

Styrylpyrazoles, Styrylisoxazoles, and Styrylisothiazoles. Novel 5-Lipoxygenase and Cyclooxygenase Inhibitors¹

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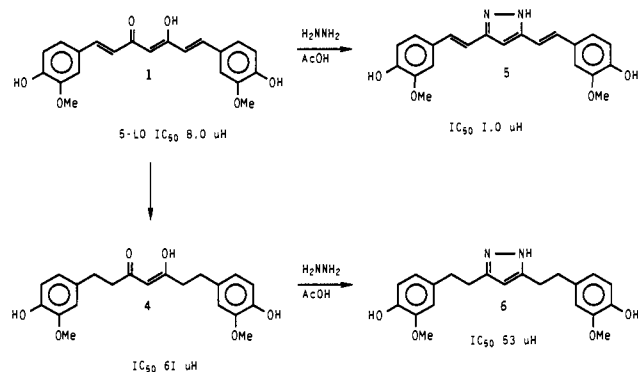
A series of styrylpyrazoles, styrylisoxazoles, and styrylisothiazoles were prepared and found to be dual inhibitors of 5-lipoxygenase and cyclooxygenase in rat basophilic leukemia cells. Compounds from this series also were found to inhibit the in vivo production of LTB₄ when dosed orally in rats. Among these compounds, di-*tert*-butylphenols **19** and **33** exhibit oral activity in various models of inflammation and, most importantly, are devoid of ulcerogenic potential.

The mammalian 5-lipoxygenase (5-LO) enzyme plays a key role in the conversion of arachidonic acid to a number of lipoxygenase products, including 5-HPETE, LTA₄, LTB₄, and the peptidoleukotrienes LTC₄, LTD₄, and LTE₄.³ The latter leukotrienes have been identified as components of SRS-A. Because these mediators have been demonstrated to possess potent chemotactic, bronchoconstrictor, and vascular leakage properties, they have been implicated as important mediators of various allergic diseases including asthma. Various lipoxygenase products (LTB₄) also exhibit proinflammatory properties in vitro and in vivo.⁴ Thus inhibition of 5-LO is currently the subject of intense research toward the discovery of novel antiallergic and antiinflammatory agents.

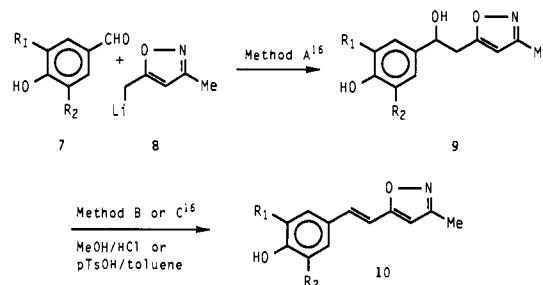
The inhibition of cyclooxygenase (CO) is a hallmark feature of virtually all marketed nonsteroidal antiinflammatory drugs (NSAIDs)^{5,6} currently in wide use for the treatment of rheumatoid arthritis and osteoarthritis. However, NSAIDs possess certain types of mechanism-based side effects including dyspepsia, gastrointestinal ulceration/bleeding, and nephrotoxicity. These side effects often limit the clinical usefulness of this therapeutic class.⁶ The inhibition of prostaglandin synthesis has been invoked as a mechanism for these side effects of NSAIDs.^{7,8} More recently, however, evidence has accumulated which implicates increased leukotriene formation in addition to prostaglandin inhibition as a more complete explanation for the NSAID side-effect profile.⁹⁻¹² Hence, the discovery of novel dual inhibitors of CO and 5-LO appears to be a fruitful approach toward the identification of safer second-generation NSAIDs.

Recently, we reported that naturally occurring diaryl-

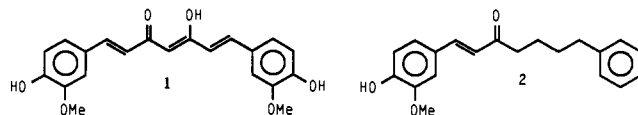
Scheme I



Scheme II

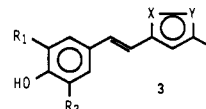


alkanoids display dual inhibition of 5-LO and CO in vitro.¹³ Namely, curcumin (**1**) and yakuchinone B (**2**) were found



to be inhibitors of both 5-LO and CO enzymes, but did not exhibit reproducible in vivo inhibition of LTB₄ biosynthesis or antiinflammatory activity.

In an effort to impart more potent in vitro inhibition and confer in vivo activity in this chemical class, the structure-activity relationships (SAR) of curcumin analogues was explored. SAR of these initial curcumin analogues led to the development of a novel series of styryl heterocycles (**3**). We now report that this series potentially

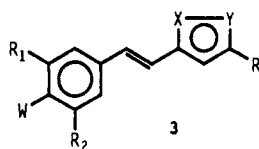


- (1) Presented in part at the 196th National ACS meeting, September 1988, Los Angeles, CA and in part at the 1988 National Medicinal Chemistry Symposium, June 1988, Minneapolis, MN.
- (2) Address correspondence to D. L. Flynn at G. D. Searle & Co., 4901 Searle Parkway, Skokie, IL 60077.
- (3) For reviews of leukotriene biosynthesis and biology, see: (a) Kreutner, W.; Siegel, M. I. *Ann. Rep. Med. Chem.* 1984, 19, 241. (b) Samuelsson, B.; Borgeat, P.; Hammarstrom, S.; Murphy, R. C. *Adv. Prostaglandin Thromboxane Res.* 1980, 6, 1. (c) Samuelsson, B.; *Prog. Lipid Res.* 1986, 25, 13.
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Table I. Substituted Styrylpyrazoles, Styrylisoxazoles, and Styrylisothiazoles



