hexadiene, 592-57-4; propiolic acid, 471-25-0; bicyclo[2.2.2]octa-2,5-diene-2-carboxylic acid, 102589-30-0; 2-(chlorocarbonyl)bicyclo[2.2.2]octa-2,5-diene, 117040-22-9; (*E,E*)-ClC(O)CH=CHCH=CHC(O)OCH$_3$, 41967-17-3; (*E*)-ClC(O)CH=CH-*p*-C$_6$H$_4$F, 13565-08-7; ClC(O)CH=CH-*m*-C$_6$H$_4$F, 39098-87-8; ClC(O)CH=CH-*p*-C$_6$H$_4$NCS, 117201-50-0; (*E*)-ClC(O)CH=CH-*p*-C$_6$H$_4$NHC(O)CH$_2$Br, 117201-51-1; (*E,E*)-ClC(O)CH=CH-*p*-C$_6$H$_4$NHC(O)CH=CHC(O)OMe, 117226-09-2; ClC(O)CF=CHC$_6$H$_5$, 117201-52-2; (*E*)-ClC(O)CH=CHC(O)C$_6$H$_5$, 117201-53-3; *trans*-ClC(O)$\overline{\text{CHOCH}}$C$_6$H$_5$, 76527-41-8; SCNC$_6$H$_{11}$, 1122-82-3; SCN-*p*-C$_6$H$_4$N$_3$, 74261-65-7; SCN-*p*-C$_6$H$_4$NHC(=S)$\overline{\text{NCH}_2\text{CH}_2}$, 117201-54-4; ClC(O)-*m*-C$_6$H$_4$CH$_2$Cl, 63024-77-1; ClC(O)C$_6$H$_3$-*m*-I-*p*-NCS, 117201-55-5; (*E*)-ClC(O)(CH$_2$)$_4$-*p*-C$_6$H$_4$NHC(O)CH=CHC(O)OMe, 117201-56-6; 2-naphthalenecarbonyl chloride, 2243-83-6; 2-thiophenesulfonyl chloride, 16629-19-9.

Antiinflammatory 2,6-Di-*tert*-butyl-4-(2-arylethenyl)phenols

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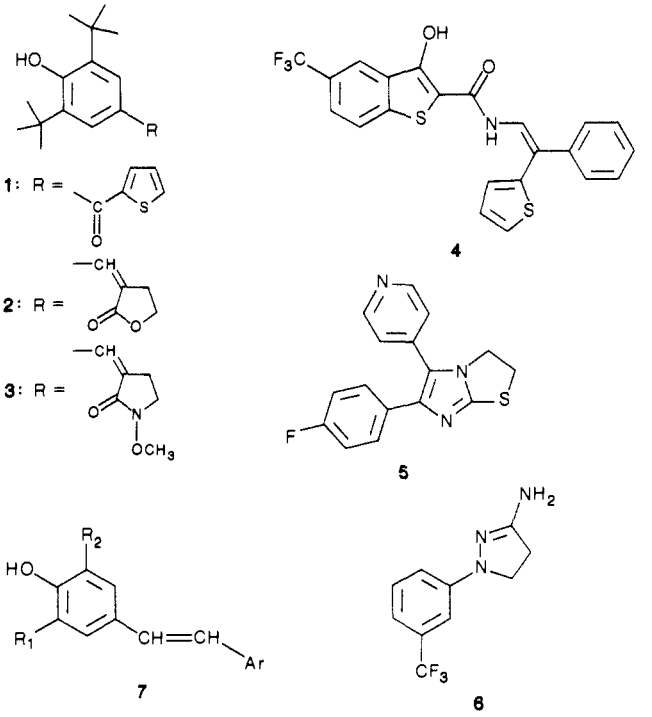
A series of 2,6-di-*tert*-butyl-4-(2-arylethenyl)phenols was prepared and examined for their ability to inhibit cyclooxygenase and 5-lipoxygenase in vitro and developing adjuvant arthritis in vivo in the rat. Structure-activity relationships are discussed. Among the best compounds is (*E*)-2,6-di-*tert*-butyl-4-[2-(3-pyridinyl)ethenyl]phenol (**7d**). It has an IC$_{50}$ of 0.67 μM for cyclooxygenase and 2.7 μM for 5-lipoxygenase and an ED$_{50}$ of 2.1 mg/kg in developing adjuvant arthritis. Additional in vivo data are reported for **7d**.

