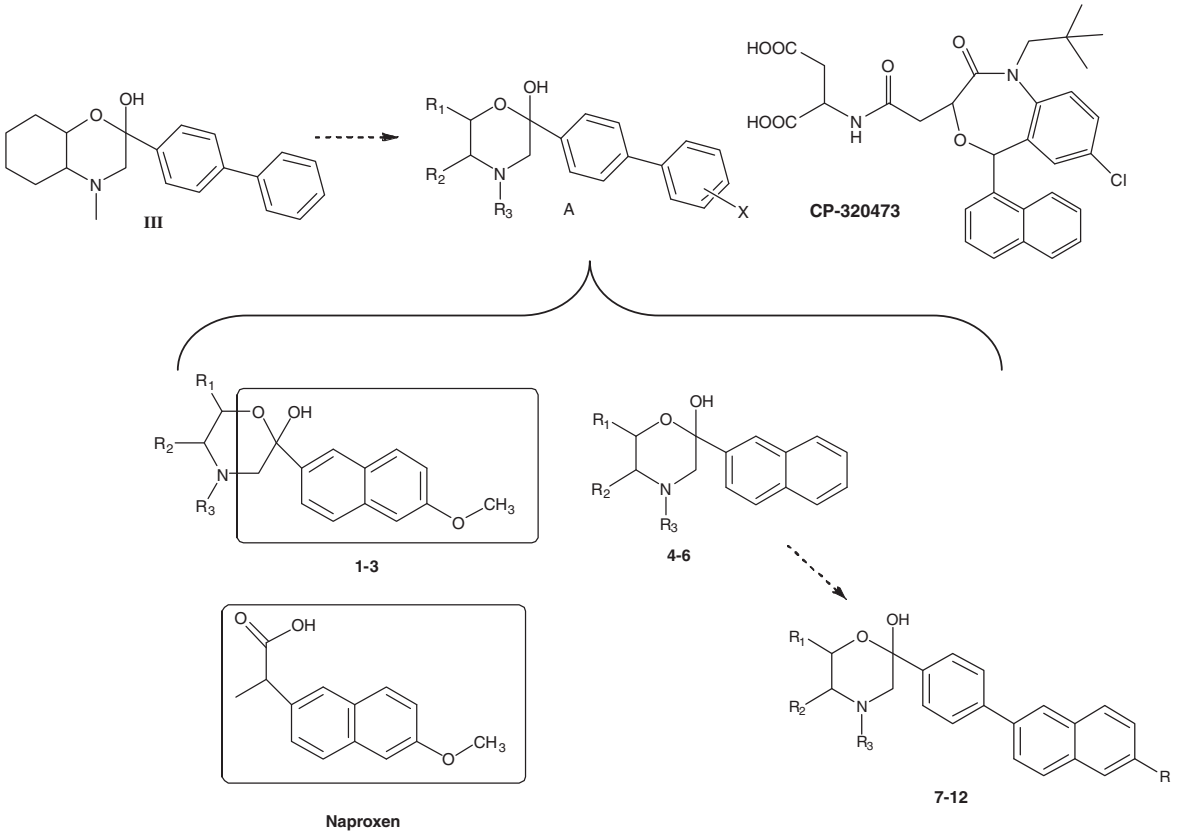


Chart 1.

previously synthesized and investigated 2-biphenyl morpholine derivatives (Chart 1, general structure A) that were shown to reduce cholesterol and triglyceride levels, acting as squalene synthase inhibitors, as well as to combine antioxidant activity.[6–8] The mode of action of two such derivatives was further investigated both in vitro[9,10] and in vivo.[11]

Oxidative stress and inflammation are major players in atherosclerosis development and subsequent cardiovascular pathology.[12] Since the potential of natural antioxidants (i.e., vitamins E and C) in cardiovascular risk reduction is controversial with several clinical studies showing a beneficial[13] and others no effect,[14] new antioxidant strategies are needed to address the oxidative stress menace in such conditions. Further, the same holds for anti-inflammatory treatment, since the overall effect and safety of various known NSAIDs (including naproxen) on cardiovascular risk outcome is also not conclusive and may vary.[15,16] However, there is an increasing realization that modulating simultaneously a multiplicity of targets[17] may be an asset in the treatment of multifactorial disorders such as atherosclerosis. We hereby designed and synthesised new aromatic morpholine derivatives aiming to incorporate in one structure not only increased antioxidant, SQS inhibition and antidyslipidemic but also anti-inflammatory activity. Thus, we incorporated a condensed aromatic system (Chart 1, compounds **1**–**6**) in order (i) to increase the conjugation effect for radical stabilization for antioxidant activity and (ii) to provide some structural similarity with a known non-steroidal anti-inflammatory drug namely naproxen. We investigated the in vitro and in vivo pharmacological activity of the synthesized derivatives and performed in silico docking studies with SQS. The latter suggested limited lipophilic interactions with the inhibitor binding site of SQS and thus new derivatives (compounds **7**–**12**) with extended aromatic substituents were designed and synthesised. The newly synthesized derivatives were evaluated and proven more potent inhibitors of SQS.

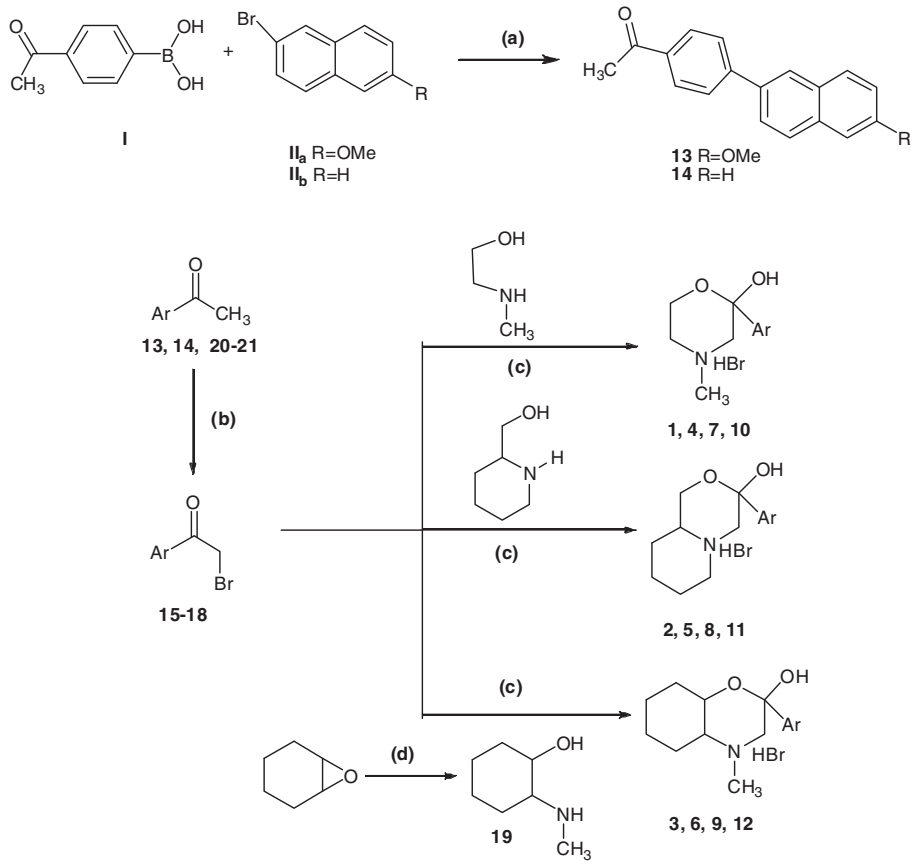
2. Results and discussion

2.1. Chemistry

The target compounds (**1**–**12**, Chart 1) were synthesised in mostly good yields and via the spontaneous cyclization of the corresponding hydroxyaminoketone intermediate to a hemiketal structure (Scheme 1). Structures and calculated[18] lipophilicity values (*c* Log *P*) are presented in Table 1. As verified by our theoretical studies hereby, as well as by previous experimental studies[19,20] stable conformations of the final compounds include equatorial position of the aromatic substituent/axial position of the OH, and chair–chair (*trans*) conformation of the fused octahydro-oxazine ring systems.

2.2. Antioxidant activity

The effect of the investigated derivatives on the non enzymatic peroxidation of hepatic microsomal membrane lipids after 45 min of incubation, expressed as IC_{50} values, is shown in Table 2. Under the same experimental conditions, 2,6-di-*tert*-butyl-4-methylphenol (BHT), a known potent antioxidant, and probucol exhibited IC_{50} values of 25 μM and >1 mM, respectively. The time course of lipid peroxidation, as affected by several concentrations of one of the most active compounds, **9**, is depicted in Figure 1. The initially designed and synthesised derivatives (**1**–**6**) did not demonstrate stronger antioxidant activity than that of the reference compound **III** (Chart 1 and Table 2). However, the performed structural modifications, that is, the extension of the conjugated system (compounds **7**–



Scheme 1. General method for the synthesis of compounds **1**–**12**. Reagents and conditions: (a) $Pd(OAc)_2$, Bu_4NBr, K_2CO_3, H_2O, Ar, 70 °C, 1.5–2 h; (b) Br_2, $CHCl_3$, rt, 2.5 h for 15 or phenyltrimethylammonium tribromide, THF/H_2O, rt, 1.5 h for 16–18; (c) (i) acetone, rt, 20–24 h; (ii) HBr/Et_2O; (d) CH_3NH_2, rt, 19 h.

