Novel Esters and Amides of Nonsteroidal Antiinflammatory Carboxylic Acids as Antioxidants and Antiproliferative Agents

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A series of phenolic antioxidant ester and amide derivatives of the nonsteroidal antiinflammatory drug naproxen was designed to have both antiinflammatory and cytoprotective activity. Compounds were evaluated in vitro both for antioxidant activity, as assessed indirectly by thiobarbituric acid reactive substance (TBARS) formation in a membrane lipid peroxidation assay, and for antiproliferative activity, as indexed by the inhibition of DNA synthesis in cultured human vascular endothelial cells. Compounds of this series exhibited potent antioxidant activity, with IC_{50} values (1.6−11.63 μM) 2−6-fold lower than that of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and 400−1300-fold lower than that of vitamin E. Structural modifications of the ester or amide substructure (**5a** and **6a**) did not affect antioxidant activity, but methylation of the 6-hydroxy substituent resulted in compound **6f** which was devoid of antioxidant activity. Although indistinguishable in antioxidant activity, the amide derivatives tended to be more potent as antiproliferative agents than the corresponding esters. The IC_{50}'s for the amide derivatives (**3, 5a**−**e, 8**) ranged from 2 to 7 μM, while the IC_{50}'s for the structurally related esters (**1, 2a**−**c, 6a**−**e**) ranged from 9 to 22 μM. Moreover, studies with compound **6a** indicate that the observed inhibition of DNA synthesis is reversible, suggesting that the antiproliferative activity is due to a cytostatic rather than cytotoxic activity of the compounds. Thus, the antioxidant−naproxen derivatives represent a novel series of agents that both protect against free-radical damage and possess cytostatic activity in vascular endothelial cells. Studies are in progress to assess the utility of these compounds as potential components of an ocular irrigating solution.

Introduction

The normal healthy eye is known to be protected by elevated levels of natural antioxidants such as ascorbate and glutathione.[1] Free radicals are produced continuously in living cells, and various defense systems are available within cells and tissues to prevent or minimize free-radical-induced damage. The primary defense system is composed of an array of enzymes which function to neutralize free radicals.[2,3] However, during tissue/cellular trauma (e.g., surgery), where some level of hypoxia/reperfusion and inflammation is always present, reactive oxygen species-mediated damage and other free-radical production can overwhelm normal cellular defense systems,[4,5] possibly resulting in ocular angiogenesis, cataract formation, and/or retinopathy.

Angiogenesis is a major contributor to a number of ocular diseases including age-related macular degeneration (ARMD), proliferative vitreoretinopathy (PVR), and diabetic retinopathy (DR), all of which may lead to blindness.[6,7] Moreover, hypoxic conditions associated both with these diseases and with their surgical alleviation facilitate the onset of angiogenesis by inducing the synthesis and secretion of growth factors, especially vascular permeability factor (VPF), also known as vascular endothelial growth factor (VEGF).[8−10] Elevated concentrations of VPF/VEGF are present in healing wounds and in inflammatory disease. Additionally, the two VPF/VEGF receptors, flt-1 and kdr, are overexpressed by microvessel endothelial cells found in wound tissue.[11−15] Thus, increases in VPF/VEGF in the eye result in induction of vascular endothelial cell proliferation and migration of newly formed microvascular beds which may disrupt normal retinal structure and function.[16−18] Many intraocular surgeries, especially in the posterior chamber of the eye, are performed to correct problems related to pathological neovascularization and associated fibroplasia.[19−24] While intraocular surgical techniques employed in the treatment of fibroangiogenic disease have improved, postsurgical complications related to inflammation, vascular leakage, and hypoxia persist and promote vascular endothelial proliferation, especially in diabetic patients.[25−27] At present, there are no therapeutic agents approved for the control of ocular angiogenesis. Because of this, postsurgical pharmacologic intervention is generally limited to control of inflammation with the use of a topical ocular agent such as a steroid or combination steroid−antibiotic.

The initial intent of our drug discovery efforts was to produce a series of antioxidant−naproxen prodrugs to serve as active ingredients of a therapeutic irrigation solution for use in ocular surgery. Unexpectedly the antioxidant−naproxen derivatives in this series were found to have antiproliferative activity as indexed by inhibition of DNA synthesis in cultured human microvascular endothelial cells. This report describes the chemistry, biological activity, and structure−activity relationships of this series of antioxidant agents.

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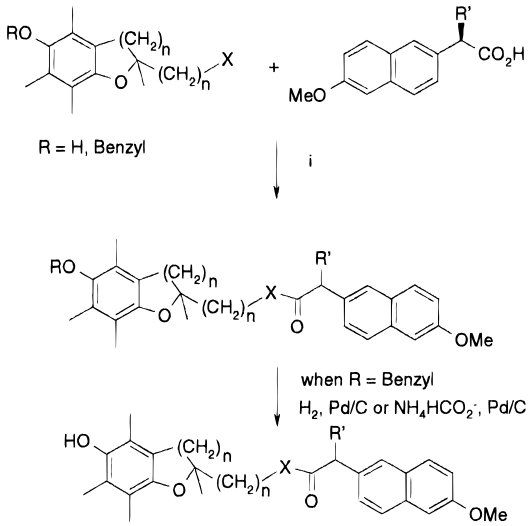
Scheme 1[a]



[a] Reagents: (i) method A—EDC, 4-DMAP; method B—EDC, pyridine, HOBT; compound **6a**—DCC, HOBT; compound **6f**—EDC, HOBT.

Chemistry

The general synthetic route used to prepare the target compounds is outlined in Scheme 1. The antioxidant alcohol could be coupled to naproxen, relying on the steric hindrance afforded by the methyl groups adjacent to the phenol hydroxyl group to prevent diacylation. However, traces of the diacylated side product and the quinone oxidation product were formed. These proved to be difficult to remove either by column chromatography or by repeated recrystallizations. Protection of the phenolic hydroxy group as a benzyl ether resulted in a cleaner reaction and an increased yield in the coupling step. Hydrogenolysis of the protected coupling product provided the target phenols.

The methoxy derivative **6f** was formed by coupling the 2-(6-methoxy-2,5,7,8-tetramethyl-2,3-dihydro-2*H*-benzo[1,2-*b*]pyran-2-yl)ethanol with naproxen. The required alcohol was made by selectively protecting the primary alcohol of (2-hydroxy-2,5,7,8-tetramethyl-2,3-dihydro-2*H*-benzo[1,2]pyran-2-yl)ethanol as the *tert*-butyl dimethylsilyl ether, then alkylating the phenolic hydroxy group with MeI and K_2CO_3, and finally deprotecting using tetra-*n*-butylammonium fluoride.

The 3-substituted benzofuran derivative **9** was prepared by the method of Zaugg et al.[28] as detailed in Scheme 2. The requisite benzofuranone **12** was prepared from trimethylquinone and diethyl methylmalonate. Protection of the phenolic OH, hydrolysis of the ester, and decarboxylation of the resulting carboxylic acid afforded the benzyloxybenzofuranone **13**. Alkylation with bromochloromethane and rearrangement of the resulting 3-methyl chloride (**14**) provided the 3-substituted dihydrobenzofuran **15** which was reduced to the benzyloxy-protected alcohol **16**. The protected alcohol was coupled with naproxen and the protecting group was removed to provide compound **9**.

Results

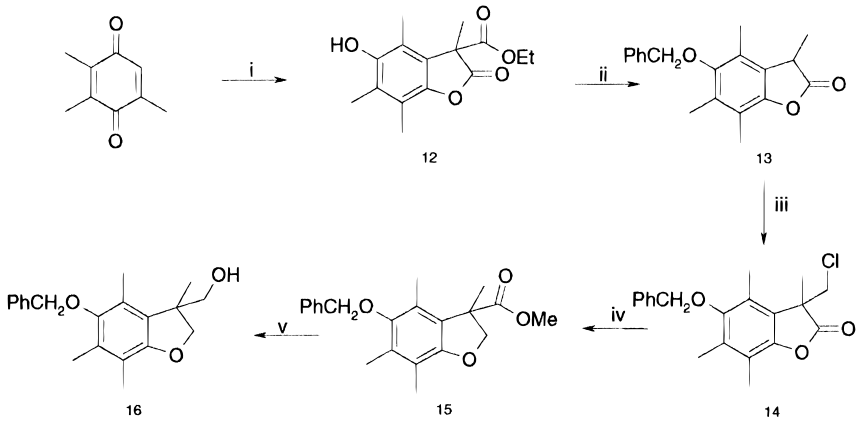
The naproxen derivatives were evaluated for antioxidant activity using a bovine heart membrane preparation. Membrane oxidation was initiated using Fe^{2+}/ascorbate, and the extent of oxidation was assessed indirectly by measuring the formation of thiobarbituric acid reactive substance (TBARS). Results are expressed as IC_{50} (concentration inhibiting the Fe^{2+}/ascorbate-induced membrane oxidation by 50%). In this group of closely related molecules, compounds **1**−**5**, **6a**−**c**,**e**, and **7**−**11** had similar antioxidant activity with IC_{50} values ranging from 1.6 to 11.63 μM (Table 1). As expected, the methoxy derivative **6f** was much less active. However, the acetyl compounds **5d** and **6e** were active as antioxidants in the bovine heart membrane preparation. This activity was a function of the facile hydrolysis of the acetyl group by the membrane preparation. HPLC analysis of the products extracted from the incubated membrane preparations following addition of **5d** and **6e** indicated that these compounds were quantitatively converted to **5a** and **6a**, respectively, during the 30-min incubation period. Compounds **5d** and **6e** did not inhibit Fe^{2+}/ascorbate-induced lipid peroxidation as measured by conjugate diene formation using a dilinoleylphosphatidylcholine preparation free of esterase activity (data not shown), providing further evidence that hydrolysis of the acetyl group is required for the compound to protect lipid membranes from oxidation.

