Discovery of Novel Phenolic Antioxidants as Inhibitors of Vascular Cell Adhesion Molecule-1 Expression for Use in Chronic Inflammatory Diseases

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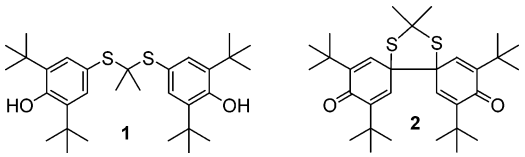
Vascular cell adhesion molecule-1 (VCAM-1) mediates recruitment of leukocytes to endothelial cells and is implicated in many inflammatory conditions. Since part of the signal transduction pathway that regulates the activation of VCAM-1 expression is redox-sensitive, compounds with antioxidant properties may have inhibitory effects on VCAM-1 expression. Novel phenolic compounds have been designed and synthesized starting from probucol (**1**). Many of these compounds demonstrated potent inhibitory effects on cytokine-induced VCAM-1 expression and displayed potent antioxidant effects in vitro. Some of these derivatives (**4o**, **4p**, **4w**, and **4x**) inhibited lipopolysaccharide (LPS)-induced secretion of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 from human peripheral blood mononuclear cells (hPBMCs) in a concentration-dependent manner in vitro and showed antiinflammatory effects in an animal model. Compounds **4ad** and **4ae** are currently in clinical trials for the treatment of rheumatoid arthritis (RA) and prevention of chronic organ transplant rejection, respectively.

Introduction

A key regulator of leukocyte trafficking to sites of inflammation, VCAM-1 has been implicated in numerous inflammatory diseases. It may be the predominant adhesive molecule responsible for migration and adherence of leukocytes to inflamed synovium in rheumatoid arthritis (RA), and soluble VCAM-1 levels have been correlated with clinical severity and progression of RA.[1,2] Increased expression of endothelial adhesion molecules including VCAM-1 has been observed during human renal, cardiac, and liver transplant rejection, and monoclonal antibodies directed against VCAM-1 have been shown to prolong allograft survival in animal models.[3]

It has been established that the activation of VCAM-1 expression on endothelial cells is partly regulated by a redox signal transduction pathway that is sensitive to inhibition by antioxidants.[4,5] In light of the role of antioxidants in the inhibition of VCAM-1 expression, we set out to look into known antioxidants for a starting point to discover novel inhibitors of VCAM-1 expression for use in chronic inflammation.

Probucol (**1**) is a powerful antioxidant with modest lipid-lowering properties.[6,7] It effectively inhibits the oxidative modification of low-density lipoprotein (LDL) independently of its lipid-lowering effect.[8] While probucol has demonstrated a reduction in incidence of restenosis in patients,[9] its clinical use is severely limited by observations that it progressively lowers the levels of HDL,[6] causes significant prolongation of the QTc interval in some patients,[10] and has limited oral bioavailability.[7] Several potentially toxic metabolites of probucol that arise from the highly reactive spiroquinone **2** have been identified.[7]



It is well-known that the strong antioxidant effects of probucol stem from the phenol groups flanked by *tert*-butyl moieties.[7] Since formation of spiroquinone **2** would require oxidation of both phenol groups of probucol, we reasoned that probucol derivatives suitably monosubstituted at one of the two phenol groups might retain the beneficial antioxidant properties (which might be required for inhibition of VCAM-1 expression) through the remaining phenol group, but could have an improved safety profile based on their inability to form the spiroquinone metabolite **2**. We have recently reported[11] our findings with probucol monoesters. A lead compound from that series, AGI-1067 (**3**), exhibited many properties desirable in a molecule to treat atherosclerosis. Compound **3** potently inhibited cytokine-induced endothelial expression of VCAM-1 and MCP-1 and smooth muscle cell proliferation in vitro, as well as progression of atherosclerosis in experimental animals. In clinical trials, **3** did not cause QTc prolongation, while probucol (**1**) did.[12]

Herein we disclose the discovery of novel probucol monoether derivatives **4** as potent inhibitors of VCAM-1 expression with antiinflammatory effects, some of which also have lipid-modulating properties. The ether linkage of this series of compounds renders better chemical

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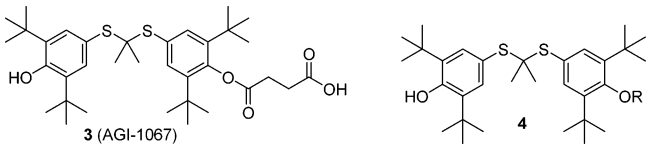


stability than the monoester series and, therefore, enables the synthesis of compounds with a wider variety of functional groups and probably also improves overall in vivo efficacy of the compounds due to potential better metabolic stability.

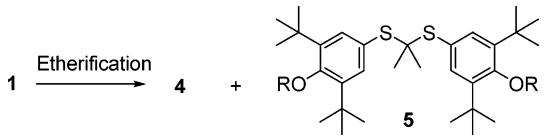
Chemistry

The synthesis of all the compounds reported herein started with alkylation of probucol (**1**) as shown in Scheme 1. Dialkylated products (**5**) were observed in almost all the alkylation reactions, but the desired monoalkylated products (**4**) could be easily isolated using routine column chromatography in moderate isolated yields (up to 40%). As shown in Scheme 2, **1** was coupled with glycidol under Mitsunobu conditions[13] to give epoxide **4a**. Hydrolysis of **4a** under basic conditions in the presence of aqueous ethanol gave **4b** and **4c**. Alternatively, in the presence of acetic acid, acetates **4d** and **4e** were obtained from **4a**. Aminolysis of **4a** was achieved with an amino acid to afford **4f** or **4g** (Scheme 2)**.** Polyether derivative **4h** was obtained in a similar manner as **4a** from **1**. A Mitsunobu reaction of a protected alcohol with probucol (**1**) gave **4i** or **4j** and subsequent deprotection afforded compound **4k** or **4l**. Coupling of probucol with a tartrate-derived diol[14] gave compound **4m**. Compound **4n**, the enantiomer of **4m**, was prepared in the same manner. Intriguingly, neither dialkylated probucol nor diether of the diol was obtained in significant amounts from this reaction. Upon hydrolysis of the protecting ortho ester, alditol **4o** or **4p** (Table 1) was obtained in moderate yields. Similarly, the coupling of probucol with D-ribonic γ-lactone ethyl ortho ester[15] (Scheme 2) gave compound **4q**, deprotection of which produced diol **4r**. Hydrolysis of **4r** gave carboxylic acid **4s**. Reduction of **4s** with lithium aluminum hydride (LAH) yielded lactol **4t** and tetraol **4u**. Reduction of lactone **4r** with LAH also led to **4t**. In a similar manner, probucol was coupled with 1,2,3,4-tetra-*O*-acetyl-β-glucopyranose to give commpound **4v**. Removal of the acetyl groups of **4v** produced lactol **4w**. Reduction of **4w** with LAH afforded pentanol **4x**.

Acrylic derivative **4y** was obtained when probucol was treated with ethyl propiolate (**6**) in the presence of triethylamine (Scheme 3). Compound **4z** (Table 1) was generated as a side product from this reaction. Reduction of compound **4y** with LAH yielded allylic alcohol **4aa**. Alternatively, hydrolysis of **4y** gave carboxylic acid **4ab**.

As shown in Scheme 4, probucol (**1**) was alkylated with ethyl iodoacetate (**7**) in the presence of potassium fluoride on alumina. Hydrolysis of the product (**4ac**) gave compound **4ad**. Compounds **4ae** and **4af** (Table 1) were obtained in a similar manner from **1**. This method failed to produce **4ag** (Table 1). Rather, **4ag** was prepared by treating probucol with β-propiolactone under basic conditions as shown in Scheme 5.[16] Coupling of **4ad** with β-alanine ethyl ester in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) gave **4ah** and subsequent hydrolysis yielded **4ai**. Compounds **4aj**, **4ak**, **4al**, and **4am** (Table 1) were obtained in a similar manner, with or without a deprotection step. Compound **4an** was prepared by treating probucol (**1**) with iodoacetonirile in the presence of potassium fluoride and compound **4ao** derived from treating **4an** with hydroxylamine. Compound **5a** (Scheme 4) was obtained as a side product from a large-scale synthesis of **4ad**.

Scheme 1



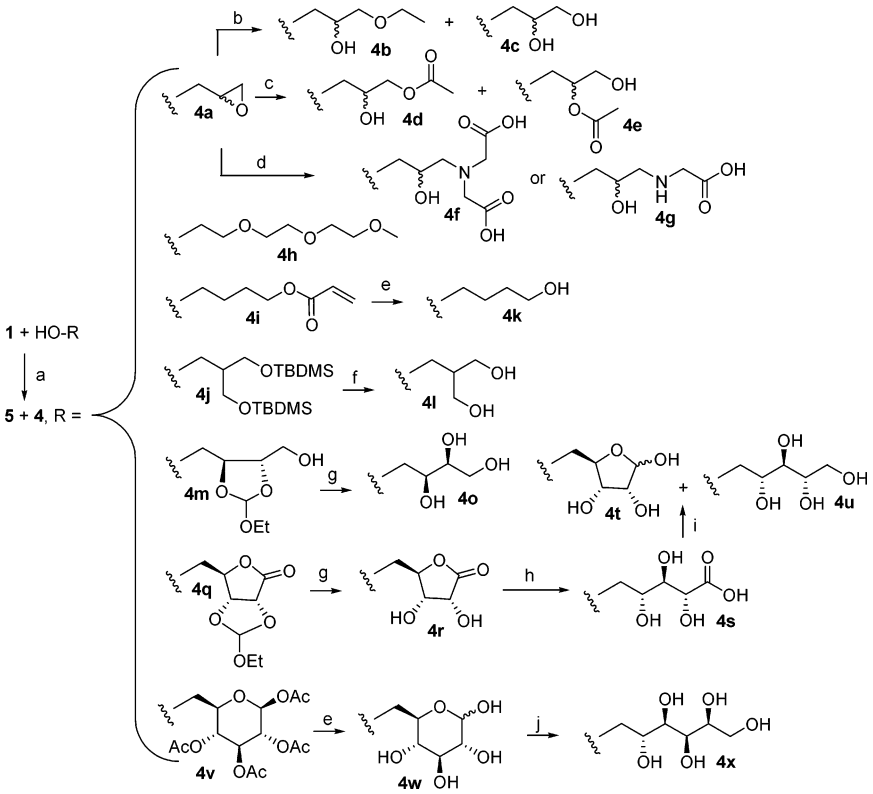
Results and Discussion

As shown in Table 1, probucol (**1**) did not show any inhibitory effect on cytokine-induced VCAM-1 expression in endothelial cells even at high concentration (100 μM). Simple ethers **4a** and **4z** and poly(ethylene glycol) ether **4h** did not show any inhibitory effects at 50 μM. Compounds with one free hydroxy group on the side chain (**4b**, **4aa**, and **4k**) showed weak potencies (IC_{50} > 50 μM). The only difference between **4b** and **4d** is the extra carbonyl group on the side chain in the latter, which probably helps increase the potency (from IC_{50} > 50 μM to IC_{50} = 41 μM) by lowering the lipophilicity of the compound. Compounds with two hydroxy groups on the side chain (**4c** and **4l**) in general showed better potency (IC_{50} = 13−30 μM) than compounds with only one hydroxy group. A comparison of **4c** with **4b** reveals that the removal of the ethyl group from **4b** and resulting decrease in lipophilicity enhances the potency of the product (**4c**) as shown in Table 1. When a methylene unit is inserted between the secondary hydroxyl and the side chain of compound **4c**, the lipophilicity of the resulting **4l** increases slightly (Figure 1), but the potency on inhibition of VCAM-1 expression increases from IC_{50} = 30 μM to 13 μM, rather than decreasing as would be predicted from the above lipophilicity−potency relationship. Another difference between these two compounds is that **4l** has two terminal primary hydroxy groups while **4c** has only one terminal primary hydroxy group. The two regioisomers **4d** and **4e** showed slightly different inhibitory effects, the one with a primary hydroxy group being more potent than the one with a secondary hydroxy group. These results indicate that other factors such as special configuration may also affect the potency of compounds.