In an effort to obtain antiinflammatory drugs with a profile superior to cyclooxygenase-inhibiting nonsteroidal antiinflammatory drugs (NSAIDs), several groups have prepared dual cyclooxygenase/5-lipoxygenase (CO/LO) inhibitors. By blocking the production of prostaglandins from arachidonic acid, CO-inhibiting NSAIDs ameliorate the primary symptoms of arthritis via their analgesic, antipyretic, and antiinflammatory properties. However, they do not prevent tissue destruction or stop the disease process.[1]

Leukotrienes, the products of 5-LO metabolism of arachidonic acid, have been associated with immediate hypersensitivity reactions, anaphylaxis, and asthma.[2] 5-LO metabolites have also been linked to the inflammatory process. Leukotriene B$_4$ (LTB$_4$, 5,12-diHETE) has been shown to be a potent chemotactic substance for neutrophils.[3,4] In addition, products of 5-LO metabolism have been shown to stimulate neutrophil degranulation,[5] resulting in the release of lysosomal enzymes and reactive oxygen species, and to increase capillary permeability,[6] contributing to edema. These responses suggest a significant role in initiating and amplifying the inflammatory response. Therefore a dual CO/LO inhibitor would affect a wider range of proinflammatory mechanisms and presumably have an improved profile.

Among the dual CO/LO inhibitors reported are the three 2,6-di-*tert*-butylphenol derivatives R-830 (**1**),[7] KME-4 (**2**),[8] and E-5110 (**3**).[9] Other structurally unrelated dual CO/LO inhibitors include L-652,343 (**4**),[10] SK&F 86002 (**5**),[11] and one of the early compounds of this class, BW 755C (**6**).[12]

The design of the 2,6-di-*tert*-butylphenol derivatives was based on their potential antioxidant activity.[13] Stimulated polymorphonuclear leukocytes and macrophages release superoxide anion, which is metabolized to other reactive oxygen species including hydrogen peroxide, hydroxyl radical, and singlet oxygen. These reactive oxygen species are thought to contribute to the inflammatory process and tissue destruction.[14] While antioxidant activity has been demonstrated for **1** in autoxidation of peanut oil and $FeCl_2$-induced peroxidation of liposomes,[7] it is not established that any of the beneficial in vivo effects are brought about by scavenging reactive oxygen species released from stimulated cells.



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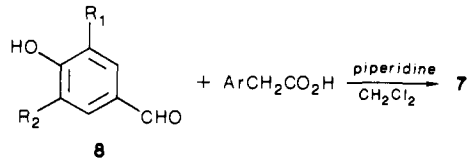
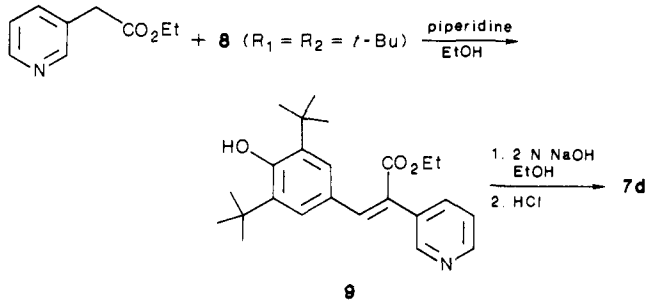
Scheme I



Scheme II



In this paper we describe our efforts on the synthesis of antiinflammatory di-*tert*-butylphenol derivatives and in particular the dual CO/LO inhibitory and antiinflammatory activity of a series of 2,6-di-*tert*-butyl-4-(2-arylethenyl)phenols (**7**).

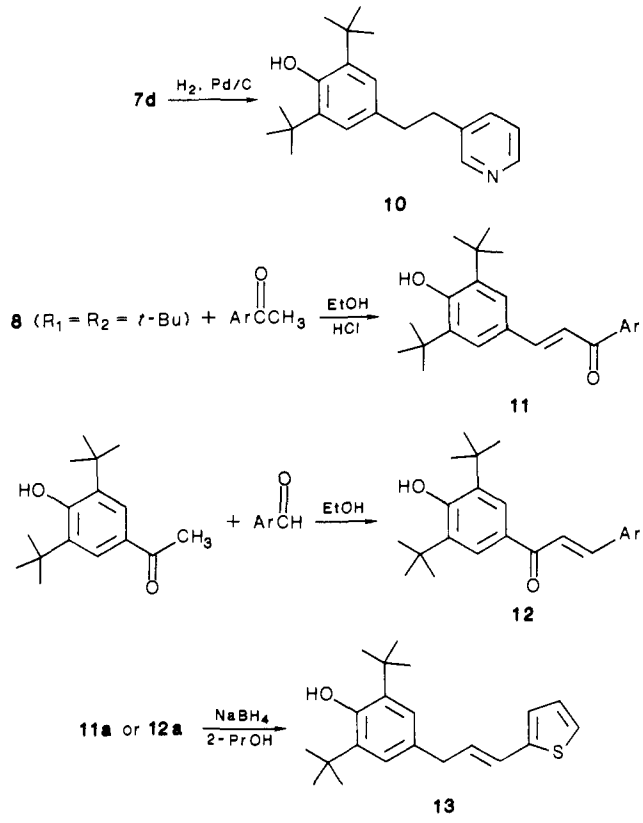
Chemistry

(Arylethenyl)phenols (**7**) were prepared by a modification of the procedure described by Cox et al.[15] (Scheme I). Coupling constants of the olefinic protons (J = 15.9–16.5 Hz) indicated all compounds **7** were in the E configuration. Starting aldehyde **8** (R_1 = H, R_2 = *tert*-butyl) was prepared by a Vilsmeier–Haack reaction of the corresponding phenol according to a published procedure.[16] Disubstituted phenols gave the corresponding aldehydes **8** ($R_1 = R_2$ = *i*-Pr, R_1 = CH_3, R_2 = *t*-Bu) in a modified Duff procedure, as described in the literature.[17] To prepare **8** ($R_1 = R_2$ = *tert*-amyl); 2,6-di-*tert*-amyl-4-methylphenol[18] was treated with bromine in aqueous HOAc.[19]