12) led consistently to a considerable (4–30-fold) improvement of their antioxidant profile. As anticipated, and in accordance to previous observations,[8] antioxidant activity seems to be favoured by conjugation or resonance effects of the further extended aromatic substituent (compare compounds **1**–**6** with **7**–**12**), with an additional contribution to this by the positive resonance (+R) effect of the OMe substituent (i.e., compare **7**–**9** with **10**–**12**). Further, lipophilicity, determined as *c* Log *P* values, in this series of derivatives seems also to correlate ($r^2 = 0.69$, $P = 0.0009$) with antioxidant activity, in agreement with the notion that the lipophilic character of antioxidants contributes to the offered inhibition of lipid peroxidation, as it may facilitate access to biological membranes.

2.3. Inhibition of rat microsomal squalene synthase

Inhibition of the activity of squalene synthase, from rat liver microsomes, by the test compounds, expressed as IC_{50} values, is shown in Table 2. Most compounds inhibited squalene synthase activity significantly and dose-dependently as shown in Figure 2 which depicts the activity of the most active compound **12**. It was first observed that the activity of the initially designed derivatives **1**–**6** was relatively low with IC_{50} values ranging from 15–155 μM. Results from our docking studies (as described below in Section 2.7) led to the development of derivatives **7**–**12** with 'elongated' hydrophobic substituents. These derivatives, with the exception of **8**, exhibited ca. 20–70-fold higher inhibitory activity. The overall increase in lipophilicity seems to favor a better interaction between the molecule and enzyme, and a mild correlation between *c* Log *P* and IC_{50} values was observed ($r^2 = 0.63$, $P = 0.004$). This is in accordance with our previous studies,[8] as well as studies on a series of 3-hydroxy-3-biaryl quinuclidines[21], that demonstrated an improved squalene synthase inhibitory activity for compounds with planar biaryl substitution while a directional requirement for the rigid biaryl side chain was identified. It is hypothesized that the aromatic substituent may act as an isoster for the isoprenyl subunits of the farnesyl chain of the endogenous enzyme substrate farnesyl pyrophosphate (FPP).

2.4. Anti-dyslipidemic activity

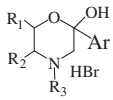
The in vivo anti-dyslipidemic activity of these derivatives is also demonstrated in Table 2. Experimental hyperlipidemia was successfully established 24 h after Triton WR 1339 administration, with an increase in plasma total cholesterol, LDL-cholesterol, and triglyceride levels of 93%, 77%, and 84%, respectively, compared to normal values.[8] All test compounds were found able to reduce the examined parameters in the plasma of hyperlipidemic rats (ca. 20–80% reduction), with activities somewhat comparable to those of reference compound **III**, with the exception of derivative **8** which had no effect on triglycerides and **7** with no effect on LDL-cholesterol. Under the same experimental conditions, probucol and simvastatin (used as anti-dyslipidemic drugs), at the same dose, reduced plasma total cholesterol by 18% and 75%, LDL-C by 18% and 70%, and triglycerides by 11% and 0%, respectively.[8]

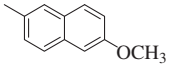
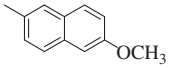
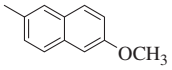
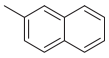
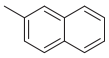
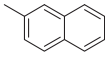
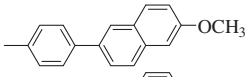
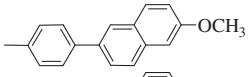
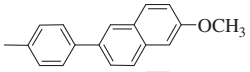
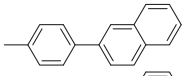
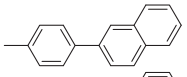
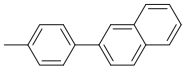
It seems that compounds **7**–**12** that incorporate an extended lipophilic substituent retained the in vivo effect of lowering lipidemic parameters. As expected, other parameters such as in vivo distribution and pharmacokinetics may affect the in vivo order of activity of these compounds as compared to their in vitro activities.

2.5. Lipoxygenase inhibition

Lipoxygenases are important inflammatory mediators implicated in acute inflammatory reaction processes. Numerous studies

Table 1
Structures of substituted hydroxymorpholins, and *c* Log *P* values



Compd	R_1	R_2	R_3	Ar	*c* Log *P*
1	H	H	CH_3		2.17
2	H	$(CH_2)_4$			3.09
3	$(CH_2)_4$		CH_3		3.60
4	H	H	CH_3		2.37
5	H	$(CH_2)_4$			3.17
6	$(CH_2)_4$		CH_3		3.80
7	H	H	CH_3		4.06
8	H	$(CH_2)_4$			4.97
9	$(CH_2)_4$		CH_3		5.49
10	H	H	CH_3		4.25
11	H	$(CH_2)_4$			5.05
12	$(CH_2)_4$		CH_3		5.69

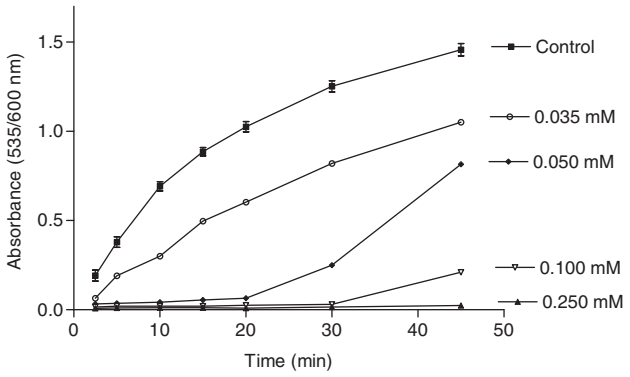


Figure 1. Representative graph of the time course of lipid peroxidation as affected by various concentrations of compound 9.

have suggested a role for this enzyme in atherosclerosis, since cholesterol-loaded macrophages contain increased levels of 12-lipoxygenase, while both mRNA and protein for 15-lipoxygenase are present in atherosclerotic lesions.[22] Thus, lipoxygenase inhibitors have been proposed as antiatherosclerotic agents.[23] Because of the built-in structural similarity of our compounds with naproxen, an active lipoxygenase inhibitor, we investigated the effect of our derivatives on soybean lipoxygenase. Soybean lipoxygenase is inhibited by non-steroidal anti-inflammatory drugs in a similar

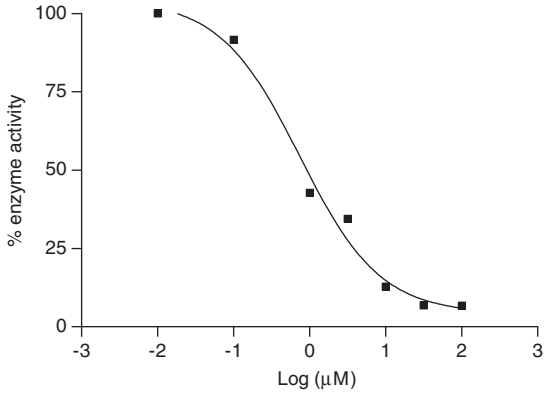


Figure 2. Representative graph showing the activity of squalene synthase as affected by various concentrations of compound **12**.

manner to that of the rat mast cell lipoxygenase and is often used as a reliable means for evaluating lipoxygenase inhibitors.[24]

Among all compounds, the methoxy-naphthyl derivatives **1**–**3** inhibited lipoxygenase activity in a concentration and time dependent manner (Fig. 3, Table 2). Their IC_{50} values, after 6 min of incubation, were 20, 53, and 84 μM, respectively, whereas the potency of the other compounds was much lower (IC_{50} values above 300 μM) (Table 2). The three active compounds **1**–**3** have incorporated in their structure a large structural moiety of naproxen (Chart 1), which is very likely to contribute to the above action. Naproxen's activity compared to other anti-inflammatory agents is particularly high, inhibiting soybean lipoxygenase with an IC_{50} value of 24 μM.[24] In contrast, no significant effect against lipoxygenase activity was noted for the respective naphthyl derivatives (**4**–**6**) or derivatives **7**–**12**, confirming not only the positive contribution of the methoxy group in the activity of **1**–**3**, but also the lack of the rest of the potential 'isosteric' moiety (Chart 1) found in the 'naproxen resembling' derivatives **1**–**3**.

2.6. In vivo anti-inflammatory activity

The anti-inflammatory activity of most derivatives (**1**–**3**, **5**, **6**, **8**–**11**) was evaluated by the method of carrageenan-induced paw edema which is a non specific inflammation maintained by the release of histamine and serotonin and later by prostaglandins.[25,26] The examined compounds were administrated ip at a dose of 300 μmol/Kg right after the injection of carrageenan.