When a third hydroxy group is introduced to the side chain of monosubstituted probucol derivatives, the inhibitory potency on VCAM-1 expression is further enhanced. Compound **4o**, having one more hydroxymethylene unit than **4c**, showed an IC_{50} value of 6 μM as compared to 30 μM for **4c** (Table 1). Interestingly, there is a difference in potency between the two enantiomers **4o** and **4p**. The *R,R*-isomer (**4p**) is 6-fold more potent than the *S,S*-isomer (**4o**). This, together with the regioisomeric selectivity discussed above, suggests a well-defined molecular interaction of these inhibitors with a yet undiscovered biological target.

It seems that the introduction of four or more hydroxy groups to the side chain of probucol ether derivatives

Scheme 2[a]



[a] Reagents and conditions: (a) triphenylphosphine, diethyl azodicarboxylate, THF, reflux; (b) 1 N NaOH, EtOH, reflux; (c) AcOH, THF, Et_3N, reflux; (d) iminodiacetic acid or glycine, Et_3N, reflux; (e) MeOH, K_2CO_3, rt; (f) Bu_4NF, THF, rt; (g) 1. MeOH, AcOH, H_2O, reflux; 2. MeOH, K_2CO_3, rt; (h) NaOH, H_2O, THF, rt; (i) LAH, THF, rt; (j) $NaBH_4$, THF, rt.

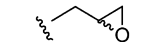
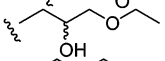
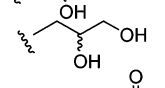
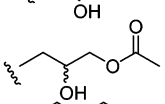
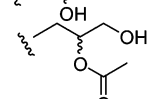
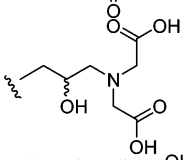
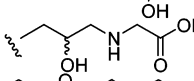
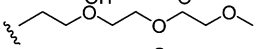
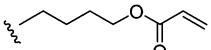
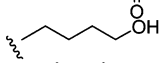
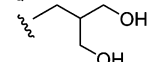
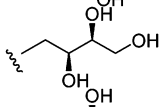
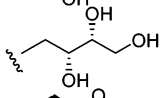
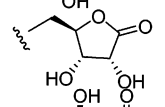
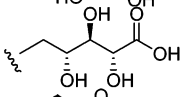
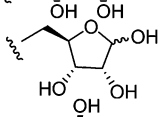
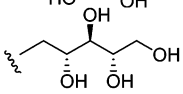
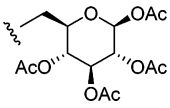
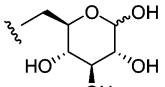
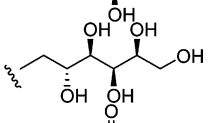
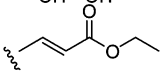
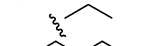
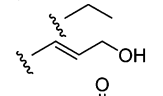
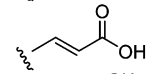
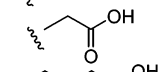
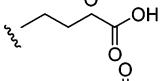
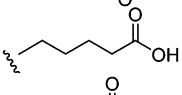
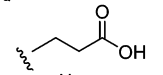
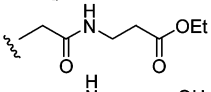
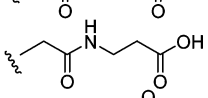
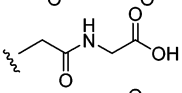
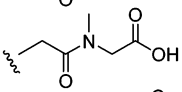
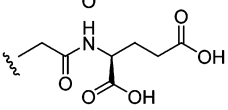
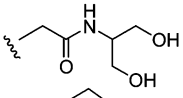
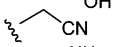
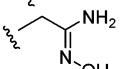
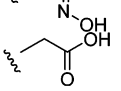
decreases the potency on VCAM-1 expression. Compound **4u**, having one more hydroxy group on the side chain than **4o** or **4p**, showed a higher IC_{50} value (13 μM). Although the stereochemistry of **4u** on the side chain is different from that of **4o** or **4p**, compound **4x**, having five hydroxy groups on the side chain and showing an IC_{50} value of 15 μM, confirms that reduction of lipophilicity beyond a certain point decreases the inhibitory potency of the compounds. The hyperbolic relationship between lipophilicity reflected by calculated logP (ClogP) of these hydroxylated compounds and their in vitro efficacy on inducible VCAM-1 expression is depicted in Figure 1.

A cyclic carbohydrate-like residue on the side chain of probucol derivatives is well tolerated and the trend of potency depending on lipophilicity remains. Compound **4r** with two hydroxy groups and a lactone residue on the side chain showed an IC_{50} value of 13 μM. When the lactone in **4r** is reduced to lactol **4t**, the potency increases to 8 μM because there are three hydroxy groups on the side chain now. When the hydroxy groups on the side chain of compound **4w** are protected with acetyl groups (compound **4v**) the potency is almost eliminated (Table 1), confirming the importance of hydroxy groups in modulating the lipophilicity of compounds.

The trend between potency and lipophilicity observed with the hydroxylated probucol derivatives is also seen with ether-linked carboxylic derivatives. Compound **4ad** showed an IC_{50} value of 10 μM (Table 1). When the side chain of **4ad** is extended by one methylene unit (compound **4ag**), the potency is increased (IC_{50} = 7 μM). However, when the side chain of **4ag** is further extended (compounds **4ae** and **4af**), the potency decreases (IC_{50} = 11 and 23 μM, respectively). The correlation between lipophilicity and efficacy of these closely related carboxylic compounds is depicted in Figure 2; compound **4ag** is in the optimal range of lipophilicity for best potency, compounds **4ad** and **4ae** are slightly off the range, and **4af** is too lipophilic to be very potent. Compound **3**, with the same length of side chain but an extra carbonyl group and hence less lipophilicity compared to **4ae**, is more potent than the latter (Table 1). This further supports the lipophilicity−potency hypothesis discussed above. Although compound **4ag** is the most potent of the four probucol derivatives of simple carboxylic acids, compounds **4ad** and **4ae** were considered for further exploration due to potential metabolic instability of and in vivo production of acrylic acid from **4ag**.

It is worth noting that when a double bond is introduced to the side chain of compound **4ag**, the potency dropped from IC_{50} = 7 μM to 30 μM (**4ab**). The change of lipophilicity alone cannot explain such a dramatic decrease in potency. Presumably, the introduction of a double bond rendered configurational changes in the molecule and, hence, altered the binding pattern of the molecule to the biological target. Therefore, the lipophilicity−potency relationship discussed above is only valid among structurally similar compounds and special configuration precedes lipophilicity in relationship with potency of compounds. The fact that the curves in Figure 1 and Figure 2 stay apart instead of superimposing with each other confirms that the lipophilicity−efficacy relationship reported herein is only valid within closely related series of compounds.

Table 1. Inhibiting Profile of Compounds on VCAM-1 Expression

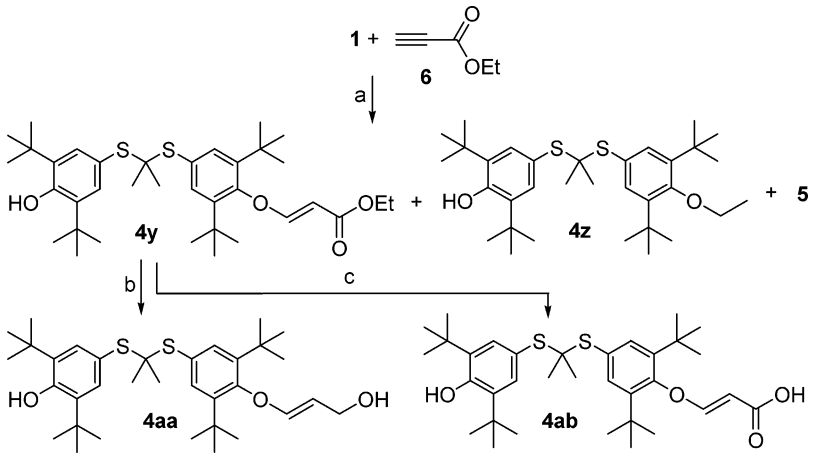
Compd	R	IC_{50} (μM)[a]
1	-	NE100
3	-	6
4a		NE50
4b		>50
4c		30
4d		41±20
4e		33
4f		NE50
4g		16±13
4h		NE50
4i		75
4k		125
4l		13±1
4o		6±3
4p		1±0.1
4r		13
4s		35±3
4t		8
4u		13±2
4v		>50
4w		10±2
4x		15±5
4y		NE50
4z		NE50
4aa		55
4ab		30
4ad		10±5
4ae		11±4
4af		23±5
4ag		7±1
4ah		NE50
4ai		6±1
4aj		6±2
4ak		7±1
4al		75
4am		6±0.4
4an		NE50
4ao		26±16
5a		>50

[a] All IC_{50} numbers reflect an average of at least two determinations, and standard deviations are given for compounds with three or more determinations; "NE50" and "NE100" stand for "no effect at 50 μM" and "no effect at 100 μM," respectively.

Probucol does not belong to either of the series of Figure 1 or Figure 2 and, therefore, cannot be predicted by either curve.

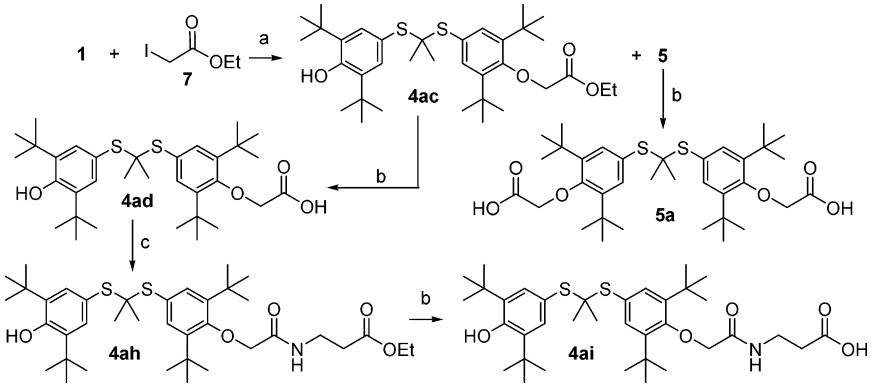
When an amide group is introduced into the side chain of compound **4ad**, the potency remained (compounds **4aj**). Further addition of a methyl group to the nitrogen (compound **4ak**) and a methylene unit into the side chain (compound **4ai**) did not affect the potency much (Table 1). This may mean that the optimal range of lipophilicity for potency is not a very narrow one in this subseries of probucol derivatives. When the terminal hydroxy group of compound **4u** is oxidized to a carboxylic acid (**4s**), the potency dropped from IC_{50} = 13 μM to 35 μM, presumably due to the decrease in lipophilicity and changes in configuration. Moreover, when **4ab** or **4ai** is esterified the potency is completely lost (**4y** or **4ah**) due to increases in lipophilicity. When an amide group is inserted into the side chain of **4l**, the potency (compound **4am**) increases from IC_{50} of 13 μM to 6 μM, possibly due to the decrease in lipophilicity brought in by the amide group. Compounds **4am** and **4aj** are both amides and equipotent, but one has a

Scheme 3[a]



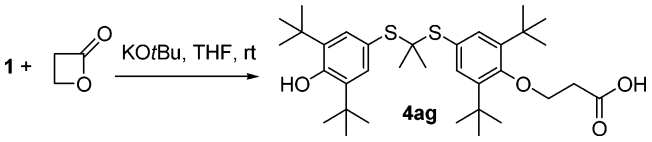
[a] Reagents and conditions: (a) Et_3N, THF, reflux; (b) LAH, THF, rt; (c) LiOH, THF, H_2O, rt.