The amides and esters **1**−**5**, **6a**−**c**,**e**, and **7**−**10** and the alcohol **11** inhibited Fe^{2+}/ascorbate-catalyzed oxidation more potently than the standards Trolox and vitamin E (2−6- and 400−1300-fold more potent, respectively). This observed difference in activity probably represents a difference in the ability of the compound to incorporate into the membranes of the bovine heart preparation rather than an inherent difference in antioxidant activity. When the antioxidant activity of the homologous benzofuran and benzopyran derivatives (**1**, **4**; **2a**, **6a**; **3**, **8**) is compared, it is evident that the expected increase in antioxidant activity, due to the increased overlap of the oxygen p orbital and the electron from the π-cloud of the aromatic ring in the benzofuran system, was not realized. There was no observed difference in the antioxidant activity of the diastereomeric pairs (**2b**,**c**; **5b**,**c**; **6b**,**c**) or the positional isomers (**1**, **9**).

The antiproliferative activity of the antioxidant compounds was assessed in cultured human lung microvascular endothelial cells (HMVEC-L). This assay uses the extent of incorporation of [^{3}H]thymidine into DNA as a measure of cell proliferative activity. Results, expressed as IC_{50} (the drug concentration producing 50% suppression of [^{3}H]thymidine incorporation observed in uninhibited controls), are shown in Table 1. Comparable results were obtained in primary cultures of human scleral fibroblasts (data not shown). The naproxen derivatives (**1**−**6, 8**−**10**) were all more potent inhibitors of cell proliferation than naproxen, vitamin E, Trolox, or the antioxidant alcohol **(11)**. These results indicate that the antiproliferative activity of the naproxen derivatives could not simply be attributed to their antioxidant activity. Within the series of compounds, the paired diastereomers (**2b**,**c**; **5b**,**c**; **6b**,**c**) were equivalent in antiproliferative activity.

The antiproliferative activity of these compounds was not attributable to their hydrolysis products generated under culture conditions. HPLC analysis of residual drug (e.g., **5a** or **6a**) after 24-h incubation in the cell

Scheme 2[a]



[a] Reagents: (i) diethyl methylmalonate, Na EtOH; (ii) (a) K_2CO_3, benzyl bromide, CH_3CN, (b) KOH, EtOH, (c) 2 N HCl; (iii) NaH, bromochloromethane, DME; (iv) NaOMe, MeOH; (v) $LiAlH_4$, THF.

culture assay indicates that less than 10% of each compound is hydrolyzed to naproxen (see Table 2). Alone or in combination, the hydrolysis products of **6a** (compound **11** and naproxen) were far less active than the parent compound in inhibiting DNA synthesis in HMVEC-L (Figure 1).

The data suggest there are some differences in the structure−activity relationships associated with antiproliferative activity and those associated with antioxidant activity. In the antioxidant assay, the ester and corresponding amide derivatives were equally active. However, the two structural classes can be distinguished on the basis of their antiproliferative activity. The amide derivatives (IC_{50}'s 2−7 μM) were slightly but consistently and reproducibly more potent than the corresponding esters (IC_{50}'s 9−22 μM) (e.g., **3** vs **2a**; **5a** vs **4**; **8** vs **6a**). Based on the HPLC data presented above, it is unlikely that the observed difference in their antiproliferative activity is due to selective metabolic stability of one structural type over the other. Additionally, the 3-furan derivative **1** was more active than the corresponding 3-furan derivative **9**, and the desmethyl derivative **7** (R′ = H) was less active than the corresponding naproxen derivative **6a** (R′ = Me).

In studies designed to characterize the antiproliferative activity of compound **6a**, the compound was shown to be a reversible inhibitor of DNA synthesis (Figure 2). When HMVEC-L were incubated with **6a** at either 5 or 25 μM, predictable inhibition was observed after 24 h. However, when **6a** was removed at 24 h and cells were exposed to medium devoid of the inhibitor (**6a**), DNA synthetic activity returned to control levels within an additional 24-h incubation period. These data demonstrating that **6a** reversibly inhibits DNA synthesis suggest that **6a** may have utility as an angiostatic agent.

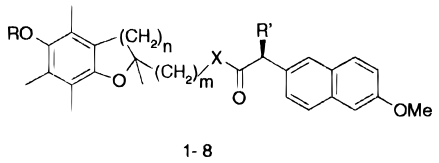
Discussion

The antioxidant ester and amide derivatives of naproxen described in this report were designed as prodrugs to deliver both naproxen (NSAID) and an antioxidant to the wound environment following metabolism. The intent of this design effort was to synthesize compounds that would have acute cytoprotective activity and would also provide a sustained release of a cyclooxygenase inhibitor to attenuate surgically induced inflammation. The use of such a therapeutic irrigating solution in ocular surgical procedures (e.g., cataract surgery or vitrectomy) would provide a unique opportunity to control the effects of trauma associated with ocular surgery because the solution acts as a temporary replacement for the aqueous (cataract) or vitreous (vitrectomy) humor. As anticipated, the synthesized compound(s) lacked intrinsic cyclooxygenase activity (**6a**, data not shown). Significantly, however, they possessed antiproliferative activity surpassing that of the individual building blocks (Table 1). Thus, the compounds of this series appear to be unique in that they possess both antioxidant and antiproliferative activity.

Vitamin E and analogous phenolic antioxidants are known to provide protection from fatty acid oxidation in the lipid bilayer of biomembranes. Other antioxidants have been reported to inhibit the inflammatory cascade by suppressing the synthesis and release of inflammatory mediators such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6).[30] An analogue of vitamin E, α-tocopherol acid succinate (α-TOCS), has been demonstrated to inhibit lipopolysaccharide (LPS) activation of nuclear factor-κB (NF-κB).[31] Additionally, α-TOCS suppresses LPS-induced TNF-α mRNA levels and secretion of TNF-α by cultured Kupffer cells.[31] Moreover, LPS does not increase TNF-α mRNA in α-TOCS-treated cells even upon removal of the α-TOCS. These findings strongly suggest that the mechanism(s) involved in tocopherol suppression of an inflammatory response involves more than the simple trapping of a free radical.[31]

The antiproliferative effects of antioxidants such as *d*-α-tocopherol on cultured vascular smooth muscle cells have been reported, with evidence supporting a mechanism involving the suppression of protein kinase C-α.[32−35] Of importance to ocular filtration surgery, *d*-α-tocopherol at 50 μM inhibits the proliferation of cultured human Tenon's capsule fibroblasts, with no apparent cytotoxicity.[36] Also, α-TOCS has been shown to inhibit phorbol ester-induced cultured bovine choroidal endothelial cell proliferation, whereas the phosphate, acetate, or nicotinate esters of α-tocopherol exhibited no inhibitory activity.[37] Similar results with α-TOCS have been reported in cultured bovine retinal pigmented epithelial cells, in which this compound suppresses cell

Table 1. In Vitro Antioxidant and Antiproliferative Activity of Naproxen−Antioxidant Derivatives



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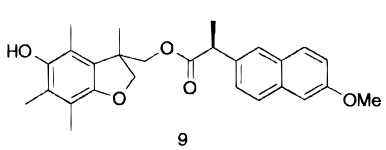

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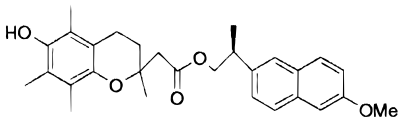

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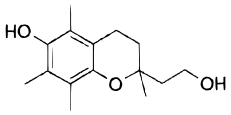