The effect of compounds **1**–**3**, **5**, **6**, **8**–**11** and naproxen on paw edema is depicted in Figure 4. All compounds demonstrated significant anti-inflammatory activity comparable to that of naproxen (51%) at the same dose,[16] with the most active being compounds

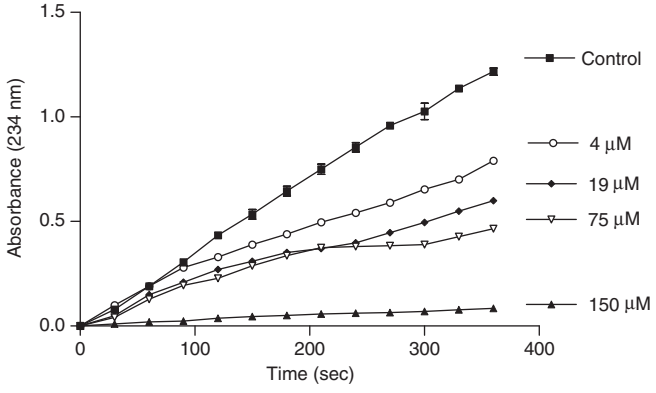


Figure 3. Effect of various concentrations of compound **1** on lipoxygenase activity.

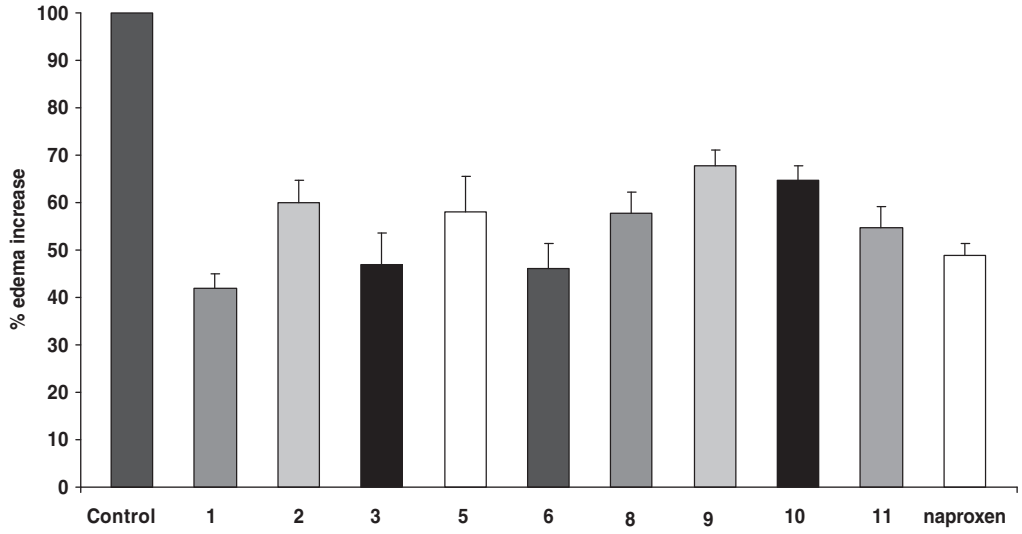


Figure 4. Anti-inflammatory effect of compounds **1**–**3**, **5**, **6**, **8**–**11** and naproxen (nap) on carrageenan-induced paw edema in rats. Results are expressed as percent inhibition of edema compared to the control group. Statistical significance (Student's *t*-test) from control: $P < 0.005$.

1, **3**, and **6** (58%, 53%, and 54% edema inhibition, respectively). The incorporated structural similarity of compounds **1**–**3** with naproxen, may contribute to their increased anti-inflammatory activity in vivo, while the naphthyl derivatives **5** and **6** also exhibited good anti-inflammatory activity, as expected, since several other naphthyl derivatives with anti-inflammatory activity have been reported in the literature.[27] Surprisingly, the activity of the 'elongated' derivatives **8**–**11** was more or less retained as far as in vivo anti-inflammatory action is concerned and comparable to compounds **1**–**6**.

2.7. Molecular docking with squalene synthase (SQS)

To gain new insights in the interactions between the initially synthesized morpholine derivatives (**1**–**6**) and squalene synthase and to design theoretically more potent SQS inhibitors, we performed rigid docking studies using the available X-ray crystal structure of SQS with a bound inhibitor. This theoretical study was performed using the software Autodock 4.[28]

In order to test Autodock's accuracy a docking run was performed on the initial co-crystallized ligand CP-320473[29] (structure in Chart 1) under the same conditions and parameters. This produced a very good prediction of the actual original binding mode of the ligand with a reference difference of 0.95 Å root mean square deviation (RMSD).

The final binding modes of the morpholine derivatives, after the respective docking runs, were selected on the basis of the highest score (best fit), corresponding to the complex with the lowest free energy of binding (ΔG_b) measured in kcal/mol. For the docking process, the lowest conformations of our morpholine derivatives were used (derived as described in Section 3).

The inhibitor binding site in SQS is a solvent exposed external cavity formed at the end of a central channel running through the protein. This cavity is partially covered from one side by a 'flap' formed by residues 50–54 with the side chain of Phe54 forming one wall of a large hydrophobic cavity under the flap. The deep hydrophobic cavity leads to an aspartate-rich opening. Both the aspartate-rich sequences and the above mentioned residues forming the 'flap' are among the most highly conserved in squalene synthases from different species.[30] The aminoacids belonging to the aspartate-rich sequence of SQS (80DTLED84) are considered to play an important role in the catalytic reaction mechanism. As specifically discussed in Pandit et al., this aspartate-rich sequence overlaps with a similar one in the structural superimposition of SQS and FPS (farnesyl pyrophosphate synthase) that belong to the same class I of isoprenoid biosynthetic enzymes.[29,31] This sequence is considered to be the binding site for the phosphate groups, via Mg^{2+}, of the substrate farnesyl pyrophosphate (FPP).

Visual inspection of the lowest binding free energies of SQS complexes with our initially synthesized morpholine derivatives (**1**–**6**) revealed a hydrogen bond between the 2-hydroxyl group of the substituted morpholine ring of all compounds studied in this series and the side chain carboxyl group of ASP80, possibly suggesting that this hydrogen bond is significant for their inhibitory action (Fig. 5a).

Other interactions suggested by the docking results were the hydrophobic interactions of the bulky hydrophobic groups of compounds **1**–**6** as they were found oriented towards the hydrophobic cavity under the 'flap' in close proximity to the *tert*-butyl group of the co-crystallized ligand CP-320473 suggesting similar hydrophobic interactions. Hydrophobic residues such as VAL175, ALA176, VAL 179, LEU 211, and LEU183, forming the inner cavity of the active site, surround the hydrophobic side chains of the compounds.

Realizing that hydrophobic interactions of our morpholine derivatives (**1**–**6**) were limited at least when compared to those of the bound inhibitor (compare the position of the naphthyl groups of the respective molecules, Fig. 5a) we sought to reach deeper in the hydrophobic cavity by elongating the hydrophobic moiety of our morpholine derivative. This was accomplished by extending this naphthyl group with the addition of an extra phenyl ring, as seen in the designed compounds **7**–**12**.

It seemed from the docking studies of the designed new derivatives that not only the previous lipophilic interactions (previously exhibited by the naphthyl moiety of compounds **1**–**6**) were maintained (via the additional phenyl ring of the new derivatives) but now also further hydrophobic interactions deeper in the hydrophobic cavity could be achieved with the re-positioning of the naphthyl moiety (Fig. 5b). So these derivatives seemed to have a better binding capacity as also estimated by their relatively lower binding free energy values.

In specific, the naphthyl and *tert*-butyl part of CP-320473 were found in the same area as the naphthyl and phenyl groups, respectively, of the best docking poses of compounds **7**–**12**, which turned out to be the most active compounds of the series (Table 2). This can be exemplified by comparing the position of the naphthyl group in compound **12** (the most active compound of this series)