Scheme 4[a]



[a] Reagents and conditions: (a) KF on alumina, DMF; (b) LiOH, THF, MeOH, H_2O; (c) β-alanine ethyl ester hydrochloride, Et_3N, EDCI, CH_2Cl_2.

Scheme 5



terminal carboxy group and the other has two terminal hydroxy groups. This again shows that neither a carboxy nor a hydroxy group is essential for inhibitory activity on VCAM-1 expression; rather, it is the correct range of lipophilicity that matters.

Too much hydrophilicity is detrimental to the inhibitory efficacy on VCAM-1 expression in the carboxylic derivatives of probucol, as well. Compared to compound **4ae**, compound **4s** with three extra hydroxy groups on the side chain is more hydrophilic and less potent in the in vitro assay with an IC_{50} value of 35 μM (Table 1). Compound **4g** has an IC_{50} value of 16 μM. When an additional carboxymethyl group is introduced to the molecule (**4f**), the potency is eliminated possibly due to a decrease in lipophilicity. Similarly, compound **4al**, with an additional propionic group than **4aj**, is dramatically less potent than the latter (Table 1). Compound **4ad** shows an IC_{50} of 10 μM on the inhibition of VCAM-1 expression. When the carboxy group of **4ad** is replaced with a cyano group or an *N*-hydroxyamidino group, the potency is completely lost (compound **4an**, no effect at 50 μM) or dramatically decreased (compound **4ao,** IC_{50} = 26 μM), presumably due to changes of lipophilicity.

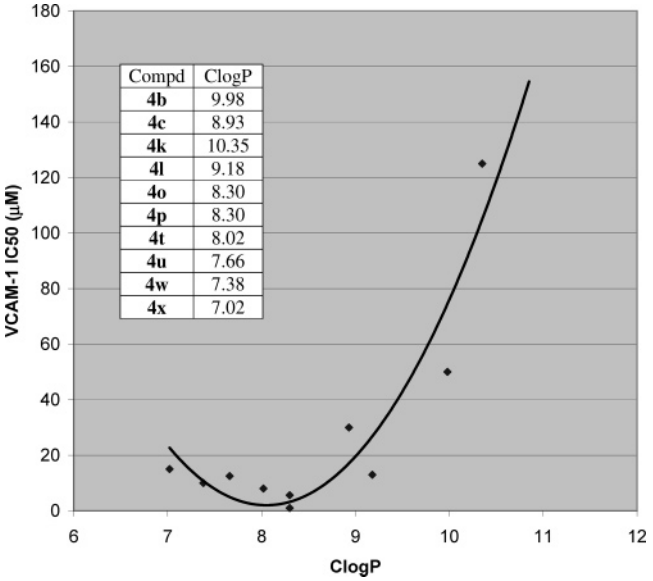


Figure 1. Correlation between calculated logP and in vitro inhibitory effect on inducible VCAM-1 expression of hydroxylated ether compounds.

In our hands probucol exhibited no effect on VCAM-1 expression at concentrations up to 100 μM. Others have reported that probucol (**1**) in vivo reduced the level of basal VCAM-1 expression and prevented its upregulation.[17] Since probucol is known to inhibit the oxidation of LDL and oxidized LDL may induce VCAM-1 expres-

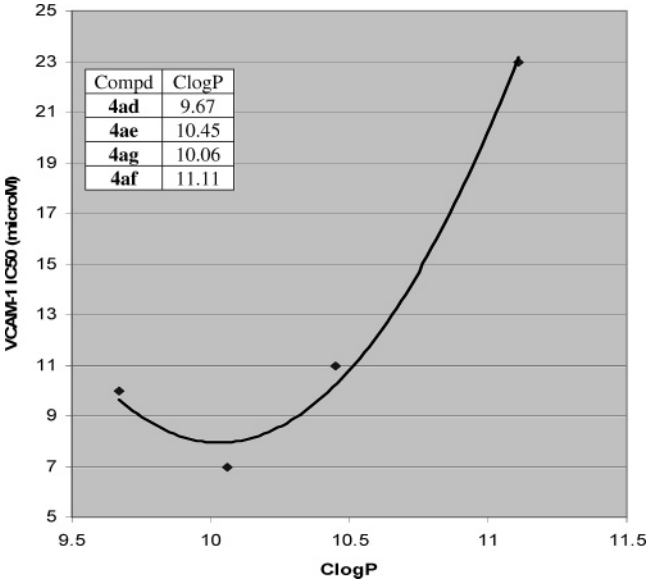


Figure 2. Correlation between calculated logP and in vitro inhibitory effect on inducible VCAM-1 expression of ether-linked carboxylic compounds.

Table 2. Antioxidant Profile of Selected Compounds against Oxidation of *N*-Benzoyl Leucomethylene Blue[a]

compd	IC_{50} (μM)
1	14 ± 2
4o	17 ± 2
4p	15 ± 5
4w	10 ± 2
4x	12 ± 3
5a	>125

[a] A literature protocol was followed;[18] all IC_{50} numbers reflect an average of at least three determinations.

sion, the in vivo inhibitory effect of probucol on VCAM-1 expression could well be due to its inhibitory effect on the oxidation of LDL rather than any direct effect on VCAM-1 expression.[17]

Although they could be defined in various ways, many antioxidants are inhibitors of lipid peroxidation. Selected compounds from this program were assayed for their ability to inhibit the hemoglobin-catalyzed oxidation of *N*-benzoyl leucomethylene blue (LMB assay)[18] by the peroxide generated from the oxidation of linoleic acid by 15-lipoxygenase. As shown in Table 2, probucol (**1**), compounds **4o**, **4p**, **4w**, and **4x** all showed similar potencies in this assay. However, compound **5a**, with both of the phenol groups of probucol substituted, did not show any antioxidant property in this assay, confirming the phenol group in these compounds as the source of the antioxidant property. Interestingly, probucol, with two phenol groups in each molecule, is not significantly more potent than its ether derivatives with only one phenol group. This may mean that after one of the two phenol groups of probucol is consumed as an antioxidant, the other one cannot be an antioxidant any more.

As reported previously,[11] when both phenol groups of probucol are acylated and, hence, the antioxidant property is eliminated, the product does not show any inhibitory effect on VCAM-1 expression. Similarly, monoether derivative **4ad** exhibits an IC_{50} value of 10 μM, while its diether analogue **5a** is inactive on VCAM-1 expression. Since **5a** does not have any antioxidant

Table 3. Inhibitory Profile of Selected Compounds on LPS-Induced Secretion of Cytokines from HPBMN Cells[a]

compd	TNF-α (IC_{50}, μM)	IL-1β (IC_{50}, μM)	IL-6 (IC_{50}, μM)
1	>40	>40	>40
4o	4.0 ± 1.0	7.7 ± 0.6	1.7 ± 0.9
4p	3.3 ± 0.3	8.0 ± 0.0	1.2 ± 0.3
4w	1.9 ± 0.7	*b*	1.6 ± 0.6
4x	1.8 ± 0.3	2.2 ± 1.6	1.0 ± 0.3
5a	>10[c]	>10[c]	>10[c]

[a] All IC_{50} numbers reflect an average of at least three determinations. [b] No dose response was observed, but the compound was as potent as **4p** at 10 μM. [c] Unable to test at higher concentrations due to insolubility of the compound.

properties as discussed above, these results further suggest the involvement of a redox pathway in VCAM-1 expression.

Cytokines are extracellular signaling proteins produced by many cell types playing a central role in human immune response, and can be categorized as either pro-inflammatory or antiinflammatory in action. TNF-α, IL-1β and IL-6 are major pro-inflammatory cytokines implicated in the pathogenesis of numerous diseases. The expression of these pro-inflammatory cytokines is also redox-regulated.[19] Selected compounds were tested for their inhibitory effects on the LPS-induced secretion of these three cytokines from hPBMCs in vitro. As shown in Table 3, compounds **4o**, **4p**, **4w**, and **4x** exhibited potent inhibitory effects on the secretion of TNF-α ($IC_{50} = 1.8-4.0$ μM) and IL-6 ($IC_{50} = 1.0-1.7$ μM). On the inhibition of IL-1β secretion, compound **4x** showed an IC_{50} value of 2.2 μM while compounds **4o** and **4p** had an IC_{50} value around 8 μM. Compound **4w** did not show a dose response in the assay, but was as potent as **4p** on the inhibition of IL-1β secretion at 10 μM. Compounds **4o**, **4p**, **4w**, and **4x** have a free phenol group in their structures, are antioxidants, and are inhibitors of VCAM-1 expression as discussed above. On the other hand, compound **5a** does not have a free phenol group, is not an antioxidant, and does not inhibit VCAM-1 expression (Table 1). Similarly **5a** did not show a potent inhibitory effect on the secretion of any of the three cytokines (Table 3). Therefore, the inhibitory effects of the compounds on cytokine secretion could also be related to their antioxidant effects. Probucol (**1**), though a strong antioxidant, was completely ineffective in inhibiting the secretion of any of the three cytokines (Table 3). This further indicates that properly designed monosubstituted probucol derivatives can enter cells or have access to cell membranes and affect intracellular signaling more readily than probucol itself. In support of these contentions, it has been found that there is a significant difference in cell uptake between probucol (**1**) and **3**.[20]

The methylated bovine serum albumin (mBSA)-induced delayed-type hypersensitivity (DTH) model of mice is often used for screening compounds with anti-inflammatory effects.[21] Four poly-hydroxylated compounds (**4o**, **4p**, **4w**, and **4x**) among others (whose results will be reported separately[23]) were tested in this model, dosed subcutaneously at 24 and 2 h prior to challenge at doses of 50 and 25 mg/kg with cyclosporin A (CSA) as positive control. As shown in Figure 3, all four compounds showed significant inhibition (58−69%) at 50 mg/kg and moderate inhibition (29−46%) at 25 mg/kg. The four compounds tested in the DTH model

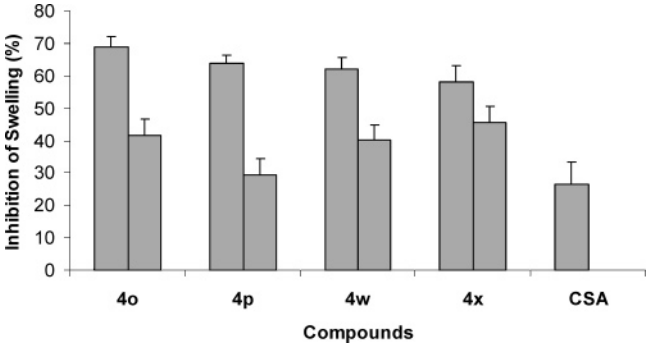


Figure 3. Inhibitory profile of compounds on paw swelling in the DTH model of inflammation (s.c. dosing; left column: 50 mg/kg, right column: 25 mg/kg dosing, CSA: 20 mg/kg).

Table 4. Effects of Selected Compounds on Cholesterol Levels in Hypercholesterolemic Hamsters

	control group		treated group		% change[a]	
compd	LDL	HDL	LDL	HDL	ΔLDL	ΔHDL
4g	241 ± 17	104 ± 7	190 ± 17	146 ± 13	−21	+ 41*
4s	262 ± 84	133 ± 10	175 ± 31	173 ± 12	−33	+30
4u	241 ± 17	104 ± 7	338 ± 10	119 ± 3	+ 40	+ 15
4w	300 ± 30	278 ± 13	9 ± 7	63 ± 5	−97*	−77*
4x	145 ± 18	153 ± 9	30 ± 5	94 ± 6	−79	−39*
4ad	227 ± 16	109 ± 6	18 ± 4	86 ± 9	−92*	−21*

[a] *p < 0.05 determined by ANOVA, followed by Fisher's PLSD Post Hoc test.

were well tolerated except for that **4o** resulted in some weight loss at the higher dose.