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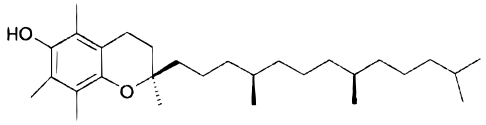
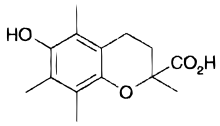


Vitamin E



Trolox

								IC$_{50}$ (μg)	
compd	R	R′	*n*	*m*	X	anitoxidant center	naproxen center	antioxidant BHMLO[a]	antiproliferation HMVEC-L[b]
1	H	Me	1	1	O	*RS*	*S*	4.83	7.3
2a	H	Me	1	2	O	*RS*	*S*	4.87	9.1
2b	H	Me	1	2	O	less polar	*S*	5.20	16.6
2c	H	Me	1	2	O	more polar	*S*	5.10	16.4
3	H	Me	1	2	NH	*RS*	*S*	5.60	1.9
4	H	Me	2	1	O	*RS*	*S*	4.36	11.3
5a	H	Me	2	1	NH	*RS*	*S*	3.99	4.3
5b	H	Me	2	1	NH	*S*	*S*	2.50	4.3
5c	H	Me	2	1	NH	*R*	*S*	4.80	4.5
5d	Ac	Me	2	1	NH	*RS*	*S*	1.60	3.3
5e	Ac	Me	2	1	NH	*S*	*S*	nd	4.0
6a	H	Me	2	2	O	*RS*	*S*	7.66	12.9
6b	H	Me	2	2	O	*R*	*S*	11.63	11.7
6c	H	Me	2	2	O	*S*	*S*	9.60	16.4
6d	H	Me	2	2	O	*R*	*R*	nd	22.0
6e	Ac	Me	2	2	O	*RS*	*S*	4.50	11.5
6f	Me	Me	2	2	O	*RS*	*S*	14000	25.0
7	H	H	2	2	O	*S*		4.50	44% (25.0)
8	H	Me	2	2	NH	*RS*	*S*	4.67	7.4
9						*RS*	*S*	5.20	19.0
10						*RS*	*S*	4.40	12.0
11						racemic		3.82	30% (25.0)
naproxen							*S*	nd	>50.0
6-methoxy-2-naphthaleneacetic acid								nd	5% (25.0)
vitamin E						*RS*		5173	22% (31.0)
Trolox						racemic		25.6	20% (25.0)

[a] In vitro inhibition of Fe^{2+}/ascorbate-induced oxidation of bovine heart membranes. [b] In vitro inhibition of proliferation measured by inhibition of [^{3}H]thymidine incorporation by HMVEC-L cells; IC$_{50}$ or percent inhibition at concentration noted; nd, not determined.

Table 2. Metabolism of Compound **6a** upon Exposure to HMVEC-L Cells: Quantification by HPLC

6a concn (μM)	exposure time (h)	naproxen produced (μM)	% conversion to naproxen
5	2	0.065 ± 0.009	1.3
5	24	0.486 ± 0.096	9.7
25	2	0.156 ± 0.013	0.6
25	24	1.742 ± 0.061	7.0

proliferation (IC$_{50}$ = 23 μM) and migration.[38] In contrast, Trolox has no reported antiproliferative activity.[37,38] Additionally, a series of 6-hydroxychroman-2-carbonitrile analogues have been studied by Boscobinik et al.[39] for inhibitory activity on smooth muscle cell proliferation. These investigators found that while α-tocopherol suppressed cell proliferation, there was little correlation between the antioxidant properties of both the tocopherols and the 6-hydroxychroman-2-carbonitrile analogues and their antiproliferative activity, suggesting that the control of cell proliferation by these compounds occurs through a nonantioxidative mechanism.[39]

Results presented in the current report clearly demonstrate that the naproxen−antioxidant derivatives also inhibit cell proliferation as measured by [^{3}H]thymidine incorporation studies. The use of a therapeutic irrigating solution containing such cytostatic agents during vitreoretinal surgery may well reduce the postsurgical fibrovascular growth, a common complication of vitrectomy procedures. Additionally, such an irrigating solution may inhibit surgically induced abnormal lens fiber growth, which is thought to lead to posterior subcapsular cataract.

Our initial objective was to design a series of naproxen prodrugs having inherent cytoprotective activity, due to inclusion of an antioxidant moiety that could serve as the active component of an ocular surgical irrigating

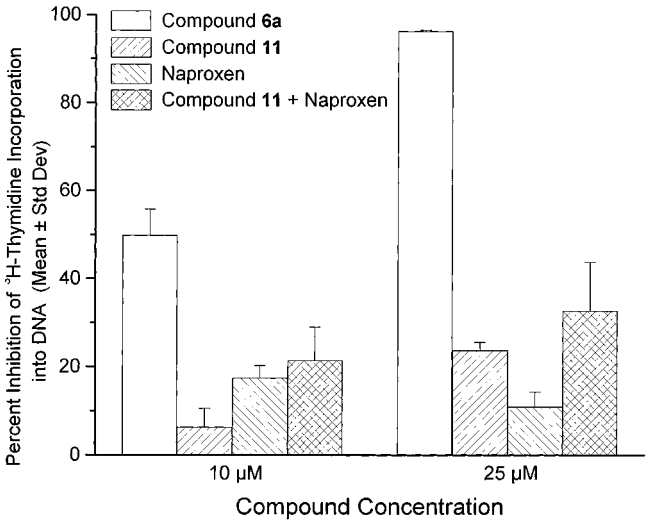


Figure 1. Inhibition of DNA synthesis in HMVEC-L by compound **6a** and by its hydrolysis products compound **11** and naproxen, alone and in combination. Cell proliferative activity was assessed by the extent of [^{3}H]thymidine incorporation into DNA. Cells were exposed to test compound(s) for 24 h; radiolabeled thymidine was added for the final 6 h of this incubation period.

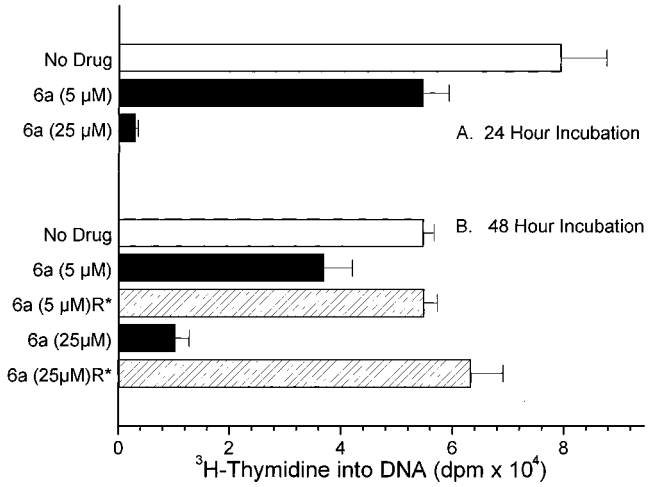


Figure 2. Inhibition of DNA synthesis in HMVEC-L by compound **6a** and the reversal of that inhibition by removal of the drug. Set A is a 24-h incubation showing relative inhibition by **6a** at medium concentrations of 5 and 25 μM. In set B, cells were incubated with **6a** for 48 h except for the two groups denoted by R*. These two groups (R*) were exposed to drug for the first 24 h, but then fresh, non-drug-containing medium was added for the second 24-h period. In all cases, cells were labeled with [^{3}H]thymidine during the last 6-h period of the incubation.

solution. In addition to their expected antioxidant activity, the naproxen−antioxidant derivatives have been shown to inhibit DNA synthesis in human vascular endothelial cells and scleral fibroblasts. Currently studies are in progress to assess the antiinflammatory activity of these compounds and their potential therapeutic utility as irrigating solution constituents capable of reducing the complications pursuant to ocular surgery.