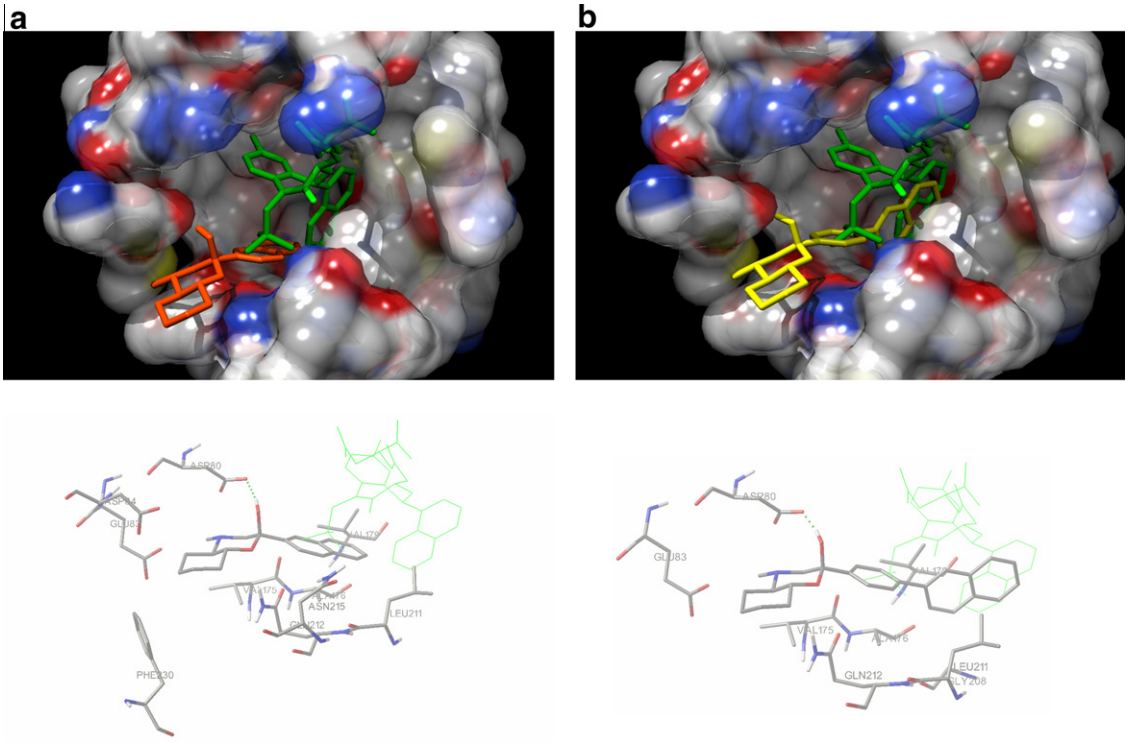


Figure 5. Binding mode visualization of compound **6** (a) and **12** (b). The binding mode of CP-320473 (green) is also included for comparison.

Table 2
Antioxidant, SQS inhibitory, lipoxygenase inhibitory, and anti-dyslipidemic effects of the investigated compounds. Autodock's binding free energy (ΔG) derived from the docking studies on SQS

Compd	Inhibition of lipid peroxidation IC_{50} (μM)	Microsomal SQS inhibition IC_{50} (μM)	ΔG_b (Kcal/mol)	Lipoxygenase inhibition IC_{50} (μM)	% Decrease compared to hyperlipidemic controls (56 μmol/kg ip)		
					TC	LDL-C	TG
1	900	155.0	−7.42	20	62[b]	24[c]	51[a]
2	390	18.7	−8.20	53	45[b]	46[a]	37[b]
3	220	40.0	−8.75	84	36[b]	33[b]	47[a]
4	640	135.0	−7.32	>300	51[a]	36[b]	40[a]
5	440	130.0	−7.95	>300	33[b]	42[b]	41[a]
6	600	15.1	−8.52	>300	43[b]	39[a]	51[a]
7	30	6.9	−9.69	>100	41[a]	9[d]	30[b]
8	65	18.0	−9.83	>100	19[b]	29[b]	6[d]
9	48	1.4	−10.58	>100	32[a]	54[b]	82[a]
10	71	1.9	−9.37	>100	37[a]	59[a]	28[b]
11	117	n.d.	−10.39	>100	49[a]	33[b]	34[b]
12	118	0.8	−10.29	>100	43[a]	48[a]	32[b]
III	450	36.0	−8.82	110	54[a]	51[b]	49[a]
Probucol	*n*	–	–	–	18[b]	18[d]	11[d]
Naproxen	*n*	–	–	24[e]	–	–	–

n.d. = not determined, *n* = above 1 mM.
[a] P <0.005.
[b] P <0.05.
[c] P <0.1.
[d] Not significant (P >0.1) (Student's test).
[e] Data from Taraporewala et al.[24]

and that of compound **6** (Fig. 5b). The naphthyl group of compound **6** is found to occupy approximately the same area (i.e., closer to the surface of the cavity) as the phenyl group of compound **12** (Fig. 5a,b) and as the *tert*-butyl hydrophobic group of CP-320473. In addition, however, compound **12** exhibits further hydrophobic interactions deeper in the hydrophobic cavity with its naphthyl moiety. Similar observations can be made when comparing binding modes of compounds **7**–**12** versus their 'shorter' counterparts **1**–**6**. Thus, these extended hydrophobic interactions, may explain the higher binding and inhibiting capacity of the former compounds. The presence of an OCH_3 group in compounds **1**–**3** and **7**–**9** did not particularly influence (i.e., increase) binding.

Comparison of the binding free energy (ΔG_b as calculated by the docking runs, Table 2) and the experimentally recorded inhibitory activity [expressed as $Log(1/IC_{50})$] indicated that lower energy values (i.e., stronger theoretical binding to SQS) correspond to lower IC_{50} values and thus more significant inhibition. Simple regression calculations between $Log(1/IC_{50})$ of all compounds and the corresponding ΔG_b revealed a significant correlation ($R^2 = 0.79$, $F = 37$, $P = 0.0001$) (Fig. 6).

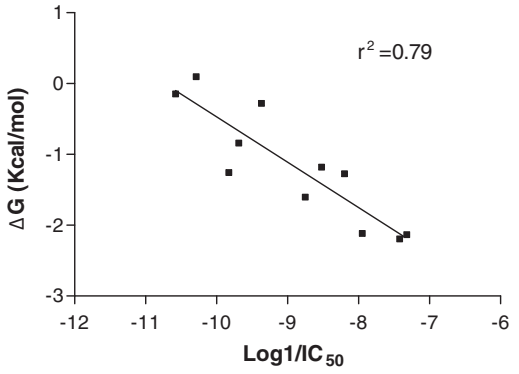


Figure 6. Correlation between the binding free energy (ΔG_b) and Log1/IC_{50} of compounds **1–12.**

2.8. Conclusion

We designed and synthesized new morpholine derivatives (**1–6**) aiming to incorporate in one structure squalene synthase inhibitory, antioxidant as well as anti-inflammatory activity. The new multiple acting molecules that combined several pharmacophore moieties showed in vivo anti-dyslipidemic and anti-inflammatory effect as well in vitro antioxidant, lipoxygenase and SQS inhibitory effect. In order to gain new insights in the interactions between these molecules and squalene synthase, we performed in silico docking studies using the X-ray crystal structure of human SQS which is available with a co-crystallized bound inhibitor. To our knowledge, this is the first time that an in silico docking study has been performed on human SQS. By visualizing and evaluating low energy docking poses, we designed and developed new derivatives (**7–12**) with 'elongated' hydrophobic moieties which, as predicted, showed increased SQS inhibitory activity, as well as antioxidant, while retaining in vivo effects. We further found a good correlation between theoretical and experimental binding of all compounds to SQS. Thus, application of findings from this docking study led to the design and development of more potent SQS inhibitors with multiple activities that are aimed against the multifactorial disorder of atherosclerosis.

3. Materials and methods

3.1. Materials

All commercially available chemicals are of the appropriate purity and purchased from standard sources. [^{3}H] FPP (21.5 Ci/mmol), NADPH, FPP, and BSA were purchased from Sigma–Aldrich (Germany). Soybean Lipoxygenase (250 U/mL) was purchased from Sigma Chemical Co. (St Louis). For the in vivo experiments, Wistar male rats (200–250 g) were used. Animals were kept in a controlled temperature room (22 ± 2 °C), having free access to laboratory chow and tap water, under a 12 h light/dark cycle.

3.2. Synthesis

Melting points (mp) were determined with a digital Electrothermal IA 9000 series apparatus and are uncorrected. Infared (IR) spectra were recorded on a Perkin–Elmer FT-IR spectrometer (Spectrum RXI). ^{1}H NMR spectra and 2D-NMR (COSY and HMQC) were obtained with a Brücker Avance DRX 400 (400 MHz) spectrometer and ^{13}C NMR spectra with a Brücker Avance DPX 200 (200 MHz) spectrometer. Elemental analyses were performed by the Service Central de Microanalyse, France and are reported in the Supplementary data.

3.2.1. General procedure for the preparation of the final compounds 1–12

The final products (Table 1) were obtained by the reaction of 2.2 mmol of 2-methylaminoethanol or 2-piperidinemethanol or *trans*-2-methylamino-cyclohexanol with 1.0 mmol of either 2-bromoacetyl-6-methoxynaphthalene (compounds **1–3**) or 2-bromoacetylnaphthalene (compounds **4–6**) or 2-bromo-1-[4-(6-methoxy-2-naphthalene)-phenyl]-ethanone (compounds **7–9**) or 2-bromo-1-[4-(2-naphthalene)-phenyl]-ethanone (compounds **10–12**), in anhydrous acetone (40 mL) at room temperature with stirring for 20–24 h. Acetone was then distilled off, ether was added to the residue, the mixture was washed with saturated sodium chloride solution, dried (K_2CO_3), and the products were isolated as hydrobromide salts.