The procedure in Scheme I was unsuitable for preparing compound **7d** on a large scale, because the product had to be purified by column chromatography. In a modified procedure suitable for scale-up (Scheme II), ethyl 3-pyridylacetate was condensed with **8** ($R_1 = R_2$ = *tert*-butyl), giving ester **9**, which was hydrolyzed and decarboxylated to **7d**.

The syntheses of compounds with connecting chains other than olefin are illustrated in Scheme III. Catalytic reduction of **7d** gave **10**. Condensation of **8** with 2- or 3-acetylthiophene in ethanolic HCl gave **11a** and **11b**, while use of 4-acetyl-2,6-di-*tert*-butylphenol[20] and 2- or 3-thiophenecarboxaldehyde under the same conditions gave **12a** and **12b**. Reduction of either **11a** or **12a** with $NaBH_4$ gave the same product **13**. The position of the olefin in **13** was confirmed by observing a nuclear Overhauser enhancement (4–5% difference) on the phenyl ring protons upon irradiation of the methylene protons (see the Experimental Section).

Scheme III



Results and Discussion

In the course of our studies on phenolic antiinflammatory derivatives, we examined the effect of structural modification of alkyl groups R, the aromatic ring Ar, and the connecting chain on general structure **7**. We began by exploring the connecting chain between the di-*tert*-butylphenol ring and a 2- or 3-thienyl group. Compounds with propenone chains (**11** and **12**) were in vitro inhibitors of CO and LO but showed no in vivo activity in the developing adjuvant arthritis model (DevAA) at 50 mg/kg. With an olefin connecting chain (**7a** and **7b**), CO and LO inhibition was retained and the compounds were quite active in DevAA (Table I). The results we obtained for **1** and **2** are included in Table I for comparison. Reduction of the olefin to an ethyl chain (**10**) destroyed CO-inhibitory and DevAA activity. Expanding the olefin to a propene chain (**13**) also sharply reduced CO-inhibitory activity.

We therefore examined an expanded series of 2,6-di-*tert*-butylphenol derivatives with an olefin connecting chain (**7a–w**). Several of these compounds have been previously reported as chemical intermediates.[15] Only those compounds with thienyl or pyridyl as substituents at Ar (**7a**, **7b**, **7d**, **7e**, and **7g**) showed appreciable LO inhibition, greater than 50% at 10 μM. Compound **7d** was the most potent LO inhibitor, IC_{50} = 2.7 μM. The compounds with a heterocyclic ring at Ar were also the most potent CO inhibitors with **7a–e** showing over 50% inhibition at 1 μM. Compound **7a** was the most potent CO inhibitor, IC_{50} = 0.063 μM. Among the compounds with a substituted phenyl ring at Ar, **7m**, which has a 4-

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Table I. Activity of Substituted Phenols