no.	W	R ₁	R ₂	X	Y	R	method (% yield)	mp, °C	formula	analysis	IC ₅₀ , μM	
											CO	5-LO
15	H	H	H	O	N	Me	A (67) C (29)	86–87 ^a	C ₁₂ H ₁₁ NO	C,H,N	>30	>30
16	OH	H	H	O	N	Me	A/B (14)	185–186	C ₁₂ H ₁₁ NO ₂	C,H,N	4.0	20.0
14	OH	OMe	H	O	N	Me	A (78), B (80)	123–127	C ₁₃ H ₁₃ O ₃ N	C,H,N	12.0	5.5
17	OH	OMe	OMe	O	N	Me	A (63), B (69), or E (42)	155–157	C ₁₄ H ₁₅ NO ₄	C,H,N	22.0	1.4
18	OH	Me	Me	O	N	Me	A (31), B (85)	158–161	C ₁₄ H ₁₅ NO ₂	C,H,N	1.2	4.5
19	OH	<i>t</i> -Bu	<i>t</i> -Bu	O	N	Me	A (72), B (69)	175–180	C ₂₀ H ₂₇ NO ₂	C,H,N	1.5	2.4
20	OH	<i>t</i> -Bu	<i>t</i> -Bu	N	O	Me	E (44)	172–174	C ₂₀ H ₂₇ NO ₂	C,H,N	0.3	2.7
21	OH	<i>t</i> -Bu	<i>t</i> -Bu	O	N	CF ₃	(90) ^b	132–134	C ₂₀ H ₂₄ F ₃ NO ₂	C,H,N	>30	>30
22	OH	<i>t</i> -Bu	<i>t</i> -Bu	N	O	CH ₂ COOH	(70) ^b	205–210	C ₂₁ H ₂₇ NO ₄	C,H,N	>30	17.0
23	OH	<i>t</i> -Bu	Me	O	N	Me	A (46), B (79)	140–152	C ₁₇ H ₂₁ NO ₂	C,H,N	7.4	2.3
24	OH	<i>t</i> -Bu	OMe	O	N	Me	A (34), B (55)	128–130	C ₁₇ H ₂₁ NO ₃	C,H,N	1.1	1.3
25	OH	Cl	Cl	O	N	Me	A (61), C (45)	184–186	C ₁₂ H ₉ Cl ₂ NO ₂	C,H,N ^c	13.8	21.0
26	OH	Br	Br	O	N	Me	A (53), B (15)	167–169	C ₁₂ H ₉ Br ₂ NO ₂	C,H,N,Br	16.0	11.0
27	OH	Br	OMe	O	N	Me	E (55)	178–180	C ₁₃ H ₁₂ BrNO ₃	C,H,N	9.3	4.0
28	OH	<i>i</i> -Pr	<i>i</i> -Pr	O	N	Me	A (56), B (98)	119–120	C ₁₈ H ₂₃ NO ₂	C,H,N	10	1.0
29	OH	OMe	H	N	NH	COOH	(68) ^b	270–271	C ₁₃ H ₁₂ O ₄ N ₂	C,H,N	>30	>30
30	OH	OMe	OMe	N	NH	Me	D (56)	153–158	C ₁₄ H ₁₆ N ₂ O ₃	C,H,N	>30	2.6
31	OH	Me	Me	N	NH	Me	F (50)	185–188	C ₁₄ H ₁₆ N ₂ O	C,H,N	4.6	0.8
32	OH	<i>i</i> -Pr	<i>i</i> -Pr	N	NH	Me	D (53)	183–184	C ₁₈ H ₂₄ N ₂ O·0.1H ₂ O	C,H,N	14.0	3.6
33	OH	<i>t</i> -Bu	<i>t</i> -Bu	N	NH	Me	D (55) or F (61)	218–223	C ₂₀ H ₂₆ N ₂ O	C,H,N	0.9	3.0
34	OH	<i>t</i> -Bu	<i>t</i> -Bu	N	NMe ^d	Me	(25) ^b	155–157	C ₂₁ H ₃₀ N ₂ O	C,H,N	7.0	1.7
35	OH	<i>t</i> -Bu	<i>t</i> -Bu	NMe ^d	N	Me	(7) ^b	120–122	C ₂₁ H ₃₀ N ₂ O	C,H,N	>30	1.4
36	OH	OMe	H	S	N	Me	G1 (35)	189–194	C ₁₃ H ₁₃ NO ₂ S	C,H,N,S	3.4	1.8
37	OH	OMe	OMe	S	N	Me	G2 (65)	202–205	C ₁₄ H ₁₅ NO ₃ S	C,H,N,S	9.0	0.2
38	OH	<i>t</i> -Bu	OMe	S	N	Me	G2 (5)	138–139	C ₁₇ H ₂₁ NO ₂ S	C,H,N,S	11.5	1.5
39	OH	<i>t</i> -Bu	OMe	N	S	Me	E (28)	156–160	C ₁₇ H ₂₁ NO ₂ S	C,H,N	0.9	1.2

^a Mixture of *E/Z* isomers (lit.⁴⁶ mp. *E* isomer 87–90 °C). ^b See the Experimental Section for methodology. ^c Anal. Cl calcd 26.75, found 26.25. ^d Regiochemistry was assigned on the basis of NOE experiments. ^e Trans geometry was assigned on the basis of coupling constant in ¹H NMR.

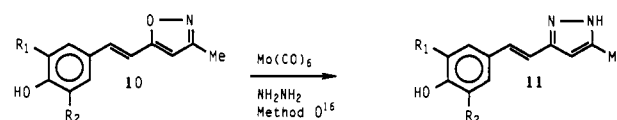
inhibits both 5-LO and CO activities in rat basophilic leukemia (RBL) cells. These agents also inhibit the *in vivo* production of LTB₄ when dosed orally in rats. Pharmacologically, this series exhibits oral activity in various models of inflammation, and most importantly is devoid of ulcerogenic potential.

Chemistry

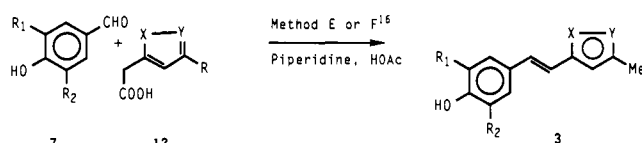
Curcumin analogues 4–6 were prepared as shown in Scheme I. Hydrogenation of curcumin (1) provided compound 4. Treatment of curcumin (1) and 4 with hydrazine hydrate in acetic acid gave the pyrazole derivatives 5 and 6, respectively.