3.2.1.1. 2-(6-Methoxy-2-naphthalene)-4-methylmorpholin-2-ol hydrobromide (1). Yield 40%, mp 148.5–150 °C. IR: 3206 (ν OH), 2720 (ν $^+$NH). ^{1}H NMR (DMSO-d_6): δ 2.72 (s, 3H, N–CH_3), 3.11 (d, J = 12.08 Hz, 1H, 3-H_{ax}), 3.19 (d, J = 10.61 Hz, 1H, 5-H_{ax}), 3.33 (m, 2H, 5-H_{eq} and –OH), 3.49 (d, J = 12.07 Hz, 1H, 3-H_{eq}), 3.80 (s, 3H, O–CH_3), 3.97 (dd, J_1 = 3.29 Hz, J_2 = 12.63 Hz, 1H, 6-H_{eq}), 4.24 (dt, J_1 = 3.21 Hz, J_2 = 12.53 Hz, 1H, 6-H_{ax}), 7.13 (dd, J_1 = 2.56 Hz, J_2 = 8.77 Hz, 1H, 7-H Ar), 7.28 (ds, J = 2.19 Hz, 1H, 5-H Ar), 7.56 (dd, J_1 = 1.56 Hz, J_2 = 8.51 Hz, 1H, 3-H Ar), 7.80 (d, J = 8.60 Hz, 1H, 4-H Ar), 7.83 (d, J = 8.97 Hz, 1H, 8-H, Ar), 7.95 (s, 1H, 1-H Ar), 9.87 (br s, 1H, NH). Anal. ($C_{16}H_{20}BrNO_3 \cdot 0.1H_2O$) C, H.

3.2.1.2. 3-(6-Methoxy-2-naphthalene)-octahydro-1,4-pyrido[2,1-*c*] oxazin-3-ol hydrobromide (2). Yield 81%, mp 191.5–192.5 °C. ^{1}H NMR ($CDCl_3$): δ 1.62–1.89 (m, 4H, 2 × 8-H and 2 × 9-H), 2.23–2.43 (m, 4H, 6-H_{ax}, –OH and 2 × 7-H), 2.72–2.78 (m, 1H, 6-H_{eq}), 2.94 (d, J = 12.71 Hz, 1H, 4-H_{ax}), 3.23–3.32 (m, 2H, 4-H_{eq} and 9α-H_{ax}), 3.35 (dd, J_1 = 3.62 Hz, J_2 = 12.12 Hz, 1H, 1-H_{eq}), 3.76 (s, 3H, O–CH_3), 4.32 (t, J = 12.46 Hz, 1H, 1-H_{ax}), 6.96–7.01 (m, 2H, 5,7-H Ar), 7.52 (dd, J_1 = 1.71 Hz, J_2 = 8.62 Hz, 1H, 3-H Ar), 7.55–7.60 (m, 2H, 4,8-H Ar), 7.93 (s, 1H, 1-H Ar), 10.72 (br s, 1H, NH). Anal. ($C_{19}H_{24}BrNO_3$) C, H.

3.2.1.3. 2-(6-Methoxy-2-naphthalene)-4-methyl-octahydro-1,4-benzoxazin-2-ol hydrobromide (3). Yield 70%, mp 158–160 °C. ^{1}H NMR ($CDCl_3$): δ 1.26–1.54 (m, 4H, 5-H_{ax}, 2 × 7-H and 8-H_{ax}), 1.81–1.99 (m, 4H, 2 × 6-H, 8-H_{eq} and –OH), 2.18–2.30 (m, 1H, 5-H_{eq}), 2.73 (d, J = 4.70 Hz, 3H, N–CH_3), 2.91–3.00 (m, 2H, 3-H_{ax} and 4α-H_{ax}), 3.61 (dd, J_1 = 2.31 Hz, J_2 = 12.55 Hz, 1H, 3-H_{eq}), 3.86 (s, 3H, O–CH_3), 4.49 (m, 1H, 8α-H_{ax}), 7.06–7.14 (m, 2H, 5,7-H Ar), 7.61 (dd, J_1 = 1.71 Hz, J_2 = 8.62 Hz, 1H, 3-H Ar), 7.64–7.71 (m, 2H, 4,8-H Ar), 8.05 (s, 1H, 1-H Ar), 11.78 (br s, 1H, NH). Anal. ($C_{20}H_{26}BrNO_3$) C, H.

3.2.1.4. 2-(2-Naphthalene)-4-methylmorpholin-2-ol hydrobromide (4). Yield 40%, mp 80–84 °C. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 2.27 (d, J = 4.16 Hz, 3H, N–CH_3), 2.40 (d, J = 11.25 Hz, 1H, 3-H_{ax}), 2.49–2.56 (m, 2H, 5-H_{ax} and –OH), 2.65 (d, J = 13.20 Hz, 1H, 5-H_{eq}), 2.98 (d, J = 11.86 Hz, 1H, 3-H_{eq}), 3.40 (dd, J_1 = 3.55 Hz, J_2 = 12.83 Hz, 1H, 6-H_{eq}), 4.16 (dt, J_1 = 3.28 Hz, J_2 = 12.84 Hz, 1H, 6-H_{ax}), 6.80–6.87 (m, 2H, 6,7-H Ar), 7.04 (dd, J_1 = 1.71 Hz, J_2 = 8.56 Hz, 1H, 3-H Ar), 7.15–7.24 (m, 3H, 4,5,8-H Ar), 7.48 (ds, J = 0.98 Hz, 1H, 1-H Ar), 10.22 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 43.53 (N–CH_3), 52.11 (5-C), 57.35 (6-C), 59.70 (3-C), 94.29 (2-C), 124.03 (3-C Ar), 125.30 (1-C Ar), 126.98 (6-C Ar), 127.19 (7-C Ar), 128.00 (8-C Ar), 128.32 (5-C Ar), 128.77 (4-C Ar), 132.76 (4α-C Ar), 133.39 (8α-C Ar), 139.03 (2-C Ar). Anal. ($C_{15}H_{18}BrNO_2 \cdot 0.2H_2O$) C, H.

3.2.1.5. 3-(2-Naphthalene)-octahydro-1,4-pyrido[2,1-*c*]oxazin-3-ol hydrobromide (5). Yield 82%, mp 193.5–194.5 °C. IR: 3276 (ν OH), 2654 (ν $^+$NH). ^{1}H NMR (DMSO-d_6): δ 1.36–1.47 (m, 2H, 8-H_{ax} and 9-H_{ax}), 1.65–1.87 (m, 4H, 2 × 7-H, 8-H_{eq} and 9-H_{eq}), 2.00 (s, 1H, -OH), 2.96 (q, J = 22.11 Hz, 1H, 6-H_{ax}), 3.18–3.28 (m, 2H, 6-H_{eq} and 4-H_{ax}), 3.40–3.48 (m, 2H, 4-H_{eq} and 9α-H_{ax}), 3.91 (dd, J_1 = 3.52 Hz, J_2 = 12.52 Hz, 1H, 1-H_{eq}), 4.01 (t, J = 12.52 Hz, 1H, 1-

H_{ax}), 7.48–7.51 (m, 2H, 6,7-H Ar), 7.63 (dd, J_1 = 1.57 Hz, J_2 = 8.61 Hz, 1H, 3-H Ar), 7.87–7.94 (m, 3H, 4,5,8-H Ar), 8.05 (s, 1H, 1-H Ar), 9.80 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 21.70 (8-C), 22.07 (7-C), 23.90 (9-C), 53.82 (6-C), 59.69 (4-C), 61.26 (9α-C), 61.87 (1-C), 94.65 (3-C), 124.11 (3-C Ar), 125.31 (1-C Ar), 126.99 (6-C Ar), 127.20 (7-C Ar), 128.00 (8-C Ar), 128.31 (5-C Ar), 128.78 (4-C Ar), 132.74 (4α-C Ar), 133.38 (8α-C Ar), 138.94 (2-C Ar). Anal. ($C_{18}H_{22}BrNO_2$) C, H.

3.2.1.6. 2-(2-Naphthalene)-4-methyl-octahydro-1,4-benzoxazin-2-ol hydrobromide (6). Yield 95%, mp 158–160 °C. IR: 3166 (ν OH), 2656 (ν $^+$NH). ^{1}H NMR (DMSO-d_6): δ 1.12–1.54 (m, 4H, 8-H_{ax}, 5-H_{ax} and 2 × 7-H), 1.73 (m, 2H, 2 × 6-H), 1.89 (d, J = 10.06 Hz, 1H, 8-H_{eq}), 2.18 (d, J = 10.43 Hz, 1H, 5-H_{eq}), 2.42 (s, 1H, -OH), 2.71 (s, 3H, N–CH_3), 3.07 (m, 1H, 4α-H_{ax}), 3.25 (m, 1H, 3-H_{ax}), 3.59 (d, J = 12.26 Hz, 1H, 3-H_{eq}), 4.06 (dt, J_1 = 3.90 Hz, J_2 = 10.98 Hz, 1H, 8α-H_{ax}), 7.47–7.50 (m, 2H, 6,7-H Ar), 7.63 (dd, J_1 = 1.47 Hz, J_2 = 8.60 Hz, 1H, 3-H Ar), 7.87–7.94 (m, 3H, 4,5,8-H Ar), 8.05 (s, 1H, 1-H Ar), 9.95 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.84 (6,7-C), 24.39 (5-C), 24.70 (8-C), 31.08 (N–CH_3), 61.26 (3-C), 66.19 (4α-C), 70.40 (8α-C), 94.34 (2-C), 124.14 (3-C Ar), 125.32 (1-C Ar), 127.02 (6-C Ar), 127.21 (7-C Ar), 128.03 (8-C Ar), 128.34 (5-C Ar), 128.78 (4-C Ar), 132.73 (4α-C Ar), 133.40 (8α-C Ar), 138.94 (2-C Ar). Anal. ($C_{19}H_{24}BrNO_2 \cdot 0.2H_2O$) C, H.