no.	R_1	R_2	Ar[a]	formula	anal.	mp,[b] °C	% inhibn LO[c] 10 μM	% inhibn LO[c] 1 μM	% inhibn CO[c] 10 μM	% inhibn CO[c] 1 μM	% inhibn Dev AA 50 mg/kg	% inhibn Dev AA 25 mg/kg	% inhibn Dev AA 5 mg/kg
1 (R-830)				$C_{19}H_{24}O_2S$	C,H,S	129–130 (127–130)	82	17	88	88		96[d]	24
2 (KME-4)				$C_{19}H_{26}O_3$	C,H	149–150 (155–156)	67	34	85	80		63[d]	39[d]
7a[e]	*t*-Bu	*t*-Bu	2T	$C_{20}H_{26}OS$	C,H,S	80–82 (80–82)	88	25	77	82	77[d]		57[d]
7b	*t*-Bu	*t*-Bu	3T	$C_{20}H_{26}OS$	C,H,S	105–107	81	9	80	78	62[d]		31
7c	*t*-Bu	*t*-Bu	2T5Cl	$C_{20}H_{25}ClOS$	C,H,Cl,S	124–125	28	19	80	70	45[d]		
7d[f]	*t*-Bu	*t*-Bu	3P	$C_{21}H_{27}NO$	C,H,N	104–105	84	26	63	68	82[d]		56[d]
7e	*t*-Bu	*t*-Bu	2P	$C_{21}H_{27}NO$	C,H,N	105–106	88	34	84	86	74[d]		36
7f	*t*-Bu	*t*-Bu	4P	$C_{21}H_{27}NO$	C,H,N	216–217 (243–245)	6		36	22	0		
7g	*t*-Bu	*t*-Bu	3PN-O	$C_{21}H_{27}NO_2$	C,H,N	199–202	59	21	73	24		26	
7h	*t*-Bu	*t*-Bu	4-MeC_6H_4	$C_{23}H_{30}O$	C,H	107–108 (98–98.5)	27	6	52	12	97[d]		55[d]
7i	*t*-Bu	*t*-Bu	4-$OMeC_6H_4$	$C_{23}H_{30}O_2$	C,H	115–116 (118–120)	12	18	6[g]	15[g]	0		
7j	*t*-Bu	*t*-Bu	3,4-$(OMe)_2C_6H_3$	$C_{24}H_{32}O_3$	C,H	101–102	20	12	20	16[g]	26		
7k	*t*-Bu	*t*-Bu	4-ClC_6H_4	$C_{22}H_{27}ClO$	C,H,Cl	121–122 (120–122)	16	6	66	31	42[d]		
7l	*t*-Bu	*t*-Bu	3,4-$Cl_2C_6H_3$	$C_{22}H_{26}Cl_2O$	C,H,Cl	137–138 (135–137)	3[h]	18	5[g]	16[g]	10		
7m	*t*-Bu	*t*-Bu	4-FC_6H_4	$C_{22}H_{27}FO$	C,H,F	87–88 (80–81)	46	23	70	52	58[d]		
7n	*t*-Bu	*t*-Bu	3-$CF_3C_6H_4$	$C_{23}H_{27}F_3O$	C,H,F	121–122	22	19	18	9	0		
7o	*t*-Bu	*t*-Bu	2-$(CO_2H)C_6H_4$	$C_{23}H_{28}O_3$	C,H	209–210		10	53	21			
7p	*t*-Bu	*t*-Bu	C_6H_5	$C_{22}H_{28}O$	C,H	85–88 (90–91)	14	12	79	34	42[d]		
7q	*t*-Bu	Me	3P	$C_{18}H_{21}NO$	C,H,N	162–164	85	75	87	68		22	
7r	*t*-Bu	H	3P	$C_{17}H_{19}NO$	C,H,N[i]	196–198	81	29	89	80		3	
7s	H	H	3P	$C_{13}H_{11}NO$	C,H,N	242–244	41		5[h]	28[g]			
7t	*t*-Am	*t*-Am	3P	$C_{23}H_{31}NO$	C,H,N	95–96		27	86	81		50[d]	
7u	*t*-Am	*t*-Am	2T	$C_{22}H_{30}OS$	C,H,S,	65.5–67		10	80	74		40[d]	
7v	*i*-Pr	*i*-Pr	3P	$C_{19}H_{23}NO$	C,H,N	136–138	87	73	81	28		17	
7w	*i*-Pr	*i*-Pr	2T	$C_{18}H_{22}OS$	C,H,S	45.5–48.5		82	76	64			
9				$C_{24}H_{31}NO_3$	C,H,N	120–121.5	83	23	78	54	24		
10				$C_{21}H_{29}NO$	C,H,N	112–114	63	13[h]	8	30[g]		3	
11a			2T	$C_{21}H_{26}O_2S$	C,H,S	195–197	84	39	75	44	8		
11b			3T	$C_{21}H_{26}O_2S$	C,H,S	187.5–190	91	53	80	56	0		
12a			2T	$C_{21}H_{26}O_2S$	C,H,S,	185–187	59	10	83	72	0		
12b			3T	$C_{21}H_{26}O_2S$	C,H,S	169–170	69	16	83	73	13		
13				$C_{21}H_{28}OS$	C,H,S	111–112	49	7[h]	61	28			

[a] 2T = 2-thienyl, 3P = 3-pyridyl, 3PN-O = 3-pyridyl *N*-oxide. [b] Melting points in parentheses are from ref 15 except for compounds 1 (ref 7), 2 (ref 16), and **7f** (ref 29). [c] IC_{50} values for standard compounds: (95% confidence limits in parentheses) LO – BW 755C, 18 μM (15–19); CO – indomethacin, 0.010 μM (0.008–0.011). Percent inhibition statistically significant compared to control by analysis of variance with Tukey's multiple comparisons test, $p < 0.05$ except where noted (*h*). [d] Decrease in paw volume statistically significant compared to control with Duncan's multiple range test for variable, $p < 0.05$. [e] IC_{50} value for CO inhibition 0.063 μM (0.057–0.070). [f] IC_{50}value for LO inhibition 2.7 μM (2.4–2.9), for CO inhibition 0.67 μM (0.61–0.73). [g] Percent stimulation. [h] Percent inhibition not statistically different from control. [i] C: calcd, 80.58; found, 79.74.

fluorophenyl group, was the most potent CO inhibitor (52% at 1 μM). The others were weak or inactive. The results in DevAA generally parallel the CO-inhibitory activity, **7a** and **7d** being the best with over 50% inhibition at 5 mg/kg. Compound **7h** was an exception having unexpectedly high activity in DevAA at 50 and 5 mg/kg; however it also showed overt toxicity (weight loss, bloody diarrhea) at 50 mg/kg. Among the pyridyl isomers **7d–f**, the 2- and 3-isomers were active in CO and LO inhibition and DevAA while the 4-isomer was inactive in all three tests. An *N*-oxide substituent (**7g**) diminished activity.