A few compounds were administered as admixtures in the chow at an approximate dose of 150 mg/kg/d to hypercholesterolemic hamsters for lipid-modulating effects. As shown in Table 4, compounds **4w**, **4x**, and **4ad** lowered LDL levels dramatically (79−97%). However, these compounds also lowered HDL levels significantly (21−77%). Compounds **4g** and **4s** showed interesting profiles. They lowered LDL levels by 21−33% and raised HDL levels by 30−41%. These results bolster our original proposal that properly modified probucol derivatives could be deprived of the detrimental HDL-lowering effect of probucol itself. Compounds **4u** and **4x** are homologous polyols. Compound **4x**, with its side chain just one carbon unit longer than that of **4u**, had a dramatic LDL-lowering effect, while the latter increased LDL levels. It is worth noting that the two compounds are also different in stereochemistry on their side chains, which cannot be ruled out as the possible cause of the different outcomes. Also interestingly, when the terminal hydroxyl group of **4u** is oxidized to the corresponding carboxylic acid (**4s**), the detrimental LDL-elevating effect is eliminated. However, it cannot be generalized that a terminal hydroxyl group is not good for LDL lowering, because **4x** showed dramatic LDL-lowering effect. Rather, certain lipophilicity and structural configuration could be required for probucol derivatives to properly bind to LDL or HDL particles to lower LDL levels or elevate HDL levels.[22] Thus, structural modification of compounds of Table 3 could lead to drug candidates with dual LDL-lowering and HDL-elevating effects.

In the mouse DTH model described above, compounds **4p**, **4x**, and **4w** also lowered total cholesterol levels in a dose-related manner while **4o** had little effect. The fact that compounds **4o** and **4p** are enantiomers and have dramatically different effects on lipid metabolism suggests that the two compounds may bind differently on lipoprotein particles and that stereochemistry plays a major role.

Based on the overall profile of potent in vitro efficacy on VCAM-1 expression, scalability of synthesis, tolerability in animals and favorable efficacy in models of inflammation, etc., a few compounds from the series reported herein were scaled to kilogram quantities and tested in animal models of chronic inflammatory diseases. Compound **4ad** (AGIX-4207) inhibited the onset of paw edema in a collagen II-sensitized rat model of RA in a dose-dependent manner when given orally. It also significantly enhanced the effect of a suboptimal dose of dexamethasone in this model.[23] Compound **4ae** (AGI-1096) inhibited arteriosclerosis in allograft recipients of rats, suggesting that it may be a promising new agent for the prevention of allograft rejection in organ transplantation.[24] Detailed pharmacological studies on these two compounds will be reported separately. Compounds **4ad** and **4ae** are currently in clinical trials for the treatment of RA and the prevention of organ transplant rejection, respectively.

In summary, a series of novel antiinflammatory compounds has been designed and synthesized starting from a well-known antioxidant, probucol (**1**), with the intention of maintaining the antioxidant properties and eliminating the potential adverse effects of QTc prolongation and HDL lowering. Many of these compounds showed potent inhibitory effects on VCAM-1 expression. A correlation between efficacy and lipophilicity has been observed. This series of monosubstituted probucol ethers showed potent antioxidant effects equivalent to probucol in vitro. Some of the derivatives also inhibited LPS-induced secretion of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 in a concentration-dependent manner in vitro and, therefore, are potential candidates or lead compounds for a wide variety of diseases, in which these cytokines are implicated. Probucol, though as strong an antioxidant in homogeneous solution, did not show any effect on the secretion of these cytokines at similar concentrations, indicating that properly designed derivatives of probucol may enter cells or have access to cell membranes more readily than probucol itself. Selected compounds have shown antiinflammatory effects in an animal model of delayed-type hypersensitivity. Some compounds have exhibited dramatic lipid-modulating effects in hypercholesterolemic hamsters. Especially of interest is the fact that some compounds elevated HDL levels, which suggests that properly modified derivatives of probucol can be deprived of the untoward HDL-lowering effect of probucol itself.

The research program reported herein has led to the discovery of two clinical candidates, i.e., compound **4ad** for RA and compound **4ae** for organ transplant rejection. By inhibiting VCAM-1 expression, a crucial component of RA pathology, compound **4ad** represents a potentially novel approach to the treatment of RA. As will be reported separately, this compound possesses numerous favorable preclinical pharmacological properties that suggest it may show therapeutic benefit for RA. Compound **4ae** addresses the serious issues of accelerated arteriosclerotic formation and inflammation at the allograft site through inhibition of VCAM-1 expression.

Used as a monotherapy or in combination with an immunosuppressant, this compound may become an effective drug for the prevention of chronic organ transplant rejection. The overall utility of this approach for beneficial therapies in chronic inflammatory diseases remains to be demonstrated with clinical trials.

Experimental Section

Chemistry. Melting points are uncorrected. ^{1}H NMR spectra were recorded on a Varian 400 MHz or a QE300 spectrometer and chemical shifts are reported in parts per million (ppm, δ) relative to tetramethylsilane as internal standard. Mass spectra were obtained on a VG 70S (for EI) or Micromass Q-TOF (for ES) instrument. Elemental analyses (C, H, N, S) were performed by Atlantic Microlabs, Norcross, Georgia. Silica gel 60 (E. Merck, 230−400 mesh) was used for preparative column chromatography. Calculated logP was obtained by using LeadScope PC Professional 2.0.11. All compounds reported herein had 95% or higher purity according to ^{1}H NMR spectra.

2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-oxiranylmethoxyphenylsulfanyl)-1-methyl-ethylsulfanyl]phenol (4a). To a solution of probucol (2.58 g, 5 mmol) in THF (50 mL) cooled to 0 °C were added glycidol (0.66 mL, 10 mmol), triphenylphosphine (2.62 g, 10 mmol), and diethyl azodicarboxylate (1.57 mL, 10 mmol). The resultant mixture was stirred under nitrogen at reflux for 48 h and then evaporated. Silica gel chromatography (hexanes/dichloromethane 4:1, 2:1) gave the title compound as a viscous residue (1.01 g, 35%) which solidified on standing, mp 139−141 °C. ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.39 (s, 1H, Ph-O*H*), 4.04 (dd, 1H, PhOCH_2), 3.75 (dd, 1H, PhOCH_2), 3.36−3.42 (m, 1H, PhOCH$_2$C*H*), 2.90 (dd, 1H, epoxide CH_2), 2.77 (dd, 1H, epoxide CH_2), 1.40−1.49 (m, 42H, *tert*-butyls, *S,S′*-isopropylidene). MS *m/z*: 671 ([M + K]$^+$, 100%).

2,6-Di-*tert*-butyl-4-{1-[3,5-di-*tert*-butyl-4-(3-ethoxy-2-hydroxypropoxy)phenylsulfanyl]-1-methyl-ethylsulfanyl}-phenol (4b) and 3-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}propane-1,2-diol (4c). To a suspension of **4a** (320 mg, 0.56 mmol) in ethanol (10 mL) was added 1 N sodium hydroxide solution (1.5 mL), and the resultant mixture was stirred at reflux for 72 h and then evaporated. The residue was distributed between ethyl acetate (50 mL) and brine (50 mL). The organic phase was washed with brine (50 mL), dried over magnesium sulfate, and evaporated. Silica gel chromatography (hexanes/dichloromethane 1:1, dichloromethane, and then dichloromethane/ethyl acetate 4:1) afforded **4c** (viscous solid, 58 mg, 18%), mp 63−65 °C and **4b** (off-white solid, 52 mg, 15%), mp 98−100 °C. **4b**: ^{1}H NMR ($CDCl_3$) δ 7.55 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.38 (s, 1H, Ph-O*H*), 4.30−4.39 [m, 1H, $CH_2CH(OH)CH_2$], 3.82 (d, 2H, PhOCH_2), 3.53−3.65 (m, 4H, CH_2OCH_2CH$_3$), 1.43−1.46 (m, 42H, *tert*-butyls and *S,S′*-isopropylidene), 1.22 (t, 3H, OCH$_2$CH_3). MS *m/z*: 657 ([M + K]$^+$, 100%). **4c**: ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.37 (s, 1H, Ph-O*H*), 4.29−4.36 [m, 1H, $CH_2CH(OH)CH_2$], 3.93 [dd, J = 8, 9 Hz, 1H, OCH_2CH(OH)], 3.83 [dd, J = 3, 11 Hz, 1H, OCH_2CH(OH)CH_2OH], 3.75 [dd, J = 4, 9 Hz, 1H, OCH_2CH(OH)], 3.66 [dd, J = 6, 11 Hz, OCH_2-CH(OH)CH_2OH], 1.45 (s, 6H, *S,S′*-isopropylidene), 1.44 (s, 18H, *tert*-butyls), 1.43 (s, 1H, *tert*-butyls). MS *m/z*: 629 ([M + K]$^+$, 80%), 353 (100%). Anal. ($C_{34}H_{54}O_4S_2\cdot H_2O$) C, H, S.

Acetic Acid 3-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}-2-hydroxypropyl Ester (4d) and Acetic Acid 2-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}-1-hydroxymethylethyl Ester (4e). To a solution of **4a** (339 mg, 0.56 mmol) in THF (15 mL) was added acetic acid (10 mL) and the mixture was stirred for 5 min. Triethylamine (10 mL) was added and the resultant mixture was stirred at reflux for 72 h and then evaporated. The residue was dissolved in dichloromethane (150 mL), washed with water (3 × 100 mL), dried over magnesium sulfate. The solution was poured onto the top of a silica gel column which was eluted with hexanes/ethyl acetate (2:1). Compounds **4d** (viscous residue, 75 mg, 21%) and **4e** (viscous residue, 6.3 mg, 2%) were obtained. **4d**: ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.38 (s, 1H, Ph-O*H*), 4.44−4.50 (m, 1H, C*H*OH), 4.20−4.31 (m, 2H, CH_2-OAc), 3.90 (dd, 1H, PhOC*H*), 3.81(dd, 1H, PhOC*H*), 2.09 (s, 3H, OAc), 1.40−1.52 (m, 42H, *tert*-butyls, *S,S′*-isopropylidene). MS *m/z*: 671 ([M + K]$^+$, 100%). **4e**: ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.53−5.46 (m, 1H, C*H*OAc), 5.39 (s, 1H, Ph-O*H*), 4.02 (dd, 1H, PhOC*H*), 3.81−3.93 (m, 1H, CH_2OH, PhOC*H*), 2.18 (s, 3H, OAc), 1.42−1.49 (m, 42H, *tert*-butyls, *S,S′*-isopropylidene). MS *m/z*: 650 ([M + NH$_4$]$^+$, 30%), 395 (100%).

[*N*-Carboxymethyl-(3-{2,6-di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]-phenoxy}-2-hydroxypropyl)amino]acetic Acid (4f). To a solution of **4a** (538 mg, 0.89 mmol) in DMF (5 mL) were added iminodiacetic acid (1.18 g, 8.9 mmol) and triethylamine (2 mL, 27 mmol), and the mixture was stirred at reflux overnight. Upon cooling to room temperature it was poured into water (100 mL), extracted with dichloromethane (3 × 100 mL), dried over magnesium sulfate, and evaporated. Silica gel chromatography (dichloromethane/methanol 40:1 to 1:1) gave the title compound as a viscous residue (121 mg, 19%). ^{1}H NMR ($CDCl_3$) δ 7.54 (s, 2H, Ph-*H*), 7.52 (d, 1H, NH), 7.44 (s, 2H, Ph-*H*), 5.40 (s, 1H, PH−O*H*), 4.23−4.30 (m, 1H, C*H*OH), 3.81−3.87 (dd, 1H, PhOC*H*), 3.65−3.72 (dd, 1H, PhOC*H*), 3.44 (s, 4H, NCH_2-COOH), 2.91−3.00 [m, 1H, (OH)CHCH_2N], 2.40−2.49 [m, 1H, (OH)CHCH_2N], 1.39−1.48 (m, 44H, *S,S′*-isopropylidene, *tert*-butyls, CH_2CH$_2$COOH). MS *m/z*: 618 ([M − 2 (COOH) + H]$^+$, 100%).