3.2.1.7. 2-[4-(6-Methoxy-2-naphthalen)-phenyl]-4-methylmorpholin-2-ol hydrobromide (7). Yield 57%, mp 157–160 °C. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 2.82 (ds, J = 3.91 Hz, 3H, –NCH_3), 3.02–3.10 (m, 1H, 5-H_{ax}), 3.21–3.29 (m, 1H, 5-H_{eq}), 3.37 (d, J = 23.48 Hz, 2H, 2 × 3-H), 3.38 (s, 1H, –OH), 3.85 (s, 3H, –OCH_3), 3.96 (dd, J_1 = 3.53 Hz, J_2 = 12.52 Hz, 1H, 6-H_{eq}), 4.47 (dt, J_1 = 3.05 Hz, J_2 = 12.52 Hz, 1H, 6-H_{ax}), 7.07–7.10 (m, 2H, 5,7-H naphthalene), 7.62–7.69 (m, 5H, 3,4,8-H naphthalene and 3,5-H phenyl), 7.72 (d, J = 9.98 Hz, 2H, 2,6-H phenyl), 7.91 (s, 1H, 1-H naphthalene) 10.25 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 43.50 (N–CH_3), 52.11 (5-C), 55.78 (O–CH_3), 57.33 (6-C), 59.77 (3-C), 94.15 (2-C), 106.21 (5-C naphthalene), 119.56 (7-C naphthalene), 125.65 (3-C naphthalene), 125.84 (8α-C naphthalene), 126.87 (1,4-C naphthalene), 127.92 (3,5-C phenyl), 129.19 (8-C naphthalene), 130.24 (2,6-C phenyl), 134.20 (2-C naphthalene), 134.87 (4α-C naphthalene), 140.36 (4-C phenyl), 141.04 (1-C phenyl), 158.03 (6-C naphthalene).Anal. ($C_{22}H_{24}BrNO_3 \cdot 2.0H_2O$) C, H.

3.2.1.8. 3-[4-(6-Methoxy-2-naphthalen)-phenyl]-octahydro-1,4-pyrido[2,1-*c*]oxazin-3-ol hydrobromide (8). Yield 67%, mp 238–239 °C. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.63–2.09 (m, 6H, 2 × 7-H, 2 × 8-H and 2 × 9-H), 2.20 (q, J = 22.22 Hz, 1H, 6-H_{ax}), 3.01 (q, J = 22.01 Hz, 1H, 6-H_{eq}), 3.14 (s, 1H, –OH), 3.28 (d, J = 12.03 Hz, 1H, 4-H_{ax}), 3.35 (d, J = 12.33 Hz, 1H, 4-H_{eq}), 3.42–3.45 (m, 1H, 9α-H_{ax}), 3.84 (dd, J_1 = 3.04 Hz, J_2 = 13.01 Hz, 1H, 1-H_{eq}), 3.87 (s, 3H, –OCH_3), 4.30 (t, J = 12.52 Hz, 1H, 1-H_{ax}), 7.09–7.10 (m, 2H, 5,7-H naphthalene), 7.61–7.76 (m, 7H, 3,4,8-H naphthalene and 2,3,5,6-H phenyl), 7.90 (s, 1H, 1-H naphthalene), 10.57 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 21.73 (8-C), 22.10 (7-C), 23.93 (9-C), 53.87 (6-C), 55.72 (O–CH_3), 59.81 (4-C), 61.33 (9α-C), 61.83 (1-C), 94.48 (3-C), 106.23 (5-C naphthalene), 119.55 (7-C naphthalene), 125.65 (3-C naphthalene), 125.84 (8α-C naphthalene), 126.89 (1,4-C naphthalene), 127.93 (3,5-C phenyl), 129.19 (8-C naphthalene), 130.23 (2,6-C phenyl), 134.21 (2-C naphthalene), 134.88 (4α-C naphthalene), 140.29 (4-C phenyl), 141.05 (1-C phenyl), 158.01 (6-C naphthalene). Anal. ($C_{25}H_{28}BrNO_3 \cdot 0.25H_2O$) C, H.

3.2.1.9. 2-[4-(6-Methoxy-2-naphthalen)-phenyl]-4-methyl-octahydro-1,4-benzoxazin-2-ol hydrobromide (9). Yield 60%, mp 106–107 °C. ^{1}H NMR (DMSO-d_6): δ 1.14–1.45 (m, 6H, 8-H_{ax}, 5-H_{ax}, 2 × 7-H and 2 × 6-H), 1.58–1.79 (m, 2H, 8-H_{eq} and 5-H_{eq}), 1.88–1.91 (m, 1H, 4α-H_{ax}), 2.20 (s, 1H, –OH), 2.72 (s, 3H, N–CH_3), 3.02 (d, J = 9.98 Hz, 1H, 3-H_{ax}), 3.19 (t, J = 10.56 Hz, 1H, 3-H_{eq}), 3.83 (s, 3H, O–CH_3), 4.00–4.06 (m, 1H, 8α-H_{ax}), 7.15 (d, J = 8.81 Hz, 1H, 7-H naphthalene), 7.31 (s, 1H, 5-H naphthalene), 7.46 (s, 1H, 8-H naphthalene), 7.62 (d, J = 8.22 Hz, 2H, 2,6-H phenyl), 7.76–7.88 (m, 4H, 3,5-H phenyl and 3,4-H naphthalene), 8.13 (s, 1H, 1-H naphthalene), 9.92 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.83 (6,7-C), 24.39 (5-C), 24.72 (8-C), 31.05 (N–CH_3), 55.73 (O–CH_3), 61.33 (3-C), 66.21 (4α-C), 70.31 (8α-C), 94.11 (2-C), 106.18 (5-C naphthalene), 119.50 (7-C naphthalene), 125.62 (3-C naphthalene), 125.85 (8α-C naphthalene), 126.91 (1,4-C naphthalene), 127.91 (3,5-C phenyl), 129.17 (8-C naphthalene), 130.19 (2,6-C phenyl), 134.17 (2-C naphthalene), 134.93 (4α-C naphthalene), 140.23 (4-C phenyl), 141.09 (1-C phenyl), 158.05 (6-C naphthalene). Anal. ($C_{26}H_{30}BrNO_3$) C, H.

3.2.1.10. 2-[4-(2-Naphthalen)-phenyl]-4-methylmorpholin-2-ol hydrobromide (10). Yield 40%, mp 116–118 °C. ^{1}H NMR DMSO-d_6 (DMSO-d_6): δ 2.73 (s, 3H, N–CH_3), 2.91 (s, 1H, –OH), 3.09 (d, J = 11.93 Hz, 1H, 3-H_{ax}), 3.15–3.21 (m, 1H, 5-H_{ax}), 3.48 (d, J = 11.93 Hz, 1H, 3-H_{eq}), 3.76 (m, 1H,5-H_{eq}), 3.96 (dd, J_1 = 2.93 Hz, J_2 = 12.52 Hz, 1H, 6-H_{eq}), 4.24 (dt, J_1 = 2.51 Hz, J_2 = 12.33 Hz, 1H, 6-H_{ax}), 7.44–7.51 (m, 2H, 6,7-H naphthalene), 7.62 (d, J = 8.22 Hz, 2H, 2,6-H phenyl), 7.80–8.04 (m, 6H, 3,5-H phenyl and 3,4,5,8-H naphthalene), 8.20 (s, 1H, 1-H naphthalene), 9.80 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 43.48 (N–CH_3), 51.99 (5-C), 57.27 (6-C), 59.77 (3-C), 94.12 (2-C), 125.45 (1-C naphthalene), 125.83 (6,7-C naphthalene), 126.75 (4-C naphthalene), 126.96 (3,5-C phenyl), 127.18 (2,6-C phenyl), 127.98 (3-C naphthalene), 128.67 (5-C naphthalene), 129.01 (8-C naphthalene), 132.82 (4α-C naphthalene), 133.76 (8α-C naphthalene), 137.21 (2-C naphthalene), 140.74 (4-C phenyl), 140.90 (1-C phenyl). Anal. ($C_{21}H_{22}BrNO_2 \cdot 0.75H_2O$) C, H.