The effect of modifying the *tert*-butyl groups was examined with **7a** and **7d** as standards. Reducing the size of one *tert*-butyl group (**7q** and **7r**) had no detrimental effect on CO or LO inhibition, but the compounds were no longer active in vivo. Removing both alkyl groups (**7s**) also destroyed the in vitro activity. Increasing the size of the alkyl groups (7t and **7u**) had no significant effect on CO or LO inhibition and in vivo activity was retained but somewhat diminished. Reducing the size of the alkyl groups to isopropyl (**7v** and **7w**) enhanced LO inhibition, but as with **7q–s**, DevAA activity was lost (**7v**). Therefore in this series the *tert*-butyl group's size appears to be crucial for in vivo activity in DevAA. This size requirement is in agreement with data published for analogs of **2**[16] and compounds related to **1**.[13]

From the data it is clear that CO inhibition alone does not guarantee in vivo activity. For example the CO inhibition of **7d** (IC_{50} = 0.67 μM) is not sufficiently different from that of **7q**, **7r** and **11a**, **11b**, **12a** and **12b** to explain the potent activity of **7d** in DevAA and the total lack of activity in the latter compounds. Apparently other factors such as absorption, distribution, or metabolism differentiate the compounds.

The loss of in vivo activity in compounds **7q**, **7r**, and **7v** may also be due to factors unrelated to in vitro activity, which was not significantly affected. One possible explanation is that alkyl groups smaller than *tert*-butyl allow rapid metabolism (for example glucuronide formation) of the phenolic OH. An adverse effect on absorption or distribution due to reduced lipophilicity is another possibility.

The better in vivo activity observed with compounds **7** having a thienyl or pyridyl ring at Ar compared to those with a phenyl ring may be directly related to the more potent CO-inhibitory activity also seen with those compounds. Although the compounds with heteroaryl Ar are also more potent LO inhibitors, it is unclear whether LO inhibition actually plays a role in the antiinflammatory activity of these compounds or of **1–6**.

Compound **7d** (BI-L-93 BS) was examined further in additional in vivo models. It had an ED_{50} of 16.5 mg/kg in carrageenan-induced paw edema in the rat and ED_{50}s of 2.1 and 4 mg/kg in DevAA and established adjuvant arthritis (EstAA), respectively. This compound also showed analgesic activity in the acetic acid writhing test

with an ED_{50} of 3.9 mg/kg. The dose producing a 50% ulcerogenic response (UD_{50}) in rats was 202 mg/kg, indicating a wide therapeutic index between antiinflammatory and ulcerogenic doses (96 for DevAA and 50 for EstAA). For comparison, in one study with eight NSAIDs, therapeutic indices from 0.6 (aspirin) to 11.5 (etodolac) were reported.[21]

Experimental Section

Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected. ^1H NMR spectra were all consistent with molecular structures and were recorded on a Bruker 250 WM spectrometer. Elemental analyses were performed at Micro-Tech Laboratories, Inc., Skokie, IL, and were within 0.4% of the calculated values unless otherwise indicated.

(*E*)-2,6-Di-*tert*-butyl-4-[2-(3-pyridinyl)ethenyl]phenol (7d) (Scheme I). Piperidine (1.64 g, 19 mmol) was added to a stirred suspension of 3-pyridylacetic acid (2.2 g, 16 mmol) in 5 mL of CH_2Cl_2. 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde (2.48 g, 10.6 mmol) was added and the mixture heated to 140 °C and the CH_2Cl_2 allowed to boil off. After 2.5 h the residue was partitioned between EtOAc and water. The organic phase was dried (Na_2SO_4) and concentrated. The residue was passed through a silica gel column and eluted with CH_2Cl_2–MeOH (95:5). The product was recrystallized from ligroin, giving 0.7 g (2.3 mmol, 21%) of **7d**: mp 104–105 °C; NMR (DMSO-d_6) δ 8.7 (d, 1 H, pyridine), 8.45 (m, 1 H, pyridine), 7.8 (m, 1 H pyridine), 7.35 (s, 2 H, Ph), 7.25 (m, 2 H, pyridine), 7.1 (d, 1 H, J = 16 Hz, olefin), 6.9 (d, 1 H, J = 16 Hz, olefin), 5.4 (s, 1 H, OH), 1.5 (s, 18 H, CH_3). Anal. ($C_{21}H_{27}NO$) C, H, N.