Experimental Section

General Procedures. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Proton NMR was obtained on a Varian VXR-200 (200 MHz) or JEOL GX-270 (270 MHz) instrument. NMR data are reported in parts per million (δ) and are referenced to TMS as the internal standard. All mass spectra were obtained on a Finnegan TSQ 46 spectrometer using chemical ionization with NH_4^+. Optical rotations were determined with a JASCO DIP-370 polarimeter. Elemental analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA) and are within ±0.4% of the theoretical values. Flash chromatography was carried out according to the method of Still[40] using Kieselgel 60 silica gel (E. Merck). Thin-layer chromatography was carried out using Kieselgel 60 F254 glass-backed plates (E. Merck). All reagents were obtained from commercial suppliers and were used without further purification.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R,S*)-(2,3-Dihydro-5-hydroxy-2,4,6,7-tetramethylbenzofuran-2-yl)methyl Ester (1). Method A. A solution of (2,3-dihydro-5-hydroxy-2,4,6,7-tetramethylbenzofuran-2-yl)methanol (0.78 g, 3.50 mmol), 6-methoxy-α-methylnaphthaleneacetic acid (0.89 g, 3.86 mmol), 4-(dimethylamino)pyridine (0.43 g, 3.51 mmol), and 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (0.67 g, 3.51 mmol) in THF (15 mL) was stirred at ambient temperature under nitrogen for 24 h. The reaction mixture was diluted with water (100 mL), and the resulting solution was extracted with EtOAc (5 × 65 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography using 2:1 to 0:1 hexanes−EtOAc as eluents. Recrystallization from methylene chloride−hexane afforded 0.24 g (15.8%) of **1** as a white solid: mp 185−187 °C; ^{1}H NMR ($CDCl_3$) δ 1.33−1.35 (d, 3H), 1.51−1.55 (d, 3H), 1.92−1.94 (s, 3H), 2.00−2.03 (d, 3H), 2.09−2.11 (d, 3H), 2.56−2.57 (d, 1H), 2.58−2.91 (d, 1H), 3.76−3.89 (m, 1H), 3.92 (s, 3H), 4.04−4.22 (m, 3H), 7.09−7.17 (m, 2H), 7.26−7.34 (m, 2H), 7.58−7.79 (m, 2H). Anal. ($C_{27}H_{30}O_5$) C, H.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R,S*)-2-(2,3-Dihydro-5-hydroxy-2,4,6,7-tetramethylbenzofuran-2-yl)ethyl Ester (2a). Synthesized by method A in 13% yield from 2-(5-hydroxy-2,4,6,7-tetramethyl-2,3-dihydrobenzofuran-2-yl)ethanol and 6-methoxy-α-methylnaphthaleneacetic acid: white solid; mp 129.5−131 °C; ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3H), 1.54−1.57 (d, 3H), 1.99 (t, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.73−2.81 (d, 1H), 2.90−2.97 (d, 1H), 3.77−3.89 (q, 1H), 3.91 (s, 3H), 4.10 (s, 1H), 4.16−4.29 (m, 2H), 7.10−7.16 (m, 2H), 7.35−7.40 (m, 2H), 7.64−7.70 (m, 2H). Anal. ($C_{28}H_{32}O_5$) C, H.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, 2-(2,3-Dihydro-5-hydroxy-2,4,6,7-tetramethylbenzofuran-2-yl)-ethyl Ester (2b,c). Semipreparative HPLC of **2a** using a Chiral Technologies (AD) column, 20 × 200 mm, eluting with 9:1 hexane−EtOH provided **2b** (less polar isomer) and **2c** (more polar isomer). **2b:** t_R = 29 min; 98.9% ee; $[\alpha]_D$ = +10.1° (c = 0.545, ethanol); ^{1}HNMR ($CDCl_3$) δ 1.24 (s, 3H), 1.57 (m, 4H), 2.00−2.17 (m, 10H), 2.67−2.97 (dd, 2H), 3.78 (m, 1H), 3.91 (s, 3H), 4.11 (s, OH), 4.23 (t, 2H), 7.11−7.70 (m, 6H). Anal. ($C_{28}H_{32}O_5$) C, H. **2c:** t_R = 33 min; 98% ee; $[\alpha]_D$ = +37.4° (c = 0.511, ethanol); ^{1}HNMR ($CDCl_3$) δ 1.25 (s, 3H), 1.57 (m, 4H), 2.00−2.17 (m, 10H), 2.73−2.98 (dd, 2H), 3.78 (m, 1H), 3.91 (s, 3H), 4.11 (s, OH), 4.23 (t, 2H), 7.11−7.70 (m, 6H). Anal. ($C_{28}H_{32}O_5$) C, H.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R,S*)-2-(2,3-Dihydro-5-hydroxy-2,4,6,7-tetramethylbenzofuran-2-yl)ethyl Amide (3). (*S*)-6-Methoxy-α-methylnaphthaleneacetic acid, (*R,S*)-2-(5-(benzyloxy)-2,3-dihydro-2,4,6,7-tetramethylbenzofuran-2-yl)ethyl amide was synthesized by method A from 2-(2-aminoethyl)-5-(benzyloxy)-2,3-dihydro-2,4,6,7-tetramethylbenzofuran hydrochloride[41] in a 86.5% yield: ^{1}H NMR ($CDCl_3$) δ 1.45 (d, 3H), 1.57 (s, 3H), 1.75−1.90 (m, 2H), 2.00 (s, 3H), 2.07 (s, 3H), 2.10 (s, 3H), 2.75−3.00 (m, 2H), 3.35−3.49 (m, 2H), 3.65 (q, 1H), 3.90 (s, 3H), 4.67 (s, 2H), 5.85 (m, 1H), 7.10−7.69 (m, 11H).

A mixture of (*S*)-6-methoxy-α-methylnaphthaleneacetic acid, (*R,S*)-2-(5-(benzyloxy)-2,3-dihydro-2,4,6,7-tetramethylbenzofuran-2-yl)ethyl amide (1.50 g, 2.79 mmol), ammonium formate (1.23 g, 19.5 mmol), and 10% Pd on carbon (0.30 g) in DMF (8 mL) was stirred at ambient temperature for 2.5 h. The reaction

mixture was filtered, and the resulting solution was partitioned between water (50 mL) and EtOAc (70 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed with brine (25 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash chromatography using a 1:0 to 1:1 solvent gradient of hexanes−EtOAc as an eluent. Crystallization of the resulting oil from a mixture of EtOAc and hexane afforded 1.12 g (89.7%) of **3** as a white solid: mp 92−93.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (d, 3H), 1.56 (s, 3H), 2.00 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 2.80−2.90 (m, 2H), 2.75−3.05 (m, 2H), 3.39−3.45 (m, 2H), 3.65 (q, 1H), 3.91 (s, 3H), 4.15 (d, 1H), 5.76 (bs 1H) 7.05−7.75 (m, 6H). Anal. ($C_{28}H_{33}NO_4$) C, H, N.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*,*S*)-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methyl Ester (4). Synthesized by method A in a 58.3% yield from (3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methylamine and 6-methoxy-α-methylnaphthaleneacetic acid: white solid; mp 103−105 °C; ^{1}H NMR ($CDCl_3$) δ 1.15 (s, 3H), 1.57−1.61 (d, 3H), 1.62−1.88 (m, 2H), 1.98−2.11 (m, 9H), 2.40−2.59 (m, 2H), 3.82−3.92 (m, 1H), 3.91 (s, 3H), 4.01−4.22 (m, 3H), 7.09−7.68 (m, 6H). Anal. ($C_{28}H_{32}O_5$) C, H.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*,*S*)-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methyl Amide (5a). Synthesized in a 58.3% yield by method A from (3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methylamine hydrochloride and 6-methoxy-α-methylnaphthaleneacetic acid: white solid; mp 128−130 °C; ^{1}H NMR ($CDCl_3$) δ 1.03−1.08 (d, 3H), 1.57−1.64 (m, 6H), 1.70 (t, 2H), 2.04−2.05 (m, 6H), 2.48−2.51 (m, 2H), 3.16−3.58 (m, 2H), 3.74 (q, 1H), 3.91 (s, 3H), 4.91 (br s, 1H), 5.75 (t, 1H), 7.01−7.19 (m, 2H), 7.29−7.40 (t, 1H), 7.52−7.81 (m, 3H). Anal. ($C_{28}H_{33}NO_4$) C, H, N.

Benzyl (*S*)-6-(Benzyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-carboxylate. To a cold solution of the commercially available (*S*)-(-)-Trolox (5.00 g, 19.9 mmol) in DMF (30 mL) was added sodium hydride (1.99 g, 49.9 mmol, 60% oil dispersion). The reaction mixture was stirred at 0 °C for 20 min. Benzyl bromide (7.50 g, 43.9 mmol) was added dropwise, and then the solution was stirred at 60 °C for 3 h. The reaction mixture was concentrated in vacuo. Water (50 mL) was added to the residue, and the resulting mixture was extracted with EtOAc (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue purified by flash column chromatography (SiO_2, hexane−EtOAc (9:1)) to afford 8.00 g (93%) of benzyl 2(*S*)-6-(benzyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-carboxylate as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.86 (s, 3H), 1.90 (m, 1H), 2.09 (s, 3H), 2.15 (s, 3H), 2.23 (s, 3H), 2.50 (m, 3H), 4.69 (s, 2H), 5.06 (dd, 2H), 7.08−7.53 (m, 10H); MS(CI) *m*/*z* 431 (M + 1).