3.2.1.11. 3-[4-(2-Naphthalen)-phenyl]-octahydro-1,4-pyrido[2,1-*c*]oxazin-3-ol hydrobromide (11). Yield 75%, mp 215–217 °C. ^{1}H NMR (DMSO-d_6): δ 1.35–1.45 (m, 2H, 8-H_{ax} and 9-H_{ax}), 1.66–1.85 (m, 4H, 2 × 7-H, 8-H_{eq} and 9-H_{eq}), 2.93 (q, J = 20.64 Hz, 1H, 6-H_{ax}), 3.15 (t, J = 10.57 Hz, 1H, 4-H_{ax}), 3.23 (d, J = 11.54 Hz, 1H, 6-H_{eq}), 3.35–3.37 (m, 1H, 9α-H_{ax}), 3.43 (d, J = 11.93 Hz, 1H, 4-H_{eq}), 3.89 (dd, J_1 = 3.33 Hz, J_2 = 12.53 Hz, 1H, 1-H_{eq}), 3.98 (t, J = 11.64 Hz, 1H, 1-H_{ax}), 7.44–7.49 (m, 2H, 6,7-H naphthalene), 7.63 (d, J = 8.41 Hz, 2H, 2,6-H phenyl), 7.80–7.89 (m, 4H, 3,5-H phenyl and 3,4-H naphthalene), 7.93–7.97 (m, 2H, 5,8-H naphthalene), 8.20 (s, 1H, 1-H naphthalene), 9.73 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 21.72 (8-C), 22.08 (7-C), 23.92 (9-C), 53.88 (6-C), 59.76 (4-C), 61.32 (9α-C), 61.84 (1-C), 94.48 (3-C), 125.47 (1-C naphthalene), 125.83 (6,7-C naphthalene), 126.76 (4-C naphthalene), 126.92 (3,5-C phenyl), 127.18 (2,6-C phenyl), 127.98 (3-C naphthalene) 128.67 (5-C naphthalene), 129.03 (8-C naphthalene), 132.82 (4α-C naphthalene), 133.75 (8α-C naphthalene), 137.20 (2-C naphthalene), 140.66 (4-C phenyl), 140.90 (1-C phenyl). Anal. ($C_{24}H_{26}BrNO_2$) C, H.

3.2.1.12. 2-[4-(2-Naphthalen)-phenyl]-4-methyl-octahydro-1,4-benzoxazin-2-ol hydrobromide (12). Yield 79%, mp 169–170 °C. ^{1}H NMR (DMSO-d_6): δ 0.98–1.43 (m, 5H, 8-H_{ax}, 5-H_{ax}, 2 × 7-H and –OH), 1.73 (m, 2H, 2 × 6-H), 1.87 (d, J = 9.39 Hz, 1H, 8-H_{eq}), 2.18 (d, J = 11.54 Hz, 1H, 5-H_{eq}), 2.71 (s, 3H, N–CH_3), 3.04 (m, 1H, 4α-H_{ax}), 3.21 (m, 1H, 3-H_{ax}), 3.56 (d, J = 12.33 Hz, 1H, 3-H_{eq}), 4.03 (dt, J_1 = 4.11 Hz, J_2 = 10.57 Hz, 1H, 8α-H_{ax}), 7.44–7.50 (m, 2H, 6,7-H naphthalene), 7.63 (d, J = 8.41 Hz, 2H, 2,6-H phenyl), 7.79–7.89 (m, 4H, 3,5-H phenyl and 3,4-H naphthalene), 7.93–7.97 (m, 2H, 5,8-H naphthalene), 8.20 (s, 1H, 1-H naphthalene), 9.93 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 23.82 (6,7-C), 24.38 (5-C), 24.70 (8-C), 31.07 (N–CH_3), 61.32 (3-C), 66.22 (4α-C), 70.33 (8α-C), 94.15 (2-C), 125.50 (1-C naphthalene), 125.83 (6,7-H naphthalene), 126.73 (4-C naphthalene), 126.97 (3,5-C phenyl), 127.18 (2,6-C

phenyl), 127.98 (3-C naphthalene), 128.66 (5-C naphthalene), 129.02 (8-C naphthalene), 132.83 (4α-C naphthalene), 133.75 (8α-C naphthalene), 137.25 (2-C naphthalene), 140.63 (4-C phenyl), 140.92 (1-C phenyl). Anal. ($C_{25}H_{28}BrNO_2$) C, H.

3.2.2. Preparation of intermediate and starting materials

3.2.2.1. General procedure for the cross-coupling reactions (13–14). To a three-necked flask were added 2 mmol of arylhalide, 2.2 mmol of 1-(4-acetyl-phenyl)-boronic acid, 0.2 mol % of $Pd(OAc)_2$, 5 mmol of powdered K_2CO_3, and 2 mmol of Bu_4NBr. The flask was flushed with argon and equipped with a rubber septum. Water (2.2 mL) was added with a syringe, and the resulting suspension was energetically stirred and degassed to remove O_2. The mixture was stirred and heated for 1.5–2 h at 70 °C under argon. It was then cooled to room temperature, diluted with water, and extracted with EtOAc. The solution was dried (Na_2SO_4), the solvent was removed in vacuum, and the crude product purified by silica gel column chromatography to yield the desirable product.

*3.2.2.1.1. 1-[4-(6-Methoxy-2-naphthalen)-phenyl]-ethanone (**13**).* Purified by flash chromatography eluting with EtOAc–petroleum ether 1:3, yield 90%, mp 186.5–188.5. ^{1}H NMR ($CDCl_3$): δ 2.66 (s, 3H, $-CH_3$), 3.96 (s, 3H, $-OCH_3$), 7.18–7.22 (m, 2H, 5,7-H naphthalene), 7.73–7.85 (m, 5H, 3,4,8-H naphthalene and 3,5-H phenyl), 8.04 (s, 1H, 1-H naphthalene), 8.07 (d, J = 8.21 Hz, 2H, 2,6-H phenyl). Anal. ($C_{19}H_{16}O_2 \cdot 0.2H_2O$) C, H.

*3.2.2.1.2. 1-[4-(2-Naphthalen)-phenyl]-ethanone (**14**)[32].* Purified by flash chromatography eluting with EtOAc–petroleum ether 1:8, yield 84%, mp 138.5–139.5 °C. ^{1}H NMR ($CDCl_3$): δ 2.61 (s, 3H, $-CH_3$), 7.44–7.50 (m, 2H, 6,7-H naphthalene), 7.71 (dd, J_1 = 1.65 Hz, J_2 = 8.60 Hz, 1H, 3-H naphthalene), 7.76 (d, J = 8.05 Hz, 2H, 3,5-H phenyl), 7.81–7.90 (m, 3H, 4,5,8-H naphthalene), 8.01 (s, 1H, 1-H naphthalene), 8.03 (d, J = 7.98 Hz, 2H, 2,6-H phenyl).

3.2.2.2. 2-Bromoacetylnaphthalene (15)[33]. Bromine (5.5 mmol) in dry chloroform (20 mL) was added dropwise to a stirred solution of the corresponding acetyl derivative 4-cyclohexyl-*a*-acetophenone (**19**) or 2-acetylnaphthalene (**20**) (5 mmol) in dry chloroform (10 mL). After the addition of bromine, the solution was stirred at room temperature for 2.5 h. The solution was washed with H_2O, 5% $NaHCO_3$, and H_2O, dried (Na_2SO_4) and concentrated. The residue was subjected to flash column chromatography on silica gel eluting with CH_2Cl_2–petroleum ether 1:2, to give the desirable product at a yield of 82%. mp 78–79.5 °C. ^{1}H NMR ($CDCl_3$): δ 4.61 (s, 2H, $-CH_2-$), 7.58–7.67 (m, 2H, 6,7-H Ar), 7.90–7.96 (m, 2H, 3,4-H Ar), 7.99–8.06 (m, 2H, 5,8-H Ar), 8.53 (s, 1H, 1-H Ar).

3.2.2.3. General procedure for the preparation of bromo-acetyl derivatives 16–18. To a solution of the acetyl derivative 2-acetyl-6-methoxynaphthalene (**21**) or 1-[4-(6-methoxy-2-naphthalen)-phenyl]-ethanone (**13**) or 1-[4-(2-naphthalen)-phenyl]-ethanone (**14**) (5 mmol) in 10 mL of anhydrous tetrahydrofuran contained in a 100 mL flask was added phenyltrimethylammonium tribromide (5 mmol) in small portions over a 30 min period. A white precipitate forms immediately. The mixture was stirred at room temperature for 1.5–2 h and then cold water was added. The crystalline precipitate was filtered, washed with water, dried, and was subjected to flash column chromatography on silica gel to give the desirable product.