(*E*)-2,6-Di-*tert*-butyl-4-[2-(3-pyridinyl)ethenyl]phenol (7d) via Ethyl 3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-(3-pyridyl)propenoate (9) (Scheme II). 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde (223 g, 0.952 mol), ethyl 3-pyridylacetate (157.2 g, 0.952 mol), piperidine (81 g, 0.952 mol), and *p*-toluenesulfonic acid (0.5 g) were combined in 2.4 L of EtOH and heated at reflux for 48 h. The reaction mixture was stirred on an ice bath and the product began to crystallize. The mixture was stored in a freezer overnight, filtered, rinsed with cold EtOH–water (1:1), and dried, giving 169.1 g of **9** (0.443 mol, 47%): mp 120–121.5 °C; NMR (DMSO-d_6) δ 8.5 (d, 1 H, pyridine), 8.35 (d, 1 H, pyridine), 7.8 (s, 1 H, olefin), 7.6 (m, 1 H, pyridine) 7.45 (m, 1 H, pyridine), 7.4 (s, 1 H, OH), 6.9 (s, 2 H, Ph), 4.5 (q, 2 H, CH_2), 1.2 (t, 3 H, CH_3), 1.15 (s, 18 H, CH_3). Anal. ($C_{24}H_{31}NO_3$) C, H, N.

A mixture of **9** (168.6 g, 0.442 mol), 850 mL of 2 N NaOH, and 850 mL of EtOH was heated at reflux for 4 h. The volume of the reaction mixture was reduced to about 1.1 L on a rotary evaporator, and then the mixture was stirred in a beaker on an ice bath while 2 N HCl (850 mL) was added slowly. The resulting solid was filtered, rinsed with water, dried, and recrystallized twice from 2-PrOH, giving **7d** (69 g). A second crop of 12.7 g was obtained for a total of 81.7 g (0.264 mol, 60%), mp 104–105 °C. Anal. ($C_{21}H_{27}NO$) C, H, N.

2,6-Di-*tert*-butyl-4-[2-(3-pyridinyl)ethyl]phenol (10). A mixture of **7d** (3 g, 9.7 mmol) and 400 mg of 10% Pd/C (50% w/w with water) in 50 mL of EtOH was shaken on a Parr hydrogenation apparatus at an initial pressure of 35 psi until no more hydrogen was taken up (1.5 h). The catalyst was filtered off and the filtrate concentrated. The residue was eluted through a silica gel column with CH_2Cl_2–MeOH (98:2) and the product recrystallized from EtOH, giving 0.53 g of **10** (1.7 mmol, 18%): mp 112–114 °C; NMR (DMSO-d_6) δ 8.4 (m, 2 H, pyridine), 7.45 (m, 1 H, pyridine), 7.2 (m, 1 H, pyridine), 6.9 (s, 2 H, Ph), 5.1 (s, 1 H, OH), 2.9 (m, 4 H, CH_2), 1.4 (s, 18 H, CH_3). Anal. ($C_{21}H_{29}NO$) C, H, N.

(*E*)-3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-1-(2-thienyl)-2-propen-1-one (11a). 3,5-Di-tert-butyl-4-hydroxybenzaldehyde (4.2 g, 18 mmol) and 2-acetylthiophene (2.72 g, 21.6 mmol) were combined in 250 mL of EtOH saturated with HCl and stirred at room temperature. After 4 h the reaction mixture was poured onto ice and water, stirred, and filtered. The product was recrystallized from EtOH, giving 4 g of **11a** (11.7 mmol, 65%): mp 195–197 °C; NMR (DMSO-d_6) δ 8.3 (d, 1 H, thiophene), 8.0 (d, 1 H, thiophene), 7.7 (d, 1 H, J = 15.9 Hz, olefin), 7.6 (d, 1 H, J = 15.9 Hz, olefin), 7.55 (s, 1 H, OH), 7.5 (s, 2 H, Ph), 7.3 (m, 1 H, thiophene) 1.45 (s, 18 H, CH_3). Anal. ($C_{21}H_{26}O_2S$) C, H, S.

(*E*)-1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-3-(2-thienyl)-2-propen-1-one (12a). 4-Acetyl-2,6-di-*tert*-butylphenol (4.47 g, 18 mmol) and 2-thiophenecarboxaldehyde (2.4 g, 21.4 mmol) were combined in 200 mL of EtOH saturated with HCl and stirred at room temperature for 5 h. The reaction mixture was poured onto ice and water and the product filtered and recrystallized twice from EtOH, giving 2.46 g of **12a** (7.2 mmol, 40%): mp 185–187 °C; NMR ($CDCl_3$) δ 7.95 (d, 1 H, J = 15.3, olefin), 7.9 (s, 2 H, Ph), 7.3 (m, 3 H, thiophene and olefin), 7.1 (m, 1 H, thiophene), 5.75 (s, 1 H, OH), 1.4 (s, 18 H, CH_3). Anal. ($C_{21}H_{26}O_2S$) C, H, S.