Isoxazolymethyl Carbanion Approach. The series of styrylisoxazoles 10 was initially prepared by making use of regioselective carbanion generation from 3,5-dimethylisoxazole¹⁵ (Scheme II, methods A–C). Thus the lithio carbanion 8 was generated and reacted with a variety of substituted benzaldehydes 7, affording styrylisoxazoles 10 in good yields after acid-catalyzed dehydration of intermediates 9. Styrylisoxazoles 10 could be converted to the corresponding styrylpyrazoles 11 by hydrogenolysis of the isoxazole using Mo(CO)₆,¹⁷ followed by ring closure with hydrazine (Scheme III, method D).

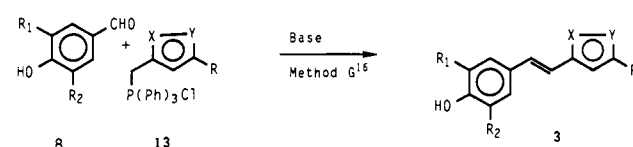
Scheme III



Scheme IV



Scheme V



Decarboxylative Knoevenagel Approach. An alternative approach to styryl heterocycles made use of the Doebner modification of the Knoevenagel reaction¹⁸ (Scheme IV, methods E and F). This sequence provided greater flexibility in that both regioisomeric pairs of styrylisoxazoles 3 could be prepared starting with the appropriate isoxazoleacetic acid (12).^{19,20} Additionally,

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Table II. Substituent Effects on in Vitro Activity

no.	ΣR^{23}	IC ₅₀ , μM		selectivity
		5-LO	CO	
15	0.0	>30	>30	
16	-0.64	20.0	4.0	0.2
14	-0.81	5.5	12.0	2.2
17	-0.98	1.4	22.0	16.0
30	-0.98	2.6	>30	>16.0
37	-0.98	0.20	9.0	45.0

styrylpyrazoles could be made directly from 3-methyl-5-pyrazoleacetic acid²¹ rather than resorting to the linear sequence from isoxazoles using Mo(CO)₆. Finally, key styrylisothiazoles were prepared from the isothiazoleacetic acid²² by this route.

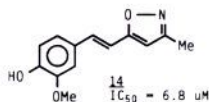
Wittig Approach. In certain cases, the Wittig reaction was employed to obtain the styryl heterocycles (Scheme V, method G).

The structures and synthetic route employed for each of the styryl heterocycles are summarized in Table I.

Results and Discussion

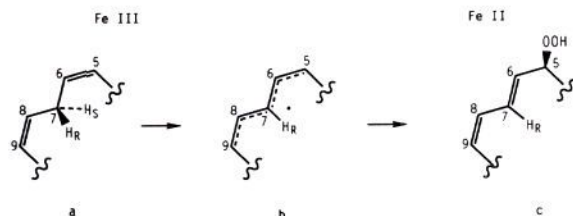
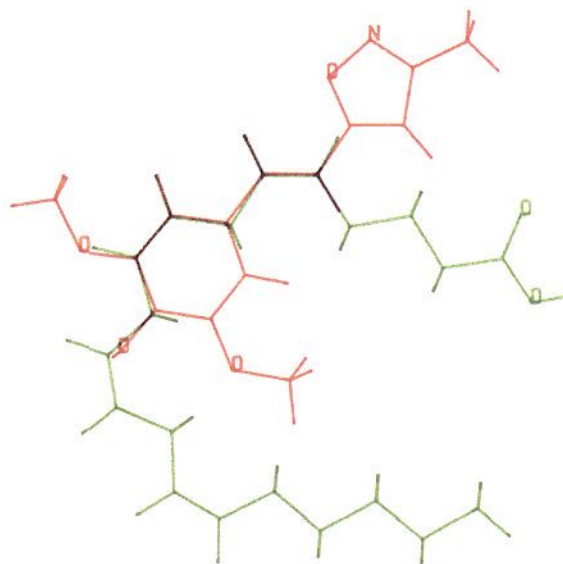
A. Initial Modifications of Curcumin. Initial exploration into the structure-activity relationships of curcumin (1) focused on the two chemical transformations shown in Scheme I. Saturation of the olefinic bonds of curcumin gave the tetrahydro derivative 4. A comparison of the human polymorphonuclear cell (PMN) 5-LO IC₅₀s¹⁴ revealed that 4 was 8-fold less potent than curcumin. Conversion of curcumin to the pyrazole analogue 5 resulted in a more potent 5-LO inhibitor, while the reduced analogue 6 was 53-fold less active than 5. Thus it would appear that at least one of the olefinic bonds of curcumin is required for potent 5-LO inhibition and that pyrazoles might retain or enhance the 5-LO inhibitory properties in this class.

Further analogue development revealed that the symmetrical structure of curcumin analogues was not essential for potent 5-LO inhibition. Thus the truncated isoxazole 14 displayed activity comparable to that of curcumin.



B. Biological Structure-Activity Relationships. 1. Biochemical SAR/Binding Model Hypothesis. Initial structure-activity relationships of the styrylheterocycles 3 focused on phenyl substituent effects on both 5-LO and CO inhibition. Table II reveals that whereas the unsubstituted analogue 15 is inactive as either a CO or 5-LO inhibitor, the para-hydroxylated analogue 16 is active as a CO (IC₅₀ = 4.0 μM) and 5-LO (IC₅₀ = 20.0 μM) inhibitor. Interestingly, as the phenyl substituents increase in their capacity to donate electron density into the styryl heterocycle framework (as measured by R²³), the 5-LO inhibitory potency increases dramatically while CO potency diminishes (cf. compounds 16, 14, and 17). The dimethoxy analogues 17, 30, and 37 are ≥ 16 -fold selective 5-LO inhibitors.

The best dual inhibitor compounds possess one or two alkyl groups, particularly the di-*tert*-butylated phenolic

**Figure 1.** Pentadienyl radical model of the 5-LO reaction.²⁸**Figure 2.** Superposition of arachidonic acid (green) and compound 17 (red).

analogues 19, 20, and 33. Among the di-*tert*-butylated phenols, the 5-LO inhibitory activity is diminished by electron-withdrawing substituents at the C-3 carbon of the isoxazole ring (cf. compounds 19 and 21).