(*S*)-6-(Benzyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyrancarboxylic Acid. To a solution comprised of benzyl (*S*)-6-(benzyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-carboxylate (8.00 g, 18.60 mmol), THF (100 mL), methanol (15 mL), and water (15 mL) was added lithium hydroxide monohydrate (4.80 g, 0.11 mol). After the solution was stirred at room temperature for 6 h, the reaction mixture was concentrated in vacuo and EtOAc (100 mL) was added to the residue. The resulting mixture was acidified with 1 N HCl. The layers were separated, and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to afford 6.3 g (99%) of 2(*S*)-6-(benzyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyrancarboxylic acid as a white solid: mp 140−142 °C; ^{1}H NMR ($CDCl_3$) δ 1.63 (s, 3H), 2.00 (m, 1H), 2.14 (m, 1H), 2.16 (s, 3H), 2.22 (s, 3H), 2.23 (s, 3H), 2.34 (m, 1H), 2.57 (m, 2H), 4.69 (s, 2H), 7.25−7.51 (m, 5H); MS(CI) *m*/*z* 341 (M+1).

(*S*)-6-(Benzyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyrancarboxylic Acid Benzyl Amide. To a solution of 2(*S*)-6-(benzyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyrancarboxylic acid (6.30 g, 18.52 mmol) in THF (100 mL) cooled to 0 °C was added 2 drops of DMF followed by oxalyl chloride (3.50 g, 27.6 mmol). The reaction mixture was stirred at 0 °C for 1 h, and then benzylamine (9.92 g, 92.6 mmol) was added dropwise. After the addition was complete the reaction mixture was allowed to warm to room temperature and was stirred for an additional 3 h. The reaction mixture was diluted with EtOAc (100 mL) and washed with 1 N aqueous HCl. The organic phase was separated, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, EtOAc−hexane (1:1)) to afford 4.96 g (63%) of the amide as an oil: ^{1}H NMR ($CDCl_3$) δ 1.63 (s, 3H), 1.85 (m, 1H), 2.10 (s, 3H), 2.16 (s, 3H), 2.22 (s, 3H), 2.44 (m, 1H), 2.61 (m, 2H), 4.40 (2dd, 2H), 4.69 (s, 2H), 6.75 (t, 1H), 7.01−7.52 (m, 10H); MS(CI) *m*/*z* 430 (M + 1).

(*S*)-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyranyl-2-yl)methylamine. To a solution of 2(*S*)-6-(benzyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyrancarboxylic acid benzyl amide (3 g, 6.99 mmol) in 100 mL of THF was added 35 mL (35 mmol) of a 1 M solution of borane in THF. The reaction mixture was warmed at reflux for 6 h. The mixture was then cooled to 0 °C. Excess borane was destroyed by adding 20 mL of methanol. The reaction mixture was concentrated in vacuo, and the residue was dissolved in 2 M aqueous HCl and warmed at reflux for 3 h. The solution was cooled to room temperature and extracted with EtOAc (2 × 50 mL). The resulting aqueous solution was neutralized with a saturated solution of bicarbonate and then extracted with EtOAc (6 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in Et_2O and treated with 1 M ethereal HCl to yield crude (*S*)-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methylamine hydrochloride salt (1.00 g).

To a solution of crude (*S*)-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methylamine hydrochloride (0.9 g, ∼2.76 mmol) in THF (50 mL) were added ammonium formate (0.68 g, 10.84 mmol) and 10% Pd/C (0.10 g). The reaction mixture was stirred at room temperature for 12 h and then filtered through a pad of Celite. The filtrate was concentrated in vacuo to give a solid which was dissolved in ethanol (5 mL) and converted to the corresponding hydrochloride salt by treatment with an ethereal HCl solution: dec > 250 °C; $[\alpha]_{405} = -18.9°$ (*c* = 0.322, methanol); ^{1}H NMR ($CDCl_3$) δ 1.18 (s, 3H), 1.8−1.9 (m, 2H), 1.97 (s, 9H), 2.50 (m, 2H), 2.98 (m, 2H), 3.32 (s, 1H), 7.6−8.2 (bs, 3H); MS(CI) *m*/*z* 236 (M + 1). Anal. ($C_{14}H_{21}N_1O_2HCl\cdot0.4H_2O$) C, H, N.

(*R*)-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methylamine Hydrochloride. Synthesized by the method described for (*S*)-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methylamine hydrochloride from (*R*)-(+)-Trolox: dec > 250 °C; $[\alpha]_{405} = +20.3°$ (*c* = 0.447, methanol); ^{1}H NMR ($CDCl_3$) δ 1.18 (s, 3H), 1.8−1.9 (m, 2H), 1.97 (s, 9H), 2.50 (m, 2H), 2.98 (m, 2H), 3.32 (s, 1H), 8.03 (bs, 3H); MS(CI) *m*/*z* 236 (M + 1). Anal. ($C_{14}H_{21}N_1O_2\cdot1HCl\cdot0.2H_2O$) C, H, N.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*S*)-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methyl Amide (5b). Method B. To a solution of (2*S*)-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methylamine hydrochloride (0.30 g, 1.10 mmol) and 6-methoxy-α-methylnaphthaleneacetic acid (0.28 g, 1.21 mmol) in THF (50 mL) were added pyridine (1 mL), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (0.50 g, 2.60 mmol), and hydroxybenzotriazole hydrate (0.40 g, 3.00 mmol). After stirring at ambient temperature for 12 h, the reaction mixture was diluted with EtOAc (100 mL), and the resulting solution was washed (saturated solution of $NaHCO_3$ and 1 N HCl), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography using 1:1 hexane−EtOAc as an eluent. Crystallization from a mixture of EtOAc and hexane afforded 0.29 g (58.8%) of **5b** as a white solid: mp 130−132 °C; $[\alpha]_D = -15.6°$ (*c* = 0.507, ethanol); ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 3H), 1.68 (m, 8H), 2.04 (2s, 6H), 2.56 (m, 2H), 3.29 (m, 1H), 3.44 (m, 1H), 3.57 (m, 1H), 3.92 (s, 3H), 4.18 (s, 1H), 5.76

(m, 1H, NH), 7.06−7.71 (m, 6H); MS(CI) *m*/*z* 448 (M + 1). Anal. ($C_{28}H_{33}NO_4$) C, H, N.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*)-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methyl Amide (5c). Synthesized by method A in 32% yield from (*R*)-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methylamine hydrochloride and 6-methoxy-α-methylnaphthaleneacetic acid: white solid; mp 130−132 °C; $[\alpha]_D = -15.5°$ (*c* = 0.526, ethanol); ^{1}H NMR ($CDCl_3$) *δ* 1.05 (s, 3H), 1.68 (m, 8H), 2.04 (2s, 6H), 2.56 (m, 2H), 3.29 (m, 1H), 3.44 (m, 1H), 3.57 (m, 1H), 3.92 (s, 3H), 4.18 (s, 1H), 5.76 (m, 1H), 7.06−7.71 (m, 6H); MSCI *m*/*z* 448 (M + 1). Anal. ($C_{28}H_{33}NO_4$) C, H, N.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*,*S*)-(6-Acetoxy-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methyl Amide (5d). Acetyl chloride (0.35 g, 4.46 mmol) was added to a solution of **5a** (1.00 g, 2.23 mmol) and triethylamine (0.91 g, 8.99 mmol) in THF (40 mL) at 0 °C. The reaction mixture was allowed to warm to ambient temperature and was stirred for 2 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in CH_2Cl_2 (60 mL). The resulting mixture was washed with water (60 mL). The organic layer was separated, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography using 1:9 hexane−EtOAc as an eluent. Crystallization from a mixture of EtOAc and hexane afforded 0.80 g (73%) of **5d** as a white solid: mp 128−130 °C; ^{1}H NMR ($CDCl_3$) *δ* 1.05 (d, 3H), 1.61−174 (m, 9H), 2.04 (m, 5H), 2.32 (s, 3H), 2.52 (m, 2H), 3.25−3.51 (m, 2H), 3.71 (q, 1H), 3.91 (s, 3H), 5.73 (m, 1H), 7.11−7.70 (m, 6H); MS(CI) *m*/*z* 490 (M + 1). Anal. ($C_{30}H_{35}NO_5$) C, H, N.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, *(S)*-(6-Acetoxy-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)methyl Amide (5e). Synthesized by the method used to prepare compound **5d** in 66% yield from **5b**: mp 122 °C; $[\alpha]_D = -29.2°$ (*c* = 0.507, ethanol); MS(CI) *m*/*z* 490 (M + 1); ^{1}H NMR ($CDCl_3$) *δ* 1.05 (s, 3H), 1.53−165 (m, 9H), 1.91 (m, 5H), 2.32 (s, 3H), 2.48 (m, 2H), 3.25−3.35 (m, 2H), 3.71 (q, 1H), 3.92 (s, 3H), 5.73 (m, 1H), 7.08−7.65 (6 H). Anal. ($C_{30}H_{35}NO_5$) C, H, N.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*,*S*)-2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl Ester (6a). A solution of 1,3-dicyclohexylcarbodiimide (0.89 g, 4.31 mmol) in CH_3CN (25 mL) was added dropwise to a stirring slurry of 6-methoxy-α-methylnaphthaleneacetic acid (0.90 g, 3.91 mmol), 2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol (0.98 g, 3.91 mmol),[42] and 1-hydroxybenzotriazole hydrate (0.59 g, 4.31 mmol) in CH_3CN (50 mL). After stirring for 18 h, the reaction mixture was concentrated. The residue was partitioned between water (30 mL) and CH_2Cl_2 (30 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were washed with water (20 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography using 4:1 hexane−EtOAc as an eluent. Recrystallization from a mixture of EtOAc−hexane afforded 0.60 g (33.1%) of **6a** as a white solid: mp 99.5−101.5 °C; ^{1}H NMR ($CDCl_3$) *δ* 1.1 (d, 3H), 1.5−1.6 (m, 3H), 1.6 (m, 2H), 1.9 (m, 2H), 2.0 (s, 6H), 2.1 (s, 3H), 2.4 (t, 2H), 3.8 (q, 2H), 3.9 (s, 3H), 4.1−4.4 (m, 2H), 7.1−7.7 (m, 6H). Anal. ($C_{29}H_{34}O_5$) C, H.