*3.2.2.3.1. 2-Bromoacetyl-6-methoxynaphthalene (**16**)[34].* Purified by flash chromatography eluting with CH_2Cl_2–petroleum ether 1:1, yield 73%, mp 108–110 °C. ^{1}H NMR ($CDCl_3$): δ 3.97 (s, 3H, $O-CH_3$), 4.56 (s, 2H, $-CH_2-$), 7.17 (ds, J = 2.27 Hz, 1H, 5-H Ar), 7.23 (dd, J_1 = 2.45 Hz, J_2 = 8.95 Hz, 1H, 3-H Ar), 7.80 (d, J = 8.70 Hz, 1H, 7-H Ar), 7.88 (d, J = 8.98 Hz, 1H, 4-H Ar), 8.01 (dd, J_1 = 1.65 Hz, J_2 = 8.66 Hz, 1H, 8-H Ar), 8.45 (s, 1H, 1-H Ar).

*3.2.2.3.2. 2-Bromo-1-[4-(6-methoxy-2-naphthalene)-phenyl]-ethanone (**17**).* Purified by flash chromatography eluting with CH_2Cl_2–petroleum ether 1:4, yield 67%, mp 170.5–171.5 °C. ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3H, $-OCH_3$), 4.43 (s, 2H, $-CH_2-$), 7.11–7.15 (m, 2H, 5,7-H naphthalene), 7.75–7.79 (m, 5H, 3,4,8-H naphthalene and 3,5-H phenyl), 7.97 (s, 1H, 1-H naphthalene), 8.03 (d, J = 8.13 Hz, 2H, 2,6-H phenyl). Anal. ($C_{19}H_{15}BrO_2 \cdot 0.5H_2O$) C, H.

*3.2.2.3.3. 2-Bromo-1-[4-(2-naphthalene)-phenyl]-ethanone (**18**)[35].* Purified by flash chromatography eluting with CH_2Cl_2–petroleum ether 1:6, yield 76%, mp 154–156 °C. ^{1}H NMR ($CDCl_3$): δ 4.43 (ds, J = 1.23 Hz, 2H, $-CH_2-$), 7.45–7.50 (m, 2H, 6,7-H naphthalene), 7.70 (dd, J_1 = 1.59 Hz, J_2 = 8.44 Hz, 1H, 1-H naphthalene), 7.77–7.90 (m, 5H, 3,5-H phenyl and 3,4,5-H naphthalene), 8.03–8.05 (m, 3H, 8-H naphthalene and 2,6-H phenyl).

3.2.2.4. trans-2-Methylamino-cyclohexanol (19). To a solution of cyclohexene oxide (55.7 mmol) in 20 mL methanol and 15 mL aqueous solution (40%) of methylamine, was stirred for 19 h at rt. The product was isolated by vacuum distillation (bp_4 66–69 °C). Yield 70%.

3.3. In vitro lipid peroxidation

Heat-inactivated hepatic microsomes from untreated rats were prepared as described.[36] The incubation mixture contained heat-inactivated (90 °C for 90 s) microsomal fraction (corresponding to 2.5 mg of hepatic protein per milliliter or 4 mM fatty acid residues[37]), ascorbic acid (0.2 mM) in Tris–HCl/KCl buffer (50 mM/150 mM, pH 7.4), and the studied compounds (10 μM–1 mM) dissolved in dimethyl sulfoxide. The reaction was initiated by the addition of a freshly prepared $FeSO_4$ solution (10 μM), and the mixture was incubated at 37 °C for 45 min. Aliquots were taken at various time intervals and lipid peroxidation was assessed by spectrophotometric (535 against 600 nm) determination of the 2-thiobarbituric acid reactive material.[8] All compounds and solvents were tested and found not to interfere with the assay. Each assay was performed in duplicate and IC_{50} values represent the mean concentration of compounds that inhibits the peroxidation of control microsomes by 50% after 45 min of incubation. All standard errors are within ten percent of the respective reported values.

3.4. In vitro squalene synthase activity assay

SQS activity was evaluated by determining the amount of [^{3}H] FPP converted to squalene as previously described.[8,38] The assay was performed in 1 mL of 50 mM phosphate buffer, pH 7.4, containing 10 mM $MgCl_2$, 0.5 mM NADPH, rat liver microsomes (18 μg protein/mL), various concentrations of the test compounds or simvastatin dissolved in ethanol, and [^{3}H] FPP (0.5 μM, 0.27 Ci/mmol) in a 10x100 mm glass screw-cap tube. All components except [^{3}H] FPP were preincubated for 10 min at 37 °C. The reaction was initiated by the addition of [^{3}H] FPP. After 10 min at 37 °C, the reaction was terminated by the addition of 1 mL 15% KOH in ethanol. The tubes were incubated at 65 °C for 30 min to solubilize proteins. The mixture was extracted with 5 mL petroleum ether for 10 min. After freezing the lower aqueous phase, the organic phase was transferred to glass tubes containing 2 mL distilled water. After washing, 1.5 mL of the upper organic phase was removed and counted with 3 mL scintillation liquid using a Beckman scintillation counter. Each assay was performed in triplicate and IC_{50} values represent the mean concentration of compounds that inhibits the activity of the enzyme by 50%. All standard errors are within 10% of the respective reported values.

3.5. In vitro lipoxygenase activity assay

Lipoxygenase activity was determined using soybean lipoxygenase (250 U/mL) and sodium linoleate (100 μM) as substrate, in

Tris–HCl buffer pH 9.0. The test compounds in 60% ethanol were added and the reaction was monitored for 6 min at 28 °C, recording absorbance at 234 nm.[24] The assay for each concentration was carried out twice. All standard errors are within 10% of the respective reported values.

3.6. In vivo evaluation of antihypercholesterolemic and antihy perlipidemic activity

An aqueous solution of Triton WR 1339 was given ip to rats (200 mg/kg) and one hour later the test compounds (56 μmol/kg), dissolved in saline, or saline only were administered ip.[39] After 24 h, blood was taken from the aorta and used for the determination of plasma total cholesterol (TC), LDL-cholesterol (LDL-C) and triglyceride (TG) levels, using commercially available kits.[8] Levels of plasma lipids were determined in duplicate while values presented are the mean from 8–10 rats (per compound). All standard errors are within 12% of the respective reported values.

3.7. In vivo inhibition of carrageenan-induced rat-paw edema[25]

For the in vivo anti-inflammatory activity, Wistar male rats (150–180 g) were injected with 0.1 mL carrageenan (2% w/v solution in physiological saline) id into the right hind paw, the left paw serving as control. The test compounds (300 μmol/kg) were given ip after the carrageenan injection, and 3.5 h later the produced edema was estimated as paw weight increase. Values are the mean from 8–10 rats (per compound). All standard errors are within 12% of the respective reported values.

3.8. Protein determination

The protein content of microsomal fractions, used in lipid peroxidation and squalene synthase inhibitory assay, was determined according to Lowry's method.[40]

3.9. Statistical analysis

Data are expressed as mean ± standard deviation. Where indicated, statistical comparisons were made using Student's *t*-test and a statistically significant difference was inferred if $P < 0.05$.

3.10. Molecular modeling

3.10.1. Preperation of ligands

Molecular modeling of the compounds was performed using SPARTAN'04, (Wavefunction, Inc. Irvine, CA. 2004). All initial structures underwent conformational analysis (by use of molecular mechanics) and the lowest energy conformers were subsequently optimized using the semiempirical AM1 method. This procedure produced lowest energy conformations that were used in the docking process.

3.10.2. Selection and preparation of protein crystal structure

The protein crystal structure of human squalene synthase 1EZF was obtained from the Brookhaven Protein Data Bank. The enzyme structure, identified by X-ray diffraction at 2.15 Å resolution, contained a co-crystallized inhibitor codenamed CP-320473 (N-{2-[*trans*-7-chloro-1-(2,2-dimethyl-propyl)-5-naphthalen-1-yl-2-oxo-1,2,3,5-tetrahydrobenzo[*e*] [1,4]oxazepin-3-yl]-acetyl}-aspartic acid) and several water molecules. For the docking studies, all water molecules were removed and the single protein chain, that is, the monomer with the respective bound inhibitor, was used. This preparation procedure was performed using Chimera.[41]

3.10.3. Docking studies

Autodock Tools[42] was used in order to set up the docking runs. All files (protein structure and ligands) were transformed in the appropriate pdbqt formats with proper gasteiger charges assigned. The active site of the protein was easily identified as the cavity containing the co-crystallized ligand CP-320473. The docking search space was defined as the lattice containing the co-crystallized ligand, covering the entire volume of the cavity. Autogrid 4 was then used in order to generate the appropriate grid lattice files for every candidate ligand to be used in the subsequent docking runs.

Docking was performed using Autodock 4's Lamarckian Genetic Algorithm search method.[28] The protein structure for this procedure was kept rigid, while the ligand (compound) structures were assigned (by Autodock Tools) the appropriate degrees of freedom in terms of their flexibility. The genetic algorithm search parameters included: 300 binding modes as the initial algorithm population and 250,000 as the maximum number of energy evaluations. These values are in accordance to the ones suggested for the number of torsions assigned to the ligands (two to three torsions in our case). Default docking parameters (e.g., random number generator, energy outside the grid, number of retries, output format, cluster analysis) were used.

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Supplementary data

Supplementary data (elemental analysis data of all novel compounds (i.e., all final and some intermediates)) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.09.008.

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