(3-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}-2-hydroxypropylamino)acetic Acid (4 g). To a suspension of **4a** (0.17 g, 0.28 mmol) in ethanol (10 mL) were added glycine (43 mg, 0.57 mmol) and triethylamine (1 mL, 13.6 mmol). The resultant mixture was stirred under nitrogen at reflux overnight and then evaporated. Silica gel chromatography (dichloromethane/methanol 10:1 to 1:1) gave the title compound as a white solid (99 mg, 55%), mp 180−184 °C (dec). ^{1}H NMR ($CDCl_3$) δ 7.52 (s, 2H, Ph-*H*), 7.43 (s, 2H, Ph-*H*), 5.37 (s, 1H, Ph-O*H*), 4.58 (br. s, 1H, $CH_2CH(OH)CH_2$), 3.60−3.84 (m, 5H, OCH_2C*H*(OH)CH$_2$NHCH_2), 3.15−3.37 [m, 2H, OCH$_2$CH(OH)-CH_2NHCH$_2$], 3.09−3.15 (m, 1H, NH), 1.43 (s, 18H, *tert*-butyls), 1.41 (s, 6H, *S,S′*-isopropylidene), 1.38 (s, 18H, *tert*-butyls). MS *m/z*: 648 ([M + H]$^+$, 60%), 410 (100%). Anal. ($C_{36}H_{57}NO_5S_2\cdot H_2O$) C, H, N, S.

2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy}phenylsulfanyl)-1-methylethylsulfanyl]phenol (4h). To a solution of probucol (2.0 g, 3.9 mmol) and tri(ethylene glycol) monomethyl ether (1.27 g, 7.7 mmol) in tetrahydrofuran (40 mL) was added triphenylphosphine (2.0 g, 7.7 mmol), and the resulting mixture was cooled to 0 °C. Diethyl azodicarboxylate (1.3 g, 7.7 mmol) was then added dropwise. The mixture was stirred at 0 °C for 30 min and allowed to warm to room temperature. The solution was ultimately warmed to 40 °C and stirred for an additional 2 h. The reaction mixture was concentrated under reduced pressure to a brown oil. Silica gel chromatography (10−30% ethyl acetate/hexanes) afforded 0.96 g (40%) of the expected ether as a viscous yellow oil. R_f 0.25 (30% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$) δ 7.53 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.36 (s, 1H, Ph-O*H*), 3.89 (br s, 4H, Ph-OCH_2CH_2O), 3.67−3.75 (m, 6H, OCH_2CH_2OCH_2), 3.56−3.58 (m, 2H, OCH_2), 3.39 (s, 3H, OCH_3), 1.42−1.44 (m, 42H, *tert*-butyls and *S,S*-isopropylidene). MS (ESI) *m/z*: 685 ([M + Na]$^+$, 100%). HRMS (ESI) calcd for $C_{38}H_{62}O_5S_2$ (M + K), 701.3676; found, 701.3649. Anal. ($C_{38}H_{62}O_5S_2$) C, H, S.

Acrylic Acid 4-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]-phenoxy}butyl Ester (4i) and 2,6-Di-*tert*-butyl-4-{1-[3,5-di-*tert*-butyl-4-(4-hydroxybutoxy)phenylsulfanyl]-1-methylethylsulfanyl}phenol (4k). To a solution of probucol

(2.58 g, 5 mmol) in THF (50 mL) were added 4-hydroxybutyl acrylate (1.0 mL, 10 mmol), triphenylphosphine (2.62 g, 10 mmol), and diethyl azodicarboxylate (1.57 mL, 10 mmol). The resultant mixture was stirred under nitrogen at reflux for 72 h and then evaporated. Silica gel chromatography (hexanes/dichloromethane 4:1) gave **4i** as a brown oily residue (0.92 g, 29%). ^{1}H NMR ($CDCl_3$) δ 7.54 (s, 2H, Ph-*H*), 7.46 (s, 2H, Ph-*H*), 6.42 [dd, 1H, OC(O)CH=C*Hcis*], 6.14 [dd, 1H, OC(O)-C*H*=CH_2], 5.84 [dd, 1H, OC(O)CH=C*Htrans*], 5.38 (s, 1H, PhO*H*), 4.23 [t, 2H, CH_2OC(O)], 3.75 (t, 2H, PhOCH_2), 1.92−2.00 (m, 2H, PhOCH_2CH_2), 1.78−1.86 (m, 2H, PhOCH_2CH_2CH_2), 1.46 (s, 6H, *S,S′*-isopropylidene), 1.45 (s, 18H, *tert*-butyls), 1.42 (s, 18H, *tert*-butyls). To a suspension of this compound (0.82 g, 1.28 mmol) in methanol (20 mL) was added potassium carbonate (0.5 g, 3.6 mmol), and the resultant mixture was stirred under nitrogen at room temperature overnight. It was poured into water (50 mL), extracted with dichloromethane (2 × 50 mL), dried over magnesium sulfate and evaporated. Silica gel chromatography (hexanes/ethyl acetate 4:1) gave **4k** as a colorless viscous residue (0.52 g, 70%) which solidified on standing, mp 106−108 °C. ^{1}H NMR ($CDCl_3$) δ 7.54 (s, 2H, Ph-*H*), 7.46 (s, 2H, Ph-*H*), 5.38 (s, 1H, Ph-O*H*), 3.71−3.77 (m, 4H, OCH_2CH_2CH_2CH_2OH), 1.92−1.99 (m, 2H, PhOCH_2CH_2), 1.1.66−1.74 (m, 2H, CH_2CH_2OH), 1.39−1.52 (m, 42H, *S,S′*-isopropylidene, *tert*-butyls). MS *m/z*: 627 ([M + K]$^+$, 85%), 351 (100%).

2-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxymethyl}propane1,3-diol (4l). To a solution of probucol (1.0 g, 1.9 mmol) and 3-(*tert*-butyldimethylsilanyloxy)-2-(*tert*-butyldimethylsilanyloxymethyl)propan-1-ol[25] (1.3 g, 3.9 mmol) in THF (20 mL) was added triphenylphosphine (1.0 g, 3.9 mmol), and the resulting mixture was cooled to 0 °C. Diethyl azodicarboxylate (0.60 g, 3.9 mmol) was then added dropwise. The mixture was stirred at 0 °C for 30 min and allowed to warm to room temperature. The solution was ultimately warmed to 50 °C and stirred for an additional 18 h. The reaction mixture was concentrated under reduced pressure to a brown oil and subjected to silica gel chromatography (100% hexanes then 1−5% CH_2Cl_2/hexanes) to afford 0.33 g (20%) of 2,6-di-*tert*-butyl-4-(1-{3,5-di-*tert*-butyl-4-[3-(*tert*-butyl-dimethyl-silanyloxy)-2-(*tert*-butyl-dimethyl-silanyloxymethyl)-propoxy]phenylsulfanyl}-1-methyl-ethylsulfanyl)phenol (**4j**) as a yellow foam (mixed with trace amounts of starting probucol). R_f 0.61 (10% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$) δ 7.53 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.35 (s, 1H, Ph-O*H*), 3.76−3.81 (m, 6H, CH_2CH(CH_3)$_3$), 2.34−2.38 (m, 1H, CH_2C*H*), 1.41−1.44 (m, 42H, *tert*-butyls and *S,S*-isopropylidene), 0.88 9s, 18H, SiC(CH_3)$_3$], 0.04 [s, 12H, Si(CH_3)$_2$]. MS (ESI) *m/z*: 855 ([M + Na]$^+$, 90%), 595 (100%). To a solution containing the above silyl ether (0.10 g, 0.12 mmol) in THF (10 mL) was added tetrabutylammonium fluoride (1 M in tetrahydrofuran, 0.4 mL), and the reaction was stirred at room temperature for 2 h. The solution was ultimately warmed to 35 °C and stirred for an additional 2 h. The reaction mixture was diluted with a 50% aqueous solution of ammonium chloride (20 mL) and extracted with methylene chloride (3 × 20 mL). The combined organic layers were washed with water (2 × 10 mL), brine (1 × 10 mL) and dried over sodium sulfate, and the solvent was removed under reduced pressure. The crude residue was purified by silica gel chromatography (ethyl acetate/hexanes 1:1) to give 0.032 g (45%) of the title compound as a pale yellow foam. R_f 0.34 (50% ethyl acetate/hexanes). ^{1}H NMR ($CDCl_3$) δ 7.55 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.36 (s, 1H, Ph-O*H*), 3.83−4.02 (m, 6H, CH_2CH(CH_2)$_2$), 2.50−2.56 (m, 1H, CH_2C*H*), 2.03−2.07 (m, 2H, CH_2O*H*); 1.42−1.44 (m, 42H, *tert*-butyls and *S,S*-isopropylidene). MS (EI) *m/z*: 604 (M$^+$, 0.1%), 279 (100%). HRMS (ESI) calcd for $C_{35}H_{56}O_4S_2$ (M + K), 643.3257; found 643.3255. Anal. ($C_{35}H_{56}O_4S_2$) C, H, S.

4-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}butane-1,2-(*S*),3(*S*)triol (4o). To a solution of probucol (5.16 g, 10 mmol) in THF (20 mL) cooled to 0 °C were added triphenylphosphine (1.3 g, 5 mmol), diethyl azodicarboxylate (0.8 mL g, 5 mmol), and 2-ethoxy-1,3-dioxolane-4(*S*),5(*S*)-dimethanol[14] (0.89 g, 5 mmol). The resultant mixture was stirred at reflux for 3 h and then evaporated. Silica gel chromatography (hexanes/ethyl acetate 4:1) gave three parts: diasteroisomer A (0.36 g), diastereoisomer B (0.22 g) and a mixture of the two (0.72 g) all as viscous residues (total yield: 39%). Diastereoisomer A: ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.89 [s, 1H, C*H*(O)$_3$], 5.37 (s, 1H, PhO*H*), 4.73−4.81 (m, 1H, PhOCH_2C*H*), 4.19−4.25 (m, 1H, C*H*CH_2OH), 3.80−4.01 (m, 3H, CH_2O), 3.62−3.96 (m, 3H, CH_2O), 2.80 (dd, J = 9, 3, 1H, OH), 1.45 (s, 6H, *S,S′*-isopropylidene), 1.44 (s, 18H, *tert*-butyls), 1.43 (s, 18H, *tert*-butyls), 1.25 (t, 3H, OCH_2C*H*3). Diasteroisomer B: ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.92 [s, 1H, C*H*(O)$_3$], 5.36 (s, 1H, PhO*H*), 4.50−4.59 (m, 1H, PhOCH_2C*H*), 4.13−4.30 (m, 3H, C*H*CH_2OH, CH_2O), 3.89−4.00 (m, 3H, CH_2O), 3.61 (quad, J = 7, 2H, OCH_2CH$_3$), 1.86 (dd, J = 6, 8, 1H, OH), 1.41−1.46 (m, 42H, *S,S′*-isopropylidene, *tert*-butyls), 1.21 (t, 3H, OCH_2CH_3). The mixture obtained above (0.72 g, 1.1 mmol) was dissolved in methanol (25 mL). Acetic acid (2 mL) and water (1 mL) were added, and the resultant mixture was stirred at reflux for 3 h and then evaporated to about 10 mL. It was poured into water (100 mL) and extracted with dichloromethane (2 × 100 mL). The organic phase was dried over magnesium sulfate and evaporated. The residue was dissolved in methanol (15 mL), and potassium carbonate (0.5 g) was added. The mixture was stirred at room temperature for 1 h and then poured into 1 N HCl solution (100 mL). It was extracted with dichloromethane (2 × 100 mL), dried over magnesium sulfate, and evaporated. Silica gel chromatography (dichloromethane/ethyl acetate 4:1) gave the title compound as a white powder (0.45 g, 66%), mp 69−71 °C. ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.36 (s, 1H, PhO*H*), 4.26−4.34 (m, 1H, PhOCH_2C*H*), 4.01 (dd, J = 9, 10, 1H, C*H*-O-), 3.72−3.86 (m, 4H,, C*H*-O-), 2.69−2.77 (m, 2H, OH), 2.15 (br. t, 1H, OH), 1.42−1.47 (m, 42H, *S,S′*-isopropylidene, *tert*-butyls). MS *m/z*: 643 ([M + Na]$^+$, 100%). Anal. ($C_{35}H_{56}O_5S_2$·1/3H_2O) C, H, S.