(*S*)-2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol. A 1 M solution of lithium aluminum hydride in THF (30.3 mL, 30.3 mmol) was added dropwise to a stirring solution of (*S*)-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)acetic acid[43] (4.0 g, 15.1 mmol) in THF (73 mL) cooled by an ice−water bath. After the addition was complete, the reaction was warmed at reflux for 2 h. The reaction mixture was cooled in an ice water bath, and water (1.1 mL), 15% aqueous sodium hydroxide (1.1 mL), and water (3.4 mL) were added sequentially. Et_2O (100 mL) was added, and the suspension was filtered through Celite. Upon concentration a solid formed which was recrystallized from Et_2O to afford 3.42 g (77.5%) of (*S*)-2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol as a white solid: 150−152 °C; $[\alpha]_{405} = -17.7°$ (*c* = 1.06, ethanol); ^{1}H NMR ($CDCl_3$) *δ* 1.28 (s, 3H), 1.70−1.98 (m, 4H), 2.02 (s, 3H), 2.07 (s, 3H), 2.16 (s, 3H), 2.50 (bs, 1H), 2.66 (t, 3H), 3.90 (m, 1H), 4.33 (s, 1H). Anal. ($C_{15}H_{22}O_3$) C, H.

(*R*)-2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol. Synthesized by the method described for (*S*)-2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol from (*R*)-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)acetic acid[43] in 82.5% yield: white solid; 150−152 °C; $[\alpha]_{405} = +17.7°$ (*c* = 1.02, ethanol); ^{1}H NMR ($CDCl_3$) *δ* 1.28 (s, 3H), 1.70−1.98 (m, 4H), 2.02 (s, 3H), 2.07 (s, 3H), 2.16 (s, 3H), 2.50 (bs, 1H), 2.66 (t, 3H), 3.90 (m, 1H), 4.33 (s, 1H). Anal. ($C_{15}H_{22}O_3$) C, H.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*)-2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl Ester (6b). Synthesized by method A in 48.6% yield from (*R*)-2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol and 6-methoxy-α-methylnaphthaleneacetic acid: white solid; mp 120−121 °C; $[\alpha]_D = +43.6°$ (*c* = 0.420%, ethanol); ^{1}H NMR ($CDCl_3$) *δ* 1.17 (s, 3H), 1.56 (d, 3H), 1.65−1.75 (m, 2H), 1.81−1.95 (m, 2H), 2.04 (s, 3H), 2.05 (s, 3H), 2.13 (s, 3H), 2.40−2.55 (m, 2H), 3.84 (q, 1H), 3.91 (s, 3H), 4.17 (s, 1H), 4.20−4.33 (m, 2H), 7.10−7.70 (m, 6H). Anal. ($C_{29}H_{34}O_5$) C, H.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*S*)-2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl Ester (6c). Synthesized by method A in 59.4% yield from (*S*)-2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol and 6-methoxy-α-methylnaphthaleneacetic acid: white solid; mp 73.5−75 °C; $[\alpha]_D = +20.1°$ (*c* = 0.413%, ethanol); ^{1}H NMR ($CDCl_3$) *δ* 1.16 (s, 3H), 1.57 (d, 3H), 1.60−1.75 (m, 2H), 1.81−1.93 (m, 2H), 2.05 (s, 6H), 2.14 (s, 3H), 2.46 (t, 2H), 3.85 (q, 1H), 3.91 (s, 3H), 4.19 (s, 1H), 4.20−4.40 (m, 2H). 7.11−7.70 (m, 6H). Anal. ($C_{29}H_{34}O_5$) C, H.

(*R*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*)-2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl Ester (6d). Synthesized by method A in 48% yield from (*R*)-2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol and 6-methoxy-α-methylnaphthaleneacetic acid: white solid; mp 116−118 °C; $[\alpha]_D = -17.0°$ (*c* = 0.405, ethanol); ^{1}H NMR ($CDCl_3$) *δ* 1.25 (s, 3H), 1.57−1.92 (m, 10H), 2.05−2.13 (m, 6H), 2.46 (dd, 2H), 3.78 (m, 1H), 3.91 (s, 3H), 4.18 (s, OH), 4.23 (m, 2H), 7.11−7.71 (m, 6H). Anal. ($C_{29}H_{34}O_5$) C, H.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*,*S*)-2-(6-Acetoxy-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl Ester (6e). Synthesized by the method used to prepare compound **5d** in 91% yield from **6a**: white solid; mp 108−110 °C; ^{1}H NMR ($CDCl_3$) *δ* 1.15 (s, 3H), 1.45−1.60 (m, 5H), 1.70−1.90 (m, 5H), 1.95 (s, 3H), 2.07 (s, 3H), 2.25 (s, 3H), 2.40 (m, 2H), 3.81 (q, 1H), 3.85 (s, 3H), 4.21−4.50 (m, 2H), 7.11−7.81 (m, 6H). Anal. ($C_{31}H_{36}O_6$) C, H.

2-(3,4-Dihydro-6-methoxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol. To a stirred solution of *tert*-butyldimethyldisilyl chloride (3.0 g, 20.2 mmol) and imidazole (3.13 g, 46.0 mmol) in DMF was added 2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol (4.6 g, 18.4 mmol). The mixture was allowed to stir 3 h and was then added to ice water (200 mL). The resulting mixture was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude residue was purified by flash chromatography using 1:9 EtOAc−hexane as an eluent to afford 3.45 g (52%) of 2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl) (*tert*-butyldimethylsilyloxy)-ethane as a brown oil: ^{1}H NMR ($CDCl_3$) *δ* 0.37 (s, 6H), 0.88 (s, 9H), 1.26 (s, 3H), 1.75−1.81 (m, 4H), 2.10 (s, 6H), 2.15 (s, 3H), 2.61 (t, 2H), 3.81 (q, 2H), 4.25 (bs, 1H).

A mixture of 2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)(*tert*-butyldimethylsilyloxy)ethane (3.45 g, 9.57 mmol), methyl iodide (10.0 g, 70.4 mmol), and K_2CO_3 (6.61 g, 47.8 mmol) in CH_3CN (50 mL) was stirred at ambient

temperature for 24 h. The reaction mixture was filtered and concentrated in vacuo. The residue was partitioned between water (50 mL) and CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. A solution of crude residue (3.0 g, ~8 mmol) and tetra-*n*-butylammonium fluoride (1 M in THF, 9.5 mL, 9.5 mmol) in THF (50 mL) was stirred at ambient temperature for 3 h. The reaction mixture was added to water (200 mL), and the resulting slurry was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with water (50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography using 3:7 EtOAc−hexane as an eluent to afford 1.3 g (51.4%) of 2-(3,4-dihydro-6-methoxy-2,5,7,8-tetramethyl-2*H*-benzopyran-2-yl)-ethanol as a tan oil which solidified upon standing: ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 3H), 1.70−1.95 (m, 4H), 2.06 (s, 3H), 2.14 (s, 3H), 2.18 (s, 3H), 2.63 (t, 2H), 3.63 (s, 3H), 3.9 (q, 2H).