(*E*)-3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-1-(2-thienyl)-propene (13). $NaBH_4$ (0.5 g, 13.2 mmol) was added slowly in portions to a suspension of **12a** (3 g, 8.8 mmol) in 125 mL of 2-propanol. The reaction was stirred 22 h at room temperature, and then another 0.2 g of $NaBH_4$ (5.3 mmol) was added. After another hour the reaction was poured onto ice and 2 N HCl, and the resulting yellow solid was filtered. The crude product was passed through a silica gel column eluting with CH_2Cl_2. From the column 0.7 g of **13** was collected. It was recrystallized from EtOH, giving 0.46 g of **13** (1.4 mmol, 16%): mp 111–112 °C; NMR ($CDCl_3$) δ 7.1 (m, 1 H, thiophene), 7.05 (s, 2 H, Ph), 6.95 (m, 2 H, thiophene), 6.55 (d, 1 H, J = 15.9 Hz, olefin), 6.2 (m, 1 H, olefin), 5.1 (s, 1 H, OH), 3.4 (d, 2H, CH_2), 1.4 (s, 18 H, CH_3). Anal. ($C_{21}H_{28}OS$) C, H, N.

The difference nuclear Overhauser effect (NOE) experiment was performed on an IBM instruments AF-270 NMR spectrometer according to the procedure of Hall and Sanders.[22] The experiment was run in a sequential manner to minimize the effects of instrumental instability. Spectra were acquired with a 2-s preirradiation time period, a 1-μs pulse width (20° tip angle), 1.36-s acquisition time, and a 10-s relaxation delay. Spectral width was 6024 Hz with 16K data points. A total of 16 scans repeated through 16 cycles were used. Upon irradiation of the methylene protons at δ 3.4, a 4–5% nuclear Overhauser enhancement was observed for the olefinic protons and the phenyl aromatic protons. No enhancement for the thiophene protons was observed.

Biological Methods. Developing Adjuvant-Induced Arthritis. Arthritis[23] was induced in male Lewis rats (150–170 g) by injection of a heat-killed *Mycobacterium butyricum*. Each animal was given 0.1 mL of a 5 mg/mL adjuvant suspension in light mineral oil injected subcutaneously into the plantar surface of the right hind foot. Ten rats were used in each test group. Animals were dosed orally with a suspension of test compound in 1% acacia in distilled water immediately after adjuvant injection and once daily for a total of 14 days. Foot volumes were measured 24 h after the final dose by mercury displacement to the level of the lateral malleolus. Mean displacement volumes and their standard errors were calculated for the noninjected hind paws. An untreated arthritic control group was used as comparison for test compound effect. Results are expressed as percent inhibition of paw swelling in the drug treated group compared to the untreated arthritic controls.

Established Adjuvant-Induced Arthritis. Arthritis was induced in male Lewis rats as described above. Rats in which arthritis became established were selected 14 days after adjuvant injection, treated orally with test compound for 14 days, and evaluated as described above.

Carrageenan-Induced Paw Edema.[24] Edema was produced in the right hind paw of male CD rats (150 ± 10 g) by the subplantar injection of 0.1 mL of a 1% carrageenan suspension in saline. Ten rats were used in each test group. Test compound or its vehicle (1% acacia in distilled water) was administered orally 1 h prior to carrageenan injection. Paw volume was determined by calculating the amount of mercury displaced after immersing

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the paw to the level of the lateral malleolus. Foot volumes were measured just prior to test compound administration and again 3 h after carrageenan injection, and the difference was designated as edema volume.

Ulcerogenicity Study. The UD_{50} for compound **7d** was determined by using the procedure of Wong et al.[25] The UD_{50} refers to that dose required to produce a 50% ulcerogenic response on a graded scale where 0% = no ulcers or redness in the stomach or intestines and 100% = major lesions in both the stomach and intestines.

Acetic Acid Writhing. The analgesic potential of **7d** was determined by using the procedure described by Hendershot and Forsaith,[26] but with acetic acid[27] in place of phenylquinone.

Cyclooxygenase-Inhibition Assay. Human platelets (10^7 platelets/0.5 mL) in Ca^{2+}-free pH 7 phosphate buffer were incubated with test compound for 15 min at 37 °C with shaking. [^{14}C]Arachidonic acid (0.25 μCi, 0.076 μg/mL) was added and the mixture incubated another 30 min. The reaction was terminated by addition of 0.025 mL of 1.0 N HCl. The mixture was then extracted with EtOAc–CH_2Cl_2 (2:3) supplemented with 12 μg/mL cold arachidonic acid to reduce degradation of the metabolites. After concentration of the organic phase, the number of microliters containing 10^5 cpm was determined and that volume applied to a silica gel plate. The plate was developed in methylene chloride–methanol–acetic acid–water (90:8:1:0.8), air-dried, and counted on a Berthold linear TLC analyzer. The integrated area of the prostaglandin–thromboxane bands was determined and measured as the percentage of the total radioactivity.