The 5-LO inhibitory activities of the present compounds are most likely mediated, at least in part, by either an antioxidant or redox mechanism.²⁴⁻²⁷ Consistent with this proposal is the observation that an unprotected *p*-hydroxy group on the phenyl ring is required for activity. The obligate nature of the olefinic linkage between the phenyl ring and the heterocycle is also structurally consistent with this mechanism. However, not all antioxidants and redox-active compounds inhibit 5-LO. For instance, we have found that catechol, *o*-aminophenol, and butylated hydroxytoluene are virtually inactive as inhibitors in our RBL-cell line. It would appear that two components are necessary for 5-LO inhibition by redox-active compounds: (1) affinity for the enzyme environment containing the catalytic iron and (2) expressed redox activity. In support of this hypothesis, it is noted that most redox-active in-

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Table III. In Vivo 5-LO Inhibition and Antiinflammatory Activity

no. ^a	R ₁	R ₂	X	Y	Z-sponge ^b		CFE ^c	
					dose, mg/kg po	% inhibn ± SEM	dose, mg/kg po	% inhibn ± SEM
36	OMe	H	S	N	50	N ^d	20	32 ± 6.5
17	OMe	OMe	O	N	40	62 ± 4	20	37 ± 5.1
30	OMe	OMe	N	NH	20	32 ± 6		NT ^e
37	OMe	OMe	S	N	50	44 ± 7		NT
19	<i>t</i> -Bu	<i>t</i> -Bu	O	N	50	67 ± 5	20	32 ± 5.8
20	<i>t</i> -Bu	<i>t</i> -Bu	N	O	50	N	50	N
33	<i>t</i> -Bu	<i>t</i> -Bu	N	NH	20	47 ± 14	30	27 ± 6.2
23	<i>t</i> -Bu	Me	O	N	50	N		NT
24	<i>t</i> -Bu	OMe	O	N	50	N	20	24 ± 4.4
31	Me	Me	N	NH	20	49 ± 10	50	N
32	<i>i</i> -Pr	<i>i</i> -Pr	N	NH	50	N		NT
25	Cl	Cl	O	N	50	N	50	N
26	Br	Br	O	N	50	N	50	N

^a Complete structural data are found in Table I. ^b Percent inhibition of LTB₄ biosynthesis at the indicated dose po ($n = 5$ or 6 animals per experimental group). ^c Percent inhibition of edema at the indicated dose po ($n = 7$ animals per experimental group). ^d N = no inhibition. ^e NT = not tested.

hibitors of 5-LO contain functional groups capable of binding iron (catechols, monomethylated catechols, *o*-aminophenols, β -dicarbonyls, 1,2-diheteroatomic rings)^{24,30,31} and hence in allowing for an initial affinity-mediated component to their mechanisms of 5-LO inhibition.

Assuming an initial affinity component to the mechanism of the presently described inhibitors, it is noted that the structures of compounds 1–3 allow for binding 5-LO in a manner consistent to that recently described for a series of arylhydroxamic acids.²⁸ Thus, the critical C5–C9 region of arachidonic acid may resemble a pentadienyl radical (W conformation shown as b in Figure 1) as a transition-state structure or as a bound intermediate at the enzyme-active site.²⁹ The styryl backbone of the styryl heterocycles is envisioned as binding at the same site as the putative substrate pentadienyl radical of arachidonic acid, while the isoxazole, pyrazole, or isothiazole ring binds to an iron center near C5, the site of substrate peroxidation.^{29,30} This binding model is illustrated in Figure 2, a superposition of arachidonic acid and 17.

Several aspects of this binding model (Figure 2) are consistent with the observed structure–activity relationships. Increasing electron density in the heterocycle by placing electron-donating substituents on the phenyl ring could result in greater iron-binding capacity in the heterocyclic ring, affording more potent 5-LO inhibition (Table II). Conversely, withdrawing electron density from the heterocycle via electron-attracting groups on the heterocyclic ring hinders iron-binding and compromises 5-LO inhibition. Finally, the observation that olefinic bond saturation leads to a drastic reduction of 5-LO inhibitory potency also supports the binding model.

2. In Vivo Activity. This styryl heterocycle series was evaluated in the rat 5 h zymosan-sponge model of in vivo LTB₄ biosynthesis.³³ The dimethoxylated styryl heterocycles 17, 30, and 37, which were among the most potent in vitro 5-LO inhibitors (Table II), also inhibited LTB₄ biosynthesis in vivo (Table III). Compound 17 inhibited

LTB₄ biosynthesis by 62% at 40 mg/kg po and compound 37 by 44% at 50 mg/kg po. The di-*tert*-butylated phenol analogues 19 and 33 also exhibited excellent in vivo inhibition of LTB₄ biosynthesis by 67% (50 mg/kg) and 47% (20 mg/kg), respectively. Interestingly, compound 20, the regioisomeric isoxazole of 19, was devoid of in vivo activity in this model.

In vivo efficacy as cyclooxygenase inhibitors was assessed by using the functional model of carrageenan footpad edema (CFE) in the rat.³⁴ The dimethoxylated styryl-isoxazole 17 inhibited edema formation by 37% at 20 mg/kg po. Otherwise, the di-*tert*-butylated phenol analogues 19 and 33 and the *tert*-butylated methoxyphenol 24 appeared to be the most promising antiinflammatory agents from this series. Compound 33 demonstrated 27% inhibition at 30 mg/kg po. Others have reported di-*tert*-butylated phenols to be new-generation NSAID dual CO/5-LO inhibitors.³⁵ Our results indicate that such di-*tert*-butylated phenols in this styryl heterocycle series are also orally active dual inhibitors. Interestingly, both di-*tert*-butylated phenols 19 and 33 were devoid of ulcerogenic potential at oral doses up to 200 mg/kg po. Taken together with the above in vivo efficacy data, these compounds compare favorably to other recently disclosed di-*tert*-butylated phenolic dual inhibitor NSAIDs such as KME-4 and E-5110³⁵ (Table IV).