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*,*S*)-2-(3,4-Dihydro-6-methoxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl Ester (6f). To a stirring slurry of 6-methoxy-α-methylnaphthaleneacetic acid (0.90 g, 3.91 mmol), 2-(3,4-dihydro-6-methoxy-2,5,7,8-tetramethyl-2*H*-benzopyran-2-yl)-ethanol (0.98 g, 3.71 mmol), and 1-hydroxybenzotriazole hydrate (0.59 g, 4.36 mmol) in CH_3CN (50 mL) was added 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (1.23 g, 6.42 mmol). After stirring for 18 h, the reaction mixture was concentrated, and the residue was partitioned between EtOAc (50 mL) and 0.1 N HCl (50 mL). The layers were separated, and the organic layer was washed with water (50 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography using 9:1 hexane−EtOAc as an eluent. Recrystallization from hexanes afforded 0.90 g (38.4%) of **6f** as a white solid: mp 76−77 °C; ^{1}H NMR ($CDCl_3$) δ 1.71 (d, 3H), 1.55−1.60 (m, 3H), 1.62 (m, 2H), 1.87 (m, 2H), 2.04 (s, 3H), 2.08 (s, 3H), 2.16 (s, 3H), 2.44 (t, 2H), 3.60 (s, 3H), 3.85 (q, 1H), 3.91 (s, 3H), 4.51−4.40 (m, 2H), 7.11−7.71 (m, 6H). Anal. ($C_{30}H_{36}O_5$) C, H.

6-Methoxynaphthaleneacetic Acid, (*S*)-2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl Ester (7). Synthesized by method A in 24% yield from (*S*)-2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethanol and 6-methoxynaphthaleneacetic acid: white solid; mp 77−80 °C; $[\alpha]_D = +9.4°$ ($c = +0.511$, ethanol); ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 3H), 1.72 (m, 2H), 1.77 (m, 2H), 2.05 (s, 6H), 2.07 (s, 3H), 2.52 (m, 2H), 3.72 (s, 2H), 3.91 (s, 3H), 4.21−4.38 (m, 3H), 7.11−7.71 (m, 6H). Anal. ($C_{28}H_{32}O_5$) C, H.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*,*S*)-2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl Amide (8). Synthesized by method B in 11.6% yield from 2-(3,4-dihydro-6-hydroxy-tetramethyl-2*H*-1-benzopyran-2-yl)ethylamine[42] and 6-methoxy-α-methylnaphthaleneacetic acid: white solid; mp 125.5−127.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.17 (d, 3H), 1.57 (s, 3H), 1.84−1.78 (m, 2H), 2.00 (s, 3H), 2.01 (m, 2H), 2.07 (s, 3H), 2.12 (s, 3H), 2.45−2.61 (m, 2H), 3.21−3.4 (m, 1H), 3.53−3.71 (m, 2H), 3.91 (s, 3H), 4.22 (d, 1H), 5.73 (m, 1H), 7.05−7.65 (m, 6H). Anal. ($C_{29}H_{35}NO_4$) C, H, N.

Ethyl 5-Hydroxy-3,4,6,7-tetramethyl-2(3*H*)-oxobenzofuran-3-carboxylate (12). To a solution formed by adding sodium (4.44 g, 193 mmol) to absolute EtOH (200 mL) was added dropwise a solution of diethyl methylmalonate (40.3 g, 231 mmol) in absolute ethanol. After the addition was complete the reaction was stirred for 50 min and a solution of trimethylquinone (29.0 g, 193 mmol) in absolute EtOH (100 mL) was added dropwise over 1.5 h. The reaction mixture was stirred for 22 h. The reaction mixture was cooled to 0 °C, and pH was adjusted to 5 (2 N NaOH). The precipitate that formed was collected by filtration and discarded. The filtrate was concentrated in vacuo, and the residue was diluted with water. The resulting mixture was extracted with EtOAc (3 × 250 mL). The combined organic extracts were washed with brine, dried ($NaSO_4$), and concentrated in vacuo to afford an oily residue. Trituration with hexane afforded 36.0 g (67%) of a white solid: mp 93.5−94.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.15−1.22 (t, 3H), 1.77 (s, 3H), 2.15 (s, 3H), 2.19 (s, 3H), 2.23 (s, 3H), 4.10−4.32 (m, 2H), 4.56 (s, 1H).

Ethyl 5-(Benzyloxy)-3,4,6,7-tetramethyl-2(3*H*)-oxobenzofuran-3-carboxylate. A mixture of ethyl 5-hydroxy-3,4,6,7-tetramethyl-2(3*H*)-oxobenzofuran-3-carboxylate (16.0 g, 65 mmol), benzyl bromide (11.1 g, 64.9 mmol), and K_2CO_3 in CH_3CN was stirred at ambient temperature for 24 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography using 9:1 hexane−EtOAc as an eluent to afford 8.30 g (34.7%) of the crude product which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.42−1.21 (t, 3H), 1.78 (s, 3H), 2.17 (s, 3H), 2.24 (s, 3H), 2.26 (s, 3H), 4.10−4.30 (m, 2H), 4.74 (s, 2H), 7.31−7.50 (m, 5H).

5-(Benzyloxy)-3,4,6,7-tetramethyl-2(3*H*)-benzofuranone (13). A mixture of ethyl 5-(benzyloxy)-3,4,6,7-tetramethyl-2(3*H*)-oxobenzofuran-3-carboxylate (5.58 g, 15.2 mmol) and KOH (4.25 g, 75.5 mmol) in absolute EtOH was stirred for 40 min. A solution of 2 N HCl was added slowly, and the mixture was stirred for 20 min. The reaction mixture was diluted with water, and the resulting mixture was extracted with EtOAc (4 × 75 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash chromatography using 10:0 to 0:10 gradient of hexane−EtOAc as an eluent to afford 3.61 g (80.4%) of 5-(benzyloxy)-3,4,6,7-tetramethyl-2(3*H*)-benzofuranone as a white solid: mp 93.5−94.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.57−1.60 (d, 3H), 2.22 (s, 3H), 2.25 (s, 6H), 3.65−3.79 (q, 1H), 4.74 (s, 2H), 7.38−7.50 (m, 5H).

Methyl 5-(Benzyloxy)-3,4,6,7-tetramethyl-2,3-dihydrobenzofuran-3-carboxylate (15). Sodium hydride (60% oil dispersion, 0.48 g, 11.9 mmol) was added to a stirring solution of 5-(benzyloxy)-3,4,6,7-tetramethyl-2(3*H*)-benzofuranone (3.52 g, 11.9 mmol) in 1,2-dimethoxyethane (50 mL), and the reaction mixture was stirred at 65 °C. After 2 h bromochloromethane (1.62 g, 12.5 mmol) was added, and the reaction mixture was stirred at 50 °C for 24 h. The precipitate that formed was collected by filtration and discarded. The filtrate was diluted with Et_2O (180 mL), and the resulting solution washed with water (2 × 50 mL) and brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in methanol (50 mL), and NaOMe (0.64 g, 11.9 mmol) was added. The reaction mixture was stirred at 60 °C for 24 h, filtered through Celite, and concentrated in vacuo. The residue was purified by flash chromatography using 10:0 to 80:20 gradient of hexane−EtOAc as an eluent to afford 2.04 g (50.7%) of a pale-yellow oil that was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.55−1.59 (d, 3H), 2.13 (s, 3H), 2.15 (s, 3H), 2.21 (s, 3H), 3.73 (s, 3H), 4.24−4.28 (d, 1H), 4.71 (s, 2H), 4.75−4.78 (d, 1H), 7.36−7.51 (m, 5H).

(5-(Benzyloxy)-2,3-dihydro-3,4,6,7-tetramethylbenzofuran-3-yl)methanol (16). To a solution of methyl 5-(benzyloxy)-3,4,6,7-tetramethyl-2(3*H*)-dihydrobenzofuran-3-carboxylate (2.02 g, 6.00 mmol) in THF was added a 1 M solution of lithium aluminum hydride (18.0 mL, 18 mmol) in THF. The reaction mixture was stirred at ambient temperature for 22 h. The reaction mixture was quenched by the sequential addition of 10% aqueous THF (30 mL), 10% NaOH (30 mL), and water (9 mL). The resulting suspension was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography using a 10:0 to 70:30 gradient of hexane−EtOAc as an eluent to afford 0.88 g (47.1%) of a white solid: mp 111−112.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 3H), 2.15 (s, 3H), 2.25 (s, 3H), 2.29 (s, 3H), 3.50 (bs, 1H), 3.65−3.77 (q, 2H), 4.08−4.12 (d, 1H), 4.55−4.59 (d, 1H), 4.74 (s, 2H), 7.40−7.59 (s, 5H). Anal. ($C_{20}H_{24}O_3$) C, H.