Inhibition was calculated by the comparison of compound (cmd) with control (cntl) activity: (1 − cmd/cntl) × 100. Screening data reported were the result of a single determination performed in duplicate. Statistical significance at the $p < 0.05$ level was determined by analysis of variance by using the General Linear Models procedure of the SAS statistical software package.[28] The IC_{50} values for the standard compound indomethacin and for the test compounds were determined by linear regression analysis using four or more concentrations. The number of determinations were as follows: indomethacin (n = 21), **7a** (n = 3), **7d** (n = 4). The median coefficient of variation based on replicate measurements for indomethacin was 11.0%.

5-Lipoxygenase-Inhibition Assay. This procedure is similar to the one described for cyclooxygenase inhibition with the following modifications. Human peripheral blood leukocytes (5×10^6 cells/0.5 mL) in pH 7.2 phosphate buffer containing Ca^{2+} (0.6 mM) and Mg^{2+} (1.0 mM) were incubated with test compound for 15 min at 37 °C with shaking. Calcium ionophone A23187 (0.25 mM, 0.01 mL) and [^{14}C]arachidonic acid (0.10 μCi in 0.025 mL of 0.01 N NaOH) were added to the cell-test compound mixture and incubated as above for 2.5 min. After termination, extraction, and concentration of the organic phase, 5×10^4 cpm was applied to a silica gel plate. The integrated area of the 5-HETE band was determined, and calculation of percent inhibition and statistical analysis were the same as described for cyclooxygenase inhibition.

The IC_{50} values for the standard compound BW 755C and for the test compounds were determined by linear regression analysis using four or more concentrations. The number of determinations were as follows: BW 755C (n = 4), **7d** (n = 4). The median coefficient of variation based on replicate measurements for BW 755C was 11.7%.

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Registry No. **7**, 116405-78-8; **7b**, 116376-60-4; **7c**, 116376-61-5; **7d**, 116376-62-6; **7e**, 116376-63-7; **7f**, 51451-68-4; **7g**, 116376-64-8; **7h**, 116376-65-9; **7i**, 116376-66-0; **7j**, 116376-67-1; **7k**, 116376-68-2; **7l**, 116376-69-3; **7m**, 116376-70-6; **7n**, 116376-71-7; **7o**, 116376-72-8; **7p**, 21449-69-4; **7q**, 116376-73-9; **7r**, 116376-74-0; **7s**, 85666-05-3; **7t**, 116376-75-1; **7u**, 116376-76-2; **7v**, 116376-77-3; **7w**, 116376-78-4; **8** ($R_1 = R_2$ = Bu-*t*), 1620-98-0; **8** (R_1 = Bu-*t*, R_2 = Me), 123-08-0; **8** ($R_1 = R_2$ = Am-*t*), 10507-96-7; **8** ($R_1 = R_2$ = Pr-*i*), 10537-86-7; **8** (R_1 = Bu-*t*, R_2 = H), 65678-11-7; **9**, 115816-06-3; **10**, 116376-79-5; **11a**, 116376-80-8; **11b**, 116376-81-9; **12a**, 116376-82-0; **12b**, 116376-83-1; **13**, 116376-84-2; $2TCH_2CO_2H$, 1918-77-0; $3TCH_2CO_2H$, 6964-21-2; $2T5ClCH_2CO_2H$, 13669-19-7; $3PCH_2CO_2H$, 501-81-5; $2PCH_2CO_2H$, 13115-43-0; **8** (R_1 = Bu-*t*, R_2 = H), 65678-11-7; $4PCH_2CO_2H$, 28356-58-3; $3PN\text{-}OCH_2CO_2H$, 67445-83-4; $4\text{-}MeC_6H_4CH_2CO_2H$, 622-47-9; $4\text{-}OMeC_6H_4CH_2CO_2H$, 104-01-8; $3,4\text{-}(OMe)_2C_6H_3CH_2CO_2H$, 93-40-3; $4\text{-}CoC_6H_4CH_2CO_2H$, 1878-66-6; $3,4\text{-}Co_2C_6H_3CH_2CO_2H$, 5807-30-7; $4\text{-}FC_6H_4CH_2CO_2H$, 405-50-5; $3\text{-}CF_3C_6H_4CH_2CO_2H$, 351-35-9; $2\text{-}(CO_2H)C_6H_4CH_2CO_2H$, 89-51-0; $C_6H_5CH_2CO_2H$, 103-82-2; ethyl 3-pyridylacetate, 39931-77-6; 2-acetylthiophene, 88-15-3; 3-acetylthiophene, 1468-83-3; 2-thiophenecarboxaldehyde, 98-03-3; 3-thiophenecarboxaldehyde, 498-62-4; 4-acetyl-2,6-di-*tert*-butylphenol, 14035-33-7; cyclooxygenase, 39391-18-9; 5-lipoxygenase, 80619-02-9.

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