Experimental Section

Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra (¹H NMR) were recorded on either a Varian EM-390 (90 MHz), a IBM WP100SY (100 MHz), a Varian XL-200 (200 MHz), or a Varian XL-300 (300 MHz) spectrometer. The peaks are described in ppm downfield of tetramethylsilane. Infrared spectra (IR) were determined on a Nicolet MX-1 FTIR. Mass spectra were obtained on either a Finnigan 4500 spectrometer or a V.G. Analytical 7070E/HF instrument. Elemental analyses were within $\pm 0.4\%$ of the theoretical values for the specified elements unless otherwise noted and were performed by the Analytical Chemistry Section of Parke-Davis Pharmaceutical Research Division. Thin-layer chromatography was carried out on 0.25-mm silica F254

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(E. Merck) glass plates. Merck silica gel 60 (230–400 mesh) was used for chromatography.

Starting benzaldehydes were either commercially available or were prepared from the corresponding 2,6-disubstituted phenols as described by van der Goot and co-workers³⁶ or Cahoy.³⁷

Chemistry. Method A. α -[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-methyl-5-isoxazoleethanol (40). A solution of *n*-butyllithium in hexane (0.103 mol, 2.5 M) was added dropwise to a solution of 3,5-dimethylisoxazole (10 g, 0.103 mol) in dry THF (100 mL) at -78°C under an argon atmosphere. After the reaction mixture was stirred at -78°C for 2 h, 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzaldehyde (12 g, 0.051 mol) in dry THF (200 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature and was stirred at room temperature for an additional 8 h. The reaction mixture was evaporated to near dryness, redissolved in ethyl acetate (500 mL), and washed with water and then brine. The organic layer was dried (Na_2SO_4) and evaporated to dryness. Flash chromatography (hexane/ethyl acetate 3:2, silica gel) gave pure α -[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-methyl-5-isoxazoleethanol (12.0 g, 72%): mp 145–147 $^{\circ}\text{C}$. Anal. ($\text{C}_{20}\text{H}_{29}\text{O}_3\text{N}$) C, H, N.

Method B. (*E*)-2,6-Bis(1,1-dimethylethyl)-4-[2-(3-methyl-5-isoxazolyl)ethenyl]phenol (19). A solution of α -[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-3-methyl-5-isoxazoleethanol (16.8 g, 0.051 mol) in saturated methanolic HCl (300 mL) was heated at reflux for 24 h. The reaction mixture was evaporated to dryness, redissolved in ethyl acetate (400 mL), and washed with 15% aqueous sodium bicarbonate and with brine. The organic layer was dried (Na_2SO_4) and evaporated to dryness. Flash chromatography (hexane/ethyl acetate 3:1, silica gel) provided 19 (11.0 g, 69%): mp 174–175 $^{\circ}\text{C}$; IR (KBr) 3627, 3365, 1645 cm^{-1} ; ^1H NMR (200 MHz, CDCl_3) δ 7.36 (s, 2 H, ArH), 7.27 (d, $J = 16$ Hz, 1 H, vinyl), 6.78 (d, $J = 16$ Hz, 1 H, vinyl), 6.06 (s, 1 H, isoxazole H-4), 5.43 (s, 1 H, OH), 2.32 (s, 3 H, CH_3), 1.48 (s, 18 H, *t*-Bu); EIMS m/z 313 (M^+). Anal. ($\text{C}_{20}\text{H}_{27}\text{O}_2\text{N}$) C, H, N.

Method C. 2,6-Dichloro-4-[2-(3-methyl-5-isoxazolyl)ethenyl]phenol (25). To a solution of α -(3,5-dichloro-4-hydroxyphenyl)-3-methyl-5-isoxazoleethanol (5.5 g, 19.1 mmol) (prepared in 61% yield from 3,5-dimethylisoxazole and 3,5-dichloro-4-hydroxybenzaldehyde according to the procedure in method A) in toluene (100 mL) was added a catalytic amount of *p*-toluenesulfonic acid. The reaction mixture was heated at reflux for 3 h with azeotropic removal of water. The solvent was evaporated and the residue was purified by flash chromatography (ethyl acetate/chloroform 1:9, silica gel) to afford 25 (2.3 g, 45%): mp 184–188 $^{\circ}\text{C}$. Anal. ($\text{C}_{12}\text{H}_9\text{Cl}_2\text{NO}_2$) C, H, N; Cl: calcd, 26.75, found 26.25.

Method D. 2,6-Bis(1,1-dimethylethyl)-4-[2-(5-methyl-1*H*-pyrazol-3-yl)ethenyl]phenol (33). A solution of 19 (2.00 g, 6.4 mmol), water (115 mg, 6.4 mmol), and molybdenum hexacarbonyl (1.27 g, 48 mmol) in acetonitrile (75 mL) was refluxed for 12 h under a nitrogen atmosphere. The reaction mixture was cooled and evaporated to dryness. The residue was dissolved in methanol (250 mL) and acidified to pH = 1 with 4 N HCl.

After the reaction mixture was stirred at room temperature for 4 h, the methanol was evaporated. The resulting aqueous solution was neutralized with 1 N NaOH and extracted with ethyl acetate. The organic layer was collected and filtered through a pad of silica gel (150 g) and further eluted with chloroform (400 mL). The combined filtrate and eluant were evaporated, taken up in a minimal amount of ethyl acetate, and passed through a silica gel pad a second time.

The resulting crude ketone (1.25 g) was suspended in a mixture of acetic acid (100 mL) and 97% hydrazine (1.0 mL). The reaction mixture was stirred at room temperature for 12 h, evaporated, diluted with water (100 mL), and stirred for 30 min. The solid was collected by filtration and purified by flash chromatography (dichloromethane/ethyl acetate 1:1, silica gel) to give 33 (1.10 g, 55%): mp 218–223 $^{\circ}\text{C}$; IR (KBr) 3615, 2959, 1645, 1585 cm^{-1} ;

NMR (100 MHz, $\text{DMSO}-d_6$) δ 7.38 (s, 1 H), 7.07 (br s, 1 H), 7.03 (d, $J = 18$ Hz, 1 H, vinyl) 6.75 (d, $J = 18$ Hz, 1 H, vinyl), 6.2 (s, 1 H, H-4 pyrazole), 2.28 (s, 3 H, CH_3), 1.43 (s, 18 H, *t*-Bu); EIMS m/z 312 (M^+). Anal. ($\text{C}_{20}\text{H}_{28}\text{N}_2\text{O}$) C, H, N.