(*S*)-6-Methoxy-α-methylnaphthaleneacetic Acid, (*R*,*S*)-(2,3-Dihydro-5-hydroxy-3,4,6,7-tetramethylbenzofuran-3-yl)methyl Ester (9). Synthesized by method A in 25% yield from (6-(benzyloxy)-2,3-dihydro-3,4,6,7-tetramethylbenzofuran-3-yl)methanol and 6-methoxy-α-methylnaphthaleneacetic acid. The ester was deprotected using the method described for compound **3** and was isolated as an oil: ^{1}H NMR ($CDCl_3$)

δ 1.30 (d, 3H), 1.54 (d, 3H), 2.05 (s, 3H), 2.08 (s, 3H), 2.12 (s, 3H), 4.80 (q, 1H), 3.92 (s, 3H), 4.10−4.40 (m, 5H), 7.10−7.70 (m, 6H). Anal. ($C_{27}H_{30}O_5$) C, H.

(*R,S*)-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)acetic Acid, (*S*)-2-(6-Methoxynaphthalen-2-yl)propyl Ester (10). To a solution of 2(*S*)-(6-methoxynaphthalen-2-yl)propanol (1.07 g, 4.97 mmol) and 2-(6-(benzyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)-acetic acid (1.6 g, 4.52 mmol) in THF (100 mL) at room temperature were added dicyclohexylcarbodiimide (1.40 g, 6.77 mmol) and 4-(dimethylamino)pyridine (0.27 g, 2.21 mmol). After stirring for 12 h, the reaction mixture was diluted with EtOAc (100 mL), and the resulting solution was washed with 1 N HCl and a saturated solution of $NaHCO_3$, dried ($MgSO_4$), and concentrated. The oily residue was dissolved in THF (50 mL), and ammonium formate (1.54 g, 24.8 mmol) and 10% Pd/C (0.25 g) were added. The reaction mixture was stirred at room temperature for 12 h and then filtered through a pad of Celite. The filtrate was concentrated, and the residue was purified by flash chromatography using 1:4 EtOAc−Hexane as an eluent to afford 0.5 g (24%) of a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3H), 1.28 (d, 3H), 1.67 (m, 1H), 2.01−2.12 (m, 9H), 2.50 (m, 2H), 2.53 (m, 2H), 3.98 (s, 3H), 4.16−4.30 (m, 5H), 7.06−7.71 (m, 6H); MS(CI) *m/z* 463 (M + 1). Anal. ($C_{29}H_{34}O_5$) C, H, N.

Assessment of Antioxidant Activity. 1. Membrane Preparation. Membranes were prepared from myocardial tissue by the method of Parinandi et al.[44] Bovine heart was obtained from Bio Resources (Richardson, TX). Atria and vessels were trimmed away. The heart was minced with scissors and then a 10% (w/v) homogenate was prepared in ice-cold incubation buffer A (25 mM HEPES, 150 mM NaCl, pH 7.4) with a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY) at a setting of 7 for 1 min. To the homogenate was added 4 M KCl in buffer A to a final concentration of 1.25 M. The homogenate was maintained on ice for 10 min with gentle stirring. The homogenate was filtered through cotton gauze and centrifuged for 20 min at 9000*g* in a Beckman J2-21M refrigerated centrifuge (Rotor JA-14) at 4 °C. The residue was suspended (10 volumes of 1.25 M KCl in buffer A), allowed to stand on ice for 10 min, and centrifuged for 20 min at 9000*g*. The supernatant was collected and subjected to centrifugation at 100000*g* for 1 h in a Beckman L5-50E ultracentrifuge. The membrane pellet was suspended in buffer A and centrifuged at 100000*g* for 1 h in a Beckman L5-50E ultracentrifuge. The resulting final membrane pellet was resuspended in buffer A. A BCA protein assay (Pierce, Rockford, IL) was performed, and the membrane preparation was then diluted with the appropriate volume of buffer A to reach a final protein concentration of 20 mg/mL. Aliquots of the preparation were stored in Eppendorf microfuge tubes and frozen at −80 °C until use.

2. Membrane Lipid Peroxidation Assay. Seventy-five (75) μL of membrane preparation (20 mg/mL), 825 μL of buffer A, and 50 μL of test compound or vehicle were added to a screw-capped test tube, and the mixture was incubated for 30 min at 37 °C. The reaction was then immediately cooled to room temperature (21 °C), and peroxidation was initiated by the addition of 50 μL of 0.1 M $FeCl_2$/1 M ascorbic acid. Control incubations were carried out in the absence of Fe^{2+}/ascorbate. Peroxidation was permitted to continue for 15 min at 21 °C in a water bath. The reaction was then stopped by the addition of 20 μL of 6% butylated hydroxytoluene (BHT; final concentration 5.4 mM) with thorough mixing. Two (2.0) mL of trichloroacetic acid/2-thiobarbituric acid/hydrochloric acid (TCA−TBA−HCl; 15%/0.375%/0.25 N) was then added to each sample and mixed thoroughly. The mixture was heated for 15 min at 80 °C in a hot water bath, cooled to room temperature, and then centrifuged at 1000*g* for 10 min at room temperature in the Beckman GPR centrifuge. The absorbance of the clear supernatant was measured at 540 nm in a EL340 Biokinetics microplate reader (Bio-Tek Instruments, Winooski, VT) against an appropriate blank. The amount of TBARS present in each sample was calculated using a standard curve prepared with malonaldehyde bis[dimethyl acetal] (100 μL of standard + 900 μL of buffer A + 2 mL of TCA−TBA−HCl) over a final concentration range of 0.5−15 nmol/mL. Lipid peroxidation was expressed as nmol of TBARS formed during the incubation period.[44] IC_{50} values were calculated using linear regression.

Assessment of Antiproliferative Activity. 1. Human Vascular Endothelial Cell Culture. Normal human lung microvascular endothelial cells (HMVEC-L 2631−2) (Clonetics Corp., San Diego, CA) were obtained from a 5-year-old male donor. These cells are positive for acetylated low-density lipoprotein uptake, expression of von Willebrand's (factor VIII) antigen, PECAM (platelet endothelial cell adhesion molecule), and Matrigel staining. Expression of α-actin occurred in less than 10% of the cells. The cells were grown in EGM-MV (endothelial growth medium−for microvascular cells; Clonetics Corp.) consisting of endothelial cell basal medium supplemented with 5% fetal bovine serum, 0.4% (v/v) bovine brain extract, 10 ng/mL human recombinant epidermal growth factor, 1 mg/mL hydrocortisone, 50 mg/mL gentamicin, and 50 ng/mL amphotericin-B (amounts indicate final concentration). Cells were used between passages 3 and 9 for all studies. Cell viability was assessed by Trypan blue.

2. DNA Inhibition Assay. Except where specifically stated, drug effects on the proliferation of HMVEC-L were evaluated as follows: Cells were grown in EGM-MV to 75−90% confluence. Thymidine incorporation into DNA was measured by a modification of the method described by Van Corven et al.[45] To initiate the assay, cells were incubated at 37 °C with fresh medium containing the test compound for a period of 18 h. [*methyl*,1′,2′-^{3}H]Thymidine (specific activity 102.7 Ci/mmol; DuPont NEN, Boston, MA) was then added to the medium to achieve 2.5 μCi in a total medium volume of 200 μL/microwell, and cell incubation was continued for 6 h. Radioactive medium was then aspirated, cells were washed once with 200 μL of cold 0.15 M NaCl, and the reaction was terminated by the addition of 200 μL of ice-cold 5.0% trichloroacetic acid (TCA). After 15 min at room temperature, the TCA was aspirated and the precipitate in each well was washed four times with 200 μL of water. The precipitate was then solubilized by incubation at 37 °C for 15 min with 150 μL of 1.0% sodium dodecyl sulfate. The [^{3}H]thymidine present in the resolubilized sample was then quantified by liquid scintillation counting. IC_{50} values were calculated using linear regression.

3. Cytostatic Activity of 6a. Because **6a** inhibited DNA synthesis in HMVEC-L (IC_{50} = 13 μM, Table 1) studies were designed to determine whether this inhibition was reversible. HMVEC-L (>80% confluent) were treated with medium supplemented with **6a** at a concentration of 5 or 25 μM in 1% DMSO or with 1% DMSO as a control. In half of the experiment, cell cultures were incubated for 18 h and then pulsed for 6 h with [^{3}H]thymidine prior to quantitation of [^{3}H]thymidine incorporation, all as described above. To assess recovery from the inhibition by **6a**, in the other half of the experiment, cells were treated with **6a** for 24 h, the medium was then replaced with fresh medium containing no **6a,** and cells were incubated for an additional 24 h. [^{3}H]Thymidine was added during the final 6 h of this second incubation period.

HPLC Analysis. Conditioned medium was analyzed for naproxen by HPLC using a Sphirisorb (10 μm, C-18) column and an isocratic mobile phase of methanol/phosphate buffer, pH 3.0 (50:50), at a flow rate of 2.0 mL/min. Naproxen was detected by UV absorption (λ = 230 nm). With an injection volume of 80 μL, the retention time for naproxen separated in this HPLC system was 14.3 min.

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