4-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]-phenoxy}butane-1,2(*R*),3(*R*)-triol (4p). This compound was prepared using the same procedure as for **4o**, mp 69−71 °C. ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.38 (s, 1H, PhO*H*), 4.25−4.32 (m, 1H, PhOCH_2C*H*), 3.96 (dd, J = 9, 10, 1H, C*H*-O-), 3.72−3.86 (m, 4H, C*H*-O-), 3.00−3.40 (m, 3H, OH), 1.46 (s, 6H, *S,S′*-isopropylidene), 1.45 (s, 18H, *tert*-butyls), 1.43 (s, 18H, *tert*-butyls). MS *m/z*: 643 ([M + Na]$^+$, 100%). Anal. ($C_{35}H_{56}O_5S_2$.1/3H_2O) C, H, S.

5-O-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenyl}-D-ribonic Acid γ-Lactone (4r). To a solution of probucol (3.0 g, 5.88 mmol) in THF (40 mL) were added triphenylphosphine (1.54 g, 5.88 mmol), diethyl azodicarboxylate (0.93 mL g, 5.88 mmol), and 2,3-*O*-(ethoxymethylene)-D-ribonic acid γ-lactone[15] (1 g, 4.89 mmol). The resultant mixture was stirred at reflux for 3 h and then evaporated. Silica gel chromatography (hexanes/ether 4:1) gave 457 mg (13%) of 5-*O*-{2,6-i-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxy-phenylsulfanyl)-1-methyl-ethylsulfanyl]-phenyl}-2,3-*O*-(ethoxymethylene)-D-ribonic acid γ-lactone as a viscous residue. ^{1}H NMR ($CDCl_3$) δ 7.57 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 6.02 [s, 1H, C*H*(O)$_3$], 5.37 (s, 1H, PhO*H*), 4.98−5.15 (m, 2H, C*H*-O-), 4.88 (br. s, 1H, C*H*-O-), 4.08 (br. s, 2H, PhOCH_2), 3.42−3.78 (m, 2H, OCH_2CH$_3$), 1.38−1.49 (m, 42H, *S,S′*-isopropylidene, *tert*-butyls), 1.18−1.36 (m, 3H, OCH2C*H*3). To a solution of this compound (450 mg, 0.64 mmol) in methanol (80 mL) was added a mixture of acetic acid/water (4:1, 10 mL), and the resultant mixture was stirred at room temperature overnight. Most of the solvent was evaporated and the remaining solution was neutralized with 1 N NaOH solution. It was extracted with dichloromethane, dried, and evaporated to give the title compound as a white solid (41 mg, 98%), mp 91−93 °C. ^{1}H NMR ($CDCl_3$) δ 7.58 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.38 (s, 1H, PhO*H*), 4.80−4.85 (m, 1H, C*H*-O-), 4.69−4.75 (m, 2H, C*H*-O-), 4.07 (br. s, 2H, PhOCH_2), 1.41−

1.50 (m, 42H, *S,S′*-isopropylidene, *tert*-butyls), 1.18−1.36 (m, 3H, OCH_2CH_3). MS *m/z*: 685 ([M + K]$^+$, 100%).

5-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}-2(*R*),3(*R*),4(*R*)-trihydroxypentanoic Acid (4s). To a solution of **4r** (200 mg, 0.31 mmol) in THF (5 mL) was added 1.2 mL of 1 N NaOH solution, and the mixture was stirred at room temperature for 3 h. The mixture was adjusted to pH = 3 with 1 N HCl, extracted with dichloromethane, dried over magnesium sulfate, and evaporated to give the title compound as a viscous solid (107 mg, 53%), mp 67−69 °C. ^{1}H NMR ($CDCl_3$) δ 7.57 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.37 (s, 1H, PhO*H*), 4.48 (dd, *J* = 8, 13 Hz, 1H, C*H*OH), 4.38 [d, *J* = 6 Hz, 1H, C*H*(OH)COOH], 4.03 (d, *J* = 6 Hz, 2H, $PhOCH_2$), 3.95 [dd, *J* = 6, 7 Hz, 1H, C*H*OH), 1.46 (s, 6H, *S,S′*-isopropylidene), 1.44 (s, 36H, *tert*-butyls). MS *m/z*: 703 ([M + K]$^+$, 100%).

5-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}pentane-1,2(*S*),3-(*S*),4(*R*)tetraol (4u) and 5-*O*-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methyl-ethylsulfanyl]phenyl}-D-ribose (4t). To a solution of **4s** (4 g, 6.16 mmol) in THF (150 mL) was added LAH (1 M in ether, 12.3 mL) slowly, and the mixture was stirred at room temperature for 2 h. Reaction was incomplete. Additional LAH solution (6.1 mL) was added and the mixture stirred for 1 h. A third charge of LAH solution (2 mL) was added again and stirred for 1 h. The reaction was carefully quenched with saturated sodium sulfate (50 mL), and the mixture was stirred overnight. Ether was added to the mixture, and it was filtered. The filtrate was dried over magnesium sulfate and evaporated. Silica gel chromatography (hexanes/ether 1:1 to 100% ether) gave **4r** (980 mg, 25%), **4t** (600 mg, 15%), and **4u** (600 mg, 15%). **4u**: Off-white solid, mp 105−110 °C. ^{1}H NMR ($CDCl_3$) δ 7.57 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.37 (s, 1H, PhO*H*), 4.38 (dd, *J* = 6, 13, 1H, C*H*-O-), 4.02 (d, *J* = 7, 2H, $PhOCH_2$), 3.82−3.88 (m, 1H, C*H*-O-), 3.86−3.90 (m, 2H, CH_2OH), 3.74 (dd, *J* = 6, 7, 1H, C*H*-O-), 1.45 (s, 6H, *S,S′*-isopropylidene), 1.44 (s, 36H, *tert*-butyls). MS *m/z*: 689 ([M + K]$^+$, 100%). **4t**: Off-white solid, mp 138−141°C. ^{1}H NMR ($CDCl_3$) δ 7.52 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.43 (d, 1H, anomeric H), 5.34 (s, 1H, PhO*H*), 4.44−4.49 (m, 1H, C*H*-O-), 4.12−4.19 (m, 2H, C*H*-O-), 3.87−3.91 (m, 2H, $PhOCH_2$), 1.37−1.45 (m, 42H, *S,S′*-isopropylidene, *tert*-butyls). MS *m/z*: 687 ([M + K]$^+$, 100%). Anal. ($C_{36}H_{56}O_6S_2$) C, H, S.

6-*O*-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenyl}-1, 2, 3, 4-tetra-*O*-acetyl-β-D-glucopyranose (4v). To a solution of probucol (1.8 g, 3.5 mmol) in THF (20 mL) were added 1,2,3,4-tetra-*O*-actyl-β-D-glucopyranose (1.0 g, 2.9 mmol), triphenylphosphine (0.92 g, 3.5 mmol), and diethyl azodicarboxylate (0.55 mL, 3.5 mmol). The resultant mixture was stirred under nitrogen at reflux for 2 h and then evaporated. Silica gel chromatography (hexanes/ethyl acetate 4:1) gave the title compound as an off-white solid (0.92 g, 31%), 149−151 °C. ^{1}H NMR ($CDCl_3$) δ 7.53 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.80 (d, 1H, anomeric H), 5.38 (s, 1H, Ph-O*H*), 5.33 (dd, 1H, C*H*OAc), 5.16 (dd, 1H, C*H*OAc), 4.90 (dd, 1H, C*H*OAc), 4.15−4.23 (m, 1H, glucose C5-*H*), 3.88 (dd, 1H, Ph-OC*H*), 3.74 (dd, 1H, Ph-OC*H*), 2.14 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.45 (s, 24H, *tert*-butyls, *S,S′*-isopropylidene), 1.38 (s, 18H, *tert*-butyls). MS *m/z*: 885 ([M + K]$^+$, 25%), 279 (100%).

6-O-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenyl}-D-glucopyranose (4w). To a suspension of **4v** (0.68 g, 0.080 mmol) in methanol (50 mL) was added potassium carbonate (1 g, 7.2 mmol), and the mixture was stirred under nitrogen at room temperature overnight. It was poured into water (200 mL), extracted with ethyl acetate (3 × 150 mL), washed with brine (100 mL), dried over magnesium sulfate, and evaporated. Silica gel chromatography (dichloromethane/methanol 10:1 to 5:1) gave the title compound as an off-white solid (0.26 g, 48%), mp 94−99 °C. ^{1}H NMR ($CDCl_3$) δ 7.52 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.36 (s, 1H, Ph-O*H*), [5.31 (br. s) and 4.78 (br. s, 1H, anomeric H)], 3.30−4.38 (br. m, 6H, $PhOCH_2$ and glucose H), 1.38−1.43 (m, 42H, *tert*-butyls, *S,S′*-isopropylidene). MS *m/z*: 701 ([M + Na]$^+$, 100%). Anal. ($C_{37}H_{58}O_7S_2 \cdot 1/3H_2O$) C, H, S.

6-*O*-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenyl}-D-glucitol (4x). To a solution of **4w** (70 mg, 0.10 mmol) in THF (5 mL) was added sodium borohydride, and the mixture was stirred under nitrogen at room temperature for 2 h. Saturated ammonium chloride (2 mL) was added and the mixture stirred for another 1 h, poured into water (50 mL), and extracted with dichloromethane (3 × 50 mL). The organic phase was dried over magnesium sulfate and evaporated. Silica gel chromatography (dichloromethane/methanol 100:12) gave the title compound as a white solid (19 mg, 27%), mp 109−113 °C. ^{1}H NMR ($CDCl_3$) δ 7.54 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.36 (s, 1H, PhO*H*), 4.35 (br. m, 1H, C*H*OH), 3.30−4.10 (m, 7H, $PhOCH_2$ and C*H*OH), 1.39 (s, 24H, *tert*-butyls, *S,S′*-isopropylidene), 1.38 (s, 18H, *tert*-butyls). MS *m/z*: 703 ([M + Na]$^+$, 100%). Anal. ($C_{37}H_{60}O_7S_2 \cdot H_2O$) C, H, S.