Method E. 2,6-Dimethoxy-4-[2-(3-methyl-5-isoxazolyl)ethenyl]phenol (17). A solution of 3-methyl-5-isoxazoleacetic acid¹⁷ (10.0 g, 71 mmol), syringaldehyde (12.9 g, 71 mmol), piperidine (0.6 g, 7 mmol), and acetic acid (0.42 g, 7 mmol) in toluene (500 mL) was heated to reflux with azeotropic removal of water for 3 h. The mixture was cooled and the solid collected by filtration. The solid was taken up in pyridine (100 mL) and heated at reflux for 4 h. The solvent was evaporated and the residue was purified by flash chromatography (methanol/chloroform 1:9, silica gel) followed by recrystallization from ethyl acetate to afford 17 (7.73 g, 42%): mp 152–155 $^{\circ}\text{C}$; IR (KBr) 3200 (br), 1647, 1606, 1517 cm^{-1} ; ^1H NMR (200 MHz, CDCl_3) δ 7.22 (d, $J = 16.3$ Hz, 1 H, vinyl), 6.78 (d, $J = 16.3$ Hz, 1 H, vinyl), 6.75 (s, 2 H, Ar), 6.07 (s, 1 H, isoxazole H-4), 5.72 (br s, 1 H, OH), 3.94 (s, 6 H, OCH_3), 2.32 (s, 3 H, CH_3); EIMS m/z 261 (M^+). Anal. ($\text{C}_{14}\text{H}_{15}\text{NO}_4$) C, H, N.

2,6-Bis(1,1-dimethylethyl)-4-[2-(5-methyl-3-isoxazolyl)ethenyl]phenol (20). According to the procedure of method E, 5-methyl-3-isoxazoleacetic acid²⁰ (0.69 g, 4.9 mmol) was reacted with 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzaldehyde (1.14 g, 4.9 mmol) to afford 20 (0.72 g, 44%): IR (KBr) 3636, 2967, 1644, 1606, 1438 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3) δ 7.33 (s, 2 H, Ar), 7.05 (d, $J = 16$ Hz, 1 H, vinyl), 6.94 (d, $J = 16$ Hz, 1 H, vinyl) 6.19 (s, 1 H, isoxazole H-4) 5.37 (s, 1 H, OH), 2.41 (s, 3 H, CH_3), 1.45 (s, 18 H, *t*-Bu); EIMS m/z 313 (M^+). Anal. ($\text{C}_{20}\text{H}_{27}\text{O}_2\text{N}$) C, H, N.

3-(1,1-Dimethylethyl)-4-hydroxy-5-methoxybenzaldehyde (41). According to the procedure of Cahoy,³⁷ a solution of 2-(1,1-dimethylethyl)-6-methoxyphenol³⁸ (28.2 g, 156 mmol) and hexamethylenetetramine (43.9 g, 313 mmol) in acetic acid (100 mL) and water (30 mL) was heated to reflux. Distillate was collected until the reaction temperature reached 130 $^{\circ}\text{C}$. At this temperature, collection of distillate was discontinued and the reaction mixture was heated at 130 $^{\circ}\text{C}$ for 6 h. The reaction mixture was poured into ice water (600 mL) and extracted with ether. The organic layer was washed with aqueous sodium bicarbonate and dried (MgSO_4). The solvent was evaporated and the residue was recrystallized from hexane to give 3-(1,1-dimethylethyl)-4-hydroxy-5-methoxybenzaldehyde (16.0 g, 49%): mp 101–102 $^{\circ}\text{C}$ (lit.³⁹ mp 103–104 $^{\circ}\text{C}$). Anal. ($\text{C}_{12}\text{H}_{16}\text{O}_3$) C, H.

Method F. 2,6-Bis(1,1-dimethylethyl)-4-[2-(5-methyl-1*H*-pyrazol-3-yl)ethenyl]phenol (33). A solution of 3-methyl-5-pyrazoleacetic acid²¹ (20.0 g, 0.14), 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (33.4 g, 0.14 mol), piperidine (1.2 g, 0.014 mol), and acetic acid (0.85 g, 0.014 mol) in toluene (1000 mL) was heated at reflux for 24 h with azeotropic removal of water. The reaction mixture was cooled and evaporated to afford a residue which was dissolved in methanol/ethyl acetate (1:9) and extracted with water (300 mL). The organic layer was evaporated and the residue recrystallized from hexane/ethyl acetate (5:1) to afford 33 (27.4 g, 61%): mp 220–224 $^{\circ}\text{C}$; IR, NMR, MS were identical with those of the material prepared by method D. Anal. ($\text{C}_{20}\text{H}_{28}\text{N}_2\text{O}$) C, H, N.

Method G1. 2-Methoxy-4-[2-(3-methyl-5-isothiazolyl)ethenyl]phenol (36). To a suspension of (4-acetoxy-3-methoxybenzyl)triphenylphosphonium chloride⁴⁰ (4.77 g, 0.01 mol) in dry THF (100 mL) and DMSO (4 mL) at 0 $^{\circ}\text{C}$ under an argon atmosphere was added sodium hydride (60% suspension oil; 0.4 g, 0.01 mol). The reaction mixture was stirred at room temperature for 30 min and 3-methylisothiazole-5-carboxaldehyde⁴¹ was added.