3-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}acrylic Acid Ethyl Ester (4y) and 2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-ethoxyphenylsulfanyl)-1-methylethylsulfanyl]-phenol (4z). To a solution of probucol (5.16 g, 10 mmol) in THF (50 mL) were added ethyl propiolate (1.2 mL, 12 mmol) and triethylamine (7 mL, 50 mmol). The resultant mixture was stirred under nitrogen at reflux for 72 h. After being cooled to room temperature, it was poured into brine (100 mL), extracted with dichloromethame (3 × 100 mL), dried over magnesium sulfate, and evaporated. Silica gel chromatography (hexanes/dichloromethane 9:1 to straight dichloromethane) gave compounds **4y** (viscous residue, 0.37 g, 6%) and **4z** (colorless solid, 509 mg, 9%), mp 138−140 °C. **4y**: ^{1}H NMR ($CDCl_3$) δ 7.63 (s, 2H, Ph-*H*), 7.43 (s, 2H, Ph-*H*), 6.40 (d, 1H, OC*H*=CH), 5.39 (s, 1H, Ph-O*H*), 5.03 (dd, 1H, OCH=C*H*), 4.26 (quad, 2H, OCH_2CH_3), 1.38−1.55 (m, 42H, *tert*-butyls, *S,S′*-isopropylidene), 1.31 (t, 3H, OCH_2CH_3). MS *m/z*: 615 ([M + H]$^+$, 50%), 377 (100%). **4z**: ^{1}H NMR ($CDCl_3$) δ 7.55 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-H), 5.38 (s, 1H, Ph-O*H*), 3.76 (quad, 2H, OCH_2CH_3), 1.38−1.51 (s, 45H, *tert*-butyls, *S,S′*-isopropylidene, OCH_2CH_3). MS *m/z*: 583 ([M + K]$^+$, 25%), 279 (100%).

2,6-Di-*tert*-butyl-4-{1-[3,5-di-*tert*-butyl-4-(3-hydroxypropenyloxy)phenylsulfanyl]-1-methylethylsulfanyl}phenol (4aa). To a solution of **4y** (65 mg, 0.1 mmol) in THF (15 mL) was added lithium aluminum hydride (1 mL, 1 M solution in THF), and the resultant mixture was stirred at room temperature overnight. The reaction was carefully quenched with saturated ammonium chloride solution (20 mL) and stirred for 0.5 h at room temperature. The mixture was extracted with dichloromethane (3 × 50 mL), dried over sodium sulfate, and evaporated. Silica gel chromatography (hexanes/ethyl acetate 4:1) gave the title compound as an oily residue (46 mg, 80%). ^{1}H NMR ($CDCl_3$) δ 7.61 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 6.00 (d, 1H, OC*H*=CH), 5.40 (s, 1H, Ph-O*H*), 4.84 (dd, 1H, OCH=C*H*), 4.45 (dd, 2H, CH_2OH), 1.35−1.48 (m, 42H, *tert*-butyls, *S,S′*-isopropylidene). MS *m/z*: 611 ([M + K]$^+$, 55%), 279 (100%).

3-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}acrylic Acid (4ab). To a solution of **4y** (0.16 g, 0.26 mmol) in THF (5 mL) were added water (2 mL) and lithium hydroxide monohydrate (42 mg, 1 mmol). The resultant mixture was stirred at reflux overnight. After being cooled to room temperature, it was poured into dichloromethane (50 mL), washed with brine, dried over magnesium sulfate, and evaporated. Silica gel chromatography (hexanes/ethyl acetate 4:1) gave the title compound as a viscous residue (22 mg, 14%) which solidified on standing, mp 213-215 °C. ^{1}H NMR ($CDCl_3$) δ 7.63 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 6.52 (d, 1H, OC*H*=CH), 5.39 (s, 1H, Ph-O*H*), 5.08 (d, 1H, OCH=C*H*), 1.47 (s, 6H, *S,S′*-isopropylidene), 1.44 (s, 18H, *tert*-butyls), 1.42 (s, 18H, *tert*-butyls). MS *m/z*: 625 ([M + K]$^+$, 75%), 159 (100%).

Acetic Acid, [4-[[1-[[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]thio]-1-methylethyl]thio]-2,6-bis(1,1-dimethylethyl)phenoxy]- (4ad). To a solution of probucol (0.5 g, 0.97 mmol) in dimethylformamide (1.5 mL) were added ethyl iodoacetate (0.31 g, 1.45 mmol) and 40% potassium fluoride on alumina (0.7 g, 4.8 mmol). The mixture was stirred for 24 h and then diluted with ether (25 mL), filtered, and washed with water (2 × 5 mL). The ether layer was dried over $MgSO_4$, filtered, and concentrated. Silica gel chromatography (hexanes/ether 5:95) yielded 160 mg of {2,6-di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}-acetic acid ethyl ester, which was dissolved in THF/H_2O/MeOH (4:1:1) (4 mL), and lithium hydroxide hydrate (50 mg) was added. The resultant mixture was stirred for 1 h and then neutralized with 1 N HCl. It was extracted with ether (2 × 10 mL), dried over $MgSO_4$, filtered, and concentrated. Silica gel chromatography (hexanes/ether 50:50) gave the title compound as a white solid (90 mg, 16%), mp 164−165 °C. ^{1}H NMR ($CDCl_3$) δ 7.55 (s, 2H, Ph-*H*), 7.40 (s, 2H, Ph-*H*), 5.35 (s, 1H, Ph-O*H*), 4.40 (s, 2H, OCH_2COOH), 1.43 (s, 6H, *S,S′*-isopropylidene), 1.41 (s, 9H, *tert*-butyl), 1.39 (s, 9H, *tert*-butyl). MS *m/z*: 613 ([M + K]$^+$, 60%), 159 (100%). Anal. ($C_{33}H_{50}O_4S_2$) C, H, S.

Butanoic Acid, 4-[4-[[1-[[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]thio]-1-methylethyl]thio]-2,6-bis(1,1-dimethylethyl)phenoxy]- (4ae). This compound was prepared using a similar procedure as for **4ad** using methyl 4-iodobutyrate and KF on alumina. Off-white solid, mp 138−139 °C. ^{1}H NMR ($CDCl_3$) δ 7.53 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.37 (s, 1H, Ph-O*H*), 3.77 (t, J = 6.8 Hz, 2H, OCH_2CH_2), 2.55 (t, J = 7.6 Hz, 2H, CH_2COOH), 2.16 (m, 2H, OCH$_2$CH_2), 1.44 (s, 24H, *tert*-butyls and *S,S′*-isopropylidene), 1.41 (s, 18H, *tert*-butyls). MS *m/z*: 641 ([M + K]$^+$, 100%). Anal. ($C_{35}H_{54}O_4S_2$) C, H, S.

5-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}pentanoic Acid (4af). This compound was prepared in similar procedure as for **4ad**. White solid, mp 60−63 °C. ^{1}H NMR ($CDCl_3$) δ 7.54 (s, 2H, Ph-*H*), 7.46 (s, 2H, Ph-*H*), 5.37 (s, 1H, Ph-O*H*), 3.73 (t, J = 7 Hz, 2H, PhOCH_2), 2.46 (t, J = 7 Hz, 2H, CH_2COOH), 1.87−1.99 (m, 2H, CH$_2$CH_2CH$_2$), 1.74−1.83 (m, 2H, CH$_2$CH_2-CH$_2$), (1.45 (s, 24H, *tert*-butyls and *S,S′*-isopropylidene), 1.42 (s, 18H, *tert*-butyls). MS *m/z*: 616 ([M + K]$^+$, 100%).

3-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}propionic Acid (4ag). To a solution of probucol (2.58 g, 5 mmol) in THF (30 mL) were added potassium *tert*-butoxide (0.56 g, 5 mmol) and β-propiolactone (0.9 g, 12.5 mmol), and the mixture was stirred at room temperature for 1.5 h. Water (100 mL) was added and the mixture was adjusted to pH = 1 with 1 N HCl solution. It was extracted with dichloromethane, washed with water, dried over magnesium sulfate, and evaporated. Silica gel chromatography (hexanes/ethyl acetate 4:1) gave the title compound as a white solid (200 mg, 68%), mp 160−163 °C. ^{1}H NMR ($CDCl_3$) δ 7.54 (s, 2H, Ph-*H*), 7.444 (s, 2H, Ph-*H*), 5.36 (s, 1H, Ph-O*H*), 4.01 (t, J = 7 Hz, 2H, PhOCH_2), 2.95 (t, J = 7 Hz, 2H, CH_2COOH), 1.44 (s, 24H, *tert*-butyls and *S,S′*-isopropylidene), 1.41 (s, 18H, *tert*-butyls). MS *m/z*: 588 ([M]$^+$, 0.1%), 279 (100%).

3-(2-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}acetylamino)propionic Acid Ethyl Ester (4ah). This compound was prepared in a similar procedure as for **4aj**. Off-white solid, mp 116−117 °C ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.43 (s, 2H, Ph-*H*), 7.31 (t, J = 6, 1H, NH), 5.37 (s, 1H, Ph-O*H*), 4.24 (s, 2H, PhOCH_2), 4.17 (quad, J = 7, 2H, OCH_2CH$_3$), 3.69 (quad, J = 6, 2H, NHCH_2), 2.61 (t, J = 6, NHCH$_2$CH_2), 1.45 (s, 6H, *S,S′*-isopropylidene), 1.44 (s, 18H, *tert*-butyls), 1.39 (s, 18H, *tert*-butyls), 1.28 (t, J = 7, 3H, OCH$_2$CH_3). MS *m/z*: 696 ([M + Na]$^+$, 100%). Anal. ($C_{38}H_{59}NO_5S_2$) C, H, N.

3-(2-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}-acetylamino)propionic Acid (4ai). This compound was prepared in a similar procedure as for **4aj**. Off-white solid, mp 183−184 °C. ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.43 (s, 2H, Ph-*H*), 7.33 (t, J = 6, 1H, NH), 5.37 (s, 1H, Ph-O*H*), 4.25 (s, 2H, PhOCH_2), 4.69 (quad, J = 6, 2H, NHCH_2), 2.68 (t, J = 6, NHCH$_2$CH_2), 1.45 (s, 6H, *S,S′*-isopropylidene), 1.44 (s, 18H, *tert*-butyls), 1.38 (s, 18H, *tert*-butyls). MS *m/z*: 668 ([M + Na]$^+$, 100%). Anal. ($C_{36}H_{55}NO_5S_2$) C, H, N, S.

(2-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}acetylamino)-acetic Acid (4aj). To a solution of **4ad** (50 mg, 0.087 mmol) in methylene chloride (0.87 mL) were added glycine ethyl ester hydrochloride (15.8 mg, 0.11 mmol), 1-(3-dimethylaminopropyl-3-ethyl carbodiimide hydrochloride (22 mg, 0.11 mmol), and 4-(dimethylamino)pyridine (28 mg, 0.23 mmol). The reaction mixture was stirred overnight, and then the methylene chloride was evaporated. The reaction was diluted with ether (10 mL), washed with water (2 × 3 mL), dried over $MgSO_4$, filtered, and concentrated. The crude mixture was purified by silica gel chromatography (ether/hexanes 50:50) to give 50 mg of (2-{2,6-di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxy-phenylsulfanyl)-1-methyl-ethylsulfanyl]phenoxy}acetylamino)acetic acid ethyl ester, which was dissolved in THF/H_2O/MeOH (2:1:1, 1 mL), lithium hydroxide monohydrate (15 mg, 0.36 mmol) was added, and the reaction was stirred for 1 h. The reaction was neutralized with 1 N HCl and extracted with ether (2 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to give the title product as a viscous residue (25 mg, 45%) which solidified, mp 91−94 °C. ^{1}H NMR ($CDCl_3$) δ 7.56 (s, 2H, Ph-*H*), 7.42 (s, 2H, Ph-*H*), 7.28 (t, 1H, NH), 5.39 (br s, 1H, Ph-O*H*), 4.31 [s, 2H, OCH_2C(O)], 4.22 (d, J = 5.2 Hz, 2H, NHCH_2COOH), 1.44 (s, 6H, *S,S′*-isopropylidene), 1.42 (s, 18H, *tert*-butyls), 1.39 (s, 18H, *tert*-butyls). MS *m/z*: 654 ([M + Na]$^+$, 100%). Anal. ($C_{35}H_{53}NO_5S_2$·1/2H_2O) C, H, N, S.

[(2-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}acetyl)-methylamino]acetic Acid (4ak). This compound was prepared in a similar procedure as for **4aj**. Off-white solid, mp 115−119 °C. ^{1}H NMR ($CDCl_3$) δ 7.58 (s, 2H, Ph-*H*), 7.45 (s, 2H, Ph-*H*), 5.37 (s, 1H, Ph-O*H*), 4.52 (s, 2H, PhOCH_2), 4.24 (s, 2H, NCH_2), 3.03 (s, 3H, NCH_3), 1.47 (s, 6H, *S,S′*-isopropylidene), 1.44 (s, 18H, *tert*-butyls), 1.43 (s, 18H, *tert*-butyls). MS *m/z*: 668 ([M + Na]$^+$, 100%). Anal. ($C_{36}H_{55}NO_5S_2$) C, H, N, S.

2-(2-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methyl-ethylsulfanyl]phenoxy}acetylamino)pentanedioic Acid (4al). This compound was prepared in a similar procedure as for **4aj**. Viscous residue. ^{1}H NMR ($CDCl_3$) δ 7.57 (s, 2H, Ph-*H*), 7.52 (d, 1H, NH), 7.42 (s, 2H, Ph-*H*), 5.37 (s, 1H, PH−O*H*), 4.81−4.88 (m, 1H, NHC*H*-COOH), 4.28 (s, 2H, PhOCH_2), 2.53−2.59 (m, 2H, CH$_2$CH_2-COOH), 1.39−1.45 (m, 44H, *S,S′*-isopropylidene, *tert*-butyls, CH_2CH$_2$COOH). MS *m/z*: 742 ([M + K]$^+$, 70%), 159 (100%). Anal. ($C_{38}H_{57}NO_7S_2$) C, H, N, S.

2-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methyl-ethylsulfanyl]phenoxy}-N−(2-hydroxy-1-hydroxymethyl-ethyl)acetamide (4am). To a solution of **4ad** (4.87 g, 8.49 mmol) in dichloromethane (200 mL) were added serinol (0.77 g, 8.49 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.26 g, 17 mmol), and the mixture was stirred at room temperature overnight. It was poured into water (100 mL), and the organic phase was dried over magnesium sulfate and evaporated. Silica gel chromatography (hexanes/ethyl acetate 1:2) gave the title compound as a white solid (2.95 g, 57%), mp 163−164 °C. ^{1}H NMR ($CDCl_3$) δ 7.57 (s, 2H, Ph-*H*), 7.49 (d, J = 7, 1 H, NH), 7.44 (s, 2H, Ph-*H*), 5.37 (s, 1H, Ph-O*H*), 4.28 (s, 2H, PhOCH_2), 4.10−4.29 (m, 1H, NHC*H*), 3.82−4.00 (m, 4H, CH_2OH), 1.45 (s, 6H, *S,S′*-isopropylidene), 2.44 (dd, J = 6, 11, 2H, OH), 1.44 (s, 18H, *tert*-butyls), 1.41 (s, 18H, *tert*-butyls). MS *m/z*: 670 ([M + Na]$^+$, 100%). Anal. ($C_{36}H_{57}NO_5S_2$) C, H, N, S.

{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}-acetonitrile (4an). To a solution of probucol (2.0 g, 3.9 mmol) and iodoacetonitrile (1.1 g, 6.8 mmol) in tetrahydrofuran (40 mL) was added potassium fluoride (1.1 g, 7.7 mmol, 40% on aluminum), and the resulting mixture was stirred at room

temperature for 1 h. The reaction mixture was filtered through a pad of Celite and rinsed with fresh portions of ethyl acetate (100 mL). The filtrate was washed with a saturated sodium bisulfite solution (4 × 75 mL) and brine (1 × 75 mL), dried over sodium sulfate, and concentrated under reduced pressure to a dark oil. Silica gel chromatography (10% ethyl acetate/hexanes) afforded 0.35 g (20%) of the title compound as a pale yellow solid, mp 139−141 °C. R_f 0.27 (10% ethyl acetate/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (s, 2H, Ph-*H*), 7.43 (s, 2H, Ph-*H*), 5.37 (s, 1H, Ph-O*H*), 4.48 (s, 2H, CH_2−CN), 1.42−1.45 (m, 42H, *tert*-butyls and *S,S′*-isopropylidene). LRMS (ESI) *m/z* (%) 555 (M + Na, 100). HRMS (ESI) calcd for $C_{33}H_{49}NO_2S_2$ (M + K), 594.2842; found, 594.2841. Anal. ($C_{33}H_{49}NO_2S_2$) C, H, N, S.

2-{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-hydroxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}-*N*-hydroxyacetamidine (4ao). Hydroxylamine hydrochloride (0.032 g, 0.47 mmol) was added to a mixture of triethylamine (0.047 g, 0.47 mmol) in 50% aqueous ethanol (0.5 mL), and the mixture was stirred for five minutes. Compound **4an** (0.20 g, 0.36 mmol) in ethanol (2.0 mL) was added, and the reaction was refluxed for 5 h, cooled to room temperature, and diluted with water (5 mL). The resulting precipitate was collected on filter paper and rinsed with water. The crude material was tritarated with hexanes to remove any residual probucol starting material and dried in vacuo to furnish 0.20 g (95%) of the title compound as a pale yellow solid, mp 185−186 °C. R_f 0.1 (10% ethyl acetate/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 2H, Ph-*H*), 7.44 (s, 2H, Ph-*H*), 5.95 (s, 1H, N−O*H*), 5.36 (s, 1H, Ph-O*H*), 4.98 (brs, 2H, NH_2), 4.33 (s, 2H, CH_2), 1.42−1.46 (m, 42H, *tert*-butyls and *S,S′*-isopropylidene). LRMS (ESI) *m/z* (%) 589 (M + H, 50), 351 (100). HRMS (ESI) calcd for $C_{33}H_{52}N_2O_3S_2$ (M + H), 589.3497; found, 589.3503. Anal. ($C_{33}H_{52}N_2O_3S_2$) C, H, N, S.

{2,6-Di-*tert*-butyl-4-[1-(3,5-di-*tert*-butyl-4-carboxymethoxyphenylsulfanyl)-1-methylethylsulfanyl]phenoxy}-acetic Acid (5a). This compound was obtained as a side product in a large-scale synthesis of **4ad**, a white solid, mp 250−253 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.48 (s, 4H, Ph-*H*), 4.25 (s, 4H, CO−CH_2−O), 1.42 (s, 6H, *S,S′*-isopropylidene), 1.37 (s, 36H, *tert*-butyls). HRMS (ESI) calcd for $C_{35}H_{52}O_6S_2$ (M + K), 671.2842; found, 671.2825. Anal. ($C_{35}H_{52}O_6S_2$) C, H, S.

In Vitro VCAM-1 Assay. Cultured primary human aortic (HAEC) or pulmonary artery (HPAEC) endothelial cells were obtained from Clonetics and were used below passage 9. Cells were seeded in 96-well plates such that they reached 90−95% confluency by the following day. On the following day cells were stimulated with TNF-α (1 ng/mL) in the presence or absence of compounds dissolved in DMSO such that the final concentration of DMSO was 0.25%. Cells were exposed to TNF-α and compounds for approximately 16 h. Then cells were examined under microscope to score for visual sign of toxicity or cell stress. The media was discarded, and cells were washed once with Hanks Balanced Salt Solution (HBSS)/phosphate-buffered saline (PBS) (1:1). Primary antibody against VCAM-1 (0.25 μg/mL in HBSS/PBS + 5% FBS) was added, and cells were incubated for 30 min at 37 °C. Cells were washed with HBSS/PBS three times, then secondary antibody Horseradish Peroxidase (HRP)-conjugated goat anti-mouse IgG (1:500 in HBSS/PBS + 5% FBS) was added, and cells were incubated for 30 min at 37 °C. Cells were then washed with HBSS/PBS four times and 3,3′,5,5′-tetramethylbenzidine (TMB) was added, and cells were incubated at room temperature in the dark until there was adequate development of blue color. The length of time of incubation was typically 5−15 min. Sulfuric acid (2 N) was added to stop the color development, and the data were collected by reading the absorbance on a BioRad ELISA plate reader at OD 450 nm. The results were expressed as IC_{50} values (the concentration of test compound required to inhibit 50% of the maximal response of the control sample stimulated by TNF-α only). IC_{50} numbers reflect an average of at least two determinations.

LMB Assay. A published procedure was followed (only the method described in Figure 4 of the reference was used).[18]

Cytokine Screening. Fresh cryopreserved hPBMCs (1 million cells/mL; Clonetics, Inc.) were pretreated with test compound for 1 h in lymphocyte growth media-3 (Clonetics, Inc.), followed by stimulation with 1 μg/mL of LPS for another 2 h in the presence of test compound. Conditioned media was collected and assayed for secreted TNF-α, IL-6 and IL-1β using commercially available human ELISA kits (R&D Systems). Samples were measured in duplicate and data presented as mean ± standard deviation. Each experiment was repeated at least three times with similar results.

DTH Model of Inflammation in Mice. Male BALB/c mice (20−25 g) were sensitized on day 0 by intradermal abdominal injection of 100 μL of a 1:1 emulsion of mBSA (5 mg/mL) and Freund's complete adjuvant. On day 7, the mice were challenged by injecting 25 μL of mBSA (5 mg/mL) into the footpad of the right hindpaw, and the left hindpaw was injected with saline. Mice were treated by subcutaneous injection of test compound 24 h and 2 h before the challenge. Twenty four hours after challenge mice were sacrificed by CO_2 inhalation. The feet were removed by cutting just above the heel with scissors, and the mass of the saline-injected foot was subtracted from the mass of the mBSA-injected foot to determine the amount of swelling that occurred in the latter. Inhibition of swelling for test compound group was calculated, taking the swelling of the vehicle-treated group as 100%.

Hamster Lipid Screening. Male Golden Syrian hamsters weighing 110−120 g (Charles River Laboratories, Wilmington, MA) were housed individually with wood chip bedding and soft nesting material. Water was provided ad libitum via an automatic watering system. Hamsters were acclimated for at least 3 days and fed standard rodent chow (Purina 5001) during that time. Before the start of the study, hamsters were weighed and distributed into control (n = 10) and treatment groups (n = 5) such that each group had similar average weights. Prior to dosing with compounds, the hamsters were made hypercholesterolemic by feeding rodent chow supplemented with 0.5% cholesterol and 10% coconut oil (Test Diet 97235, Harlan Teklad, Madison, WI) for one week. Subsequently, the hamsters were fed admixtures of Test Diet by first forming a suspension of the compound in 0.5% methylcellulose/0.1% Tween-20 (Sigma) with a Polytron homogenizer (PT2100, Kinematica, Lucerne, Switzerland). An amount of this suspension was then added to 20 g of powdered chow so as to administer a dose of test compound to the animals of 150 mg/kg/d. The chow/compound mixture was then rolled into balls, transferred to stainless steel bowls, and placed at the bottom of the cages. Chow was usually entirely consumed by the next day. The animals were dosed with compounds for two weeks. At the completion of the study, hamsters were fasted overnight by removing chow from cheek pouches and transferring the animals to clean cages. Hamsters were then anesthetized intraperitoneally with a solution of ketamine (100 mg/kg) and xylazine (10 mg/kg). Once the animals were fully sedated but still respiring, blood was collected for lipid analysis by cardiac puncture or via the abdominal aorta using a 3 mL syringe and 18 gauge needle. Plasma was fractionated by fast phase liquid chromatography, and cholesterol levels in the different lipoprotein fractions were determined by an enzymatic assay as described previously.[26] Statistical significance between treated and control groups was determined by the Dunnett's test. An experimental group was considered statistically different than control when $p < 0.05$.

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