The reaction mixture was stirred at room temperature for 3 h and was poured into a saturated solution of ammonium chloride (250 mL). The product was extracted into ethyl acetate (2 \times 300 mL). The crude acetoxy intermediate was separated from tri-

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phenylphosphine oxide by flash chromatography (25% EtOAc/CH₂Cl₂, silica). The intermediate acetoxy derivative (2.2 g) was dissolved in methanol (200 mL) and treated with sodium methoxide (0.8 g) at 0 °C for 1 h. The reaction mixture was evaporated and the residue was suspended in water, neutralized with acetic acid, and extracted with ethyl acetate. The organic layer was dried (MgSO₄) and evaporated to give crude **36** as a mixture of cis and trans isomers. Crude **36** was dissolved in saturated methanolic HCl (500 mL) and heated at reflux for 3 h to equilibrate to the trans isomer. Recrystallization from isopropyl ether gave pure trans isomer **36** (0.87 g, 35%): mp 189–194 °C; IR (KBr) 3400–2800 (br), 1628, 1591, 1524 cm⁻¹; ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.37 (s, 1 H, OH), 7.31 (d, *J* = 16.2 Hz, 1 H, vinyl), 7.25 (d, *J* = 1.8 Hz, 1 H, Ar, C₂-H), 7.19 (s, 1 H, isothiazole H-4), 7.09 (d, *J* = 16.2 Hz, 1 H, vinyl), 7.02 (dd, *J* = 1.8, *J* = 8 Hz, 1 H, Ar, C₆-H), 6.78 (d, *J* = 8 Hz, 1 H, ArC₅-H), 3.83 (s, 3 H, OMe). Anal. (C₁₃H₁₃NO₂S) C, H, N, S.

[(3-Methyl-5-isothiazolyl)methyl]triphenylphosphonium Chloride. A solution of 5-(chloromethyl)-3-methylisothiazole⁴² (5.2 g, 35 mmol) and triphenylphosphine (9.25 g, 35 mmol) in toluene (100 mL) was heated at reflux for 2 days. The resulting precipitate was collected by filtration to give the desired phosphonium chloride (13.1 g, 91%): mp >250 °C; ¹H NMR (DMSO-*d*₆) δ 8.1–7.6 (m, 15 H, Ar), 6.83 (d, *J* = 3 Hz, 1 H, isothiazole H-4), 5.82 (d, *J* = 16.5 Hz, 1 H, CH₂), 2.33 (s, 3 H, CH₃).

Method G2. 2,6-Dimethoxy-4-[2-(3-methyl-5-isothiazolyl)ethenyl]phenol (37). To a solution of [(3-methyl-5-isothiazolyl)methyl]triphenylphosphonium chloride (3.0 g, 7.3 mmol) in DMSO (75 mL) under an argon atmosphere was added potassium *tert*-butoxide (0.82 g, 7.3 mmol). The reaction mixture was stirred at room temperature for 1 h and 4-acetoxy-3,5-dimethoxybenzaldehyde (1.6 g, 7.2 mmol) was added. The reaction mixture was stirred at room temperature for 1 h and was poured into a saturated solution of ammonium chloride. The intermediate acetoxy derivative was extracted into ethyl acetate (2 × 200 mL). Workup as described in method G1 provided pure **37** (1.3 g, 65%): mp 202–205 °C (from isopropyl ether); IR (KBr) 3450 (br), 3100 (br), 1631, 1599, 1518, 1330, 1115; ¹H NMR (90 MHz, DMSO-*d*₆) δ 8.93 (s, 1 H, OH), 7.42 (d, *J* = 16.5 Hz, 1 H, vinyl), 7.20 (s, 1 H, isothiazole H-4), 7.08 (d, *J* = 16.5 Hz, 1 H, vinyl), 6.97 (s, 2 H, Ar), 3.83 (s, 6 H, OMe), 2.40 (s, 3 H, CH₃); EIMS *m/z* 277 (M⁺). Anal. (C₁₄H₁₅NO₃S) C, H, N, S.

Method H. 3,5-Bis[β-(4-hydroxy-3-methoxyphenyl)ethenyl]pyrazole (5). To a solution of curcumin (5.93 g, 16.1 mmol) in ethanol (50 mL) and butanol (50 mL) was added hydrazine hydrate (0.81 g, 16.1 mmol) and acetic acid (0.5 mL). The reaction mixture was heated at 60 °C for 24 h. The solvent was evaporated and the residue purified by flash chromatography (EtOAc, silica) to give a red solid. Recrystallization from methanol/water provided **5** (0.3 g, 5%) as a hydrate: mp 211–214 °C; IR (KBr) 1594, 1559, 1513, 1280 cm⁻¹; ¹H NMR (DMSO-*d*₆) δ 12.82 (s, 1 H, NH), 9.18 (s, 2 H, OH), 7.45–6.70 (m, Ar), 7.06 (d, 2 H, *J* = 16 Hz), 6.92 (d, 2 H, *J* = 16 Hz), 6.63 (s, 1 H, pyrazole H-4), 3.84 (s, 6 H, OMe); EIMS *m/z* 363 (M⁺), 364 (M + 1). Anal. (C₂₁H₂₀N₂O₄·0.1H₂O) C, H, N.

3,5-Bis[β-(4-hydroxy-3-methoxyphenyl)ethyl]pyrazole (6). According to the procedure of method H, 1,7-bis(4'-hydroxy-3'-methoxyphenyl)-3,5-heptadione⁴³ was reacted with hydrazine hydrate to afford **6** in 65% yield: mp 125–128 °C; IR (KBr) 3300, 1603, 1570, 1526, 1454, 1280 cm⁻¹; ¹H NMR (CDCl₃) δ 6.8–6.4 (m, 6 H, Ar), 5.73 (s, 1 H, pyrazole H-4), 3.75 (s, 6 H, OMe), 2.77 (s, 8 H, methylene); EIMS *m/z* 368 (M⁺). Anal. (C₂₁H₂₄N₂O₄) C, H, N.

5-(Chloromethyl)-3-(trifluoromethyl)isoxazole. A solution of 3-(trifluoromethyl)-5-(hydroxymethyl)isoxazole⁴⁴ (0.7 g, 4.2 mmol) and triphenylphosphine (1.43 g, 5.4 mmol) in carbon tetrachloride (50 mL) and dichloromethane (20 mL) was heated at reflux for 6 h. The reaction mixture was cooled and filtered through a pad of silica gel. Concentration of the eluent afforded 5-(chloromethyl)-3-(trifluoromethyl)isoxazole (0.64 g, 82%) of

sufficient purity for conversion to the phosphonium salt.

[[3-(Trifluoromethyl)-5-isoxazolyl]methyl]triphenylphosphonium Chloride. A solution of triphenylphosphine (1.02 g, 3.9 mmol) and 5-(chloromethyl)-3-(trifluoromethyl)isoxazole (0.72 g, 3.9 mmol) in toluene (40 mL) was heated at reflux for 24 h. Upon cooling, a white precipitate formed and was collected by filtration to afford the desired phosphonium salt in 60% yield: mp 245–250 °C.

2,6-Bis(1,1-dimethylethyl)-4-[2-[3-(trifluoromethyl)-5-isoxazolyl]ethenyl]phenol (21). To a solution of [[3-(trifluoromethyl)-5-isoxazolyl]methyl]triphenylphosphonium chloride (1.05 g, 2.35 mmol) in dimethyl sulfoxide (5 mL) was added potassium *tert*-butoxide (264 mg, 2.35 mmol) and the reaction mixture was stirred at room temperature for 45 min under an argon atmosphere. 3,5-Bis(1,1-dimethylethyl)-4-hydroxybenzaldehyde (250 mg, 1.06 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was poured over saturated aqueous ammonium chloride and extracted with ethyl acetate. The organic layer was washed with water, dried (MgSO₄), and evaporated. Flash chromatography (hexane/ethyl acetate, silica) afforded **21** as a mixture of cis and trans isomers. The crude mixture of isomers was dissolved in methanolic HCl and heated at reflux for 12 h to give pure **21** as the trans isomer (350 mg, 90%): mp 132–134 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.43 (s, 2 H, Ar), 7.40 (d, *J* = 16.4 Hz, 1 H, vinyl), 6.83 (d, *J* = 16.4 Hz, 1 H, vinyl), 6.44 (s, 1 H, isoxazole H-4), 5.52 (s, 1 H, OH), 1.49 (s, 18 H, *t*-Bu); EIMS 367 (M⁺). Anal. (C₂₀H₂₄N₂O₂F₃) C, H, N.

Ethyl 6-(4-Hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenoate (42). To a freshly prepared solution of sodium ethoxide in ethanol (50 mL) (prepared from 1.8 g of sodium metal) was added an ethanolic solution of 1-(4-hydroxy-3-methoxyphenyl)-1-buten-3-one⁴⁵ (5.00 g, 0.026 mol) under an argon atmosphere.

The reaction mixture was stirred at room temperature for 10 min and diethyl oxalate (3.8 g, 0.026 mol) was added dropwise. The reaction mixture was stirred at room temperature for 5 h and then was acidified with concentrated HCl. The reaction mixture was diluted to 400 mL with water and stirred at 0 °C for 1 h to obtain ethyl 6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenoate (4.8 g, 63%) as a precipitate which was not purified but used directly in the next reaction: mp 97–98 °C; ¹H NMR (90 MHz, CDCl₃) δ 7.74 (d, *J* = 16 Hz, 1 H, vinyl), 7.4–6.8 (m, 3 H, Ar), 6.57 (s, 1 H, =CH enol form), 6.55 (d, *J* = 16 Hz, 1 H, vinyl), 4.43 (q, *J* = 7 Hz, 2 H, CH₂), 3.95 (s, 3 H, OCH₃), 1.42 (t, *J* = 7 Hz, 3 H, CH₃).

Ethyl 3-[2-(4-Hydroxy-3-methoxyphenyl)ethenyl]-1H-pyrazole-5-carboxylate (43). According to the procedure of method H, ethyl 6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenoate (2.00 g, 6.8 mmol) was reacted with hydrazine hydrate to afford ethyl 3-[2-(3-methoxy-4-hydroxyphenyl)ethenyl]-1H-pyrazole-5-carboxylate (0.8 g, 40%): mp 99–101 °C; IR (KBr) 3319, 1697, 1595, 1522 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.1–6.9 (m, 6 H, Ar, vinyl, pyrazole), 4.41 (q, 2 H, CH₂), 3.95 (s, 3 H, OMe), 1.41 (t, 3 H, CH₃); EIMS *m/z* 288 (M⁺). Anal. (C₁₅H₁₆N₂O₄) C, H, N.

3-[2-(4-Hydroxy-3-methoxyphenyl)ethenyl]-1H-pyrazole-5-carboxylic Acid (29). Ethyl 3-[2-(4-hydroxy-3-methoxyphenyl)ethenyl]-1H-pyrazole-5-carboxylate (0.49 g, 1.7 mmol) was added to a solution of KOH (0.38 g, 6.8 mmol) in 50 mL of EtOH and the reaction mixture was heated to reflux overnight. After the reaction mixture was cooled to room temperature, it was diluted to 200 mL with water and acidified to pH = 4 with aqueous HCl. The resulting white precipitate was collected by filtration and dried over P₂O₅ to give **29** (0.3 g, 68%): mp 270–271 °C dec; IR (KBr) 3550–2400 (br), 1715, 1646, 1597, 1516; ¹H NMR (200 MHz, DMSO-*d*₆) δ 7.21–6.78 (m, 6 H), 3.84 (s, 3 H, OMe); EIMS *m/z* 288 (M⁺). Anal. (C₁₃H₁₂O₄N₂) C, H, N.

3-[2-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]ethenyl]-5-(carboxymethyl)isoxazole (22). A solution of *n*-

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Table IV. Comparison of 19 and 33 with KME-4 and E-5110

no.	IC ₅₀ , μM		Z-sponge ^a		CFE ^b ID ₂₅ , mg/kg po	UD ₅₀ ^c
	5-LO	CO	dose, mg/kg po	% inhibn ± SEM		
19	2.4	1.5	50	67 ± 5	24	0% at 200 ^c
33	3.0	0.9	20	47 ± 14	15	0% at 200 ^c
KME-4	2.5	0.15	30	N	23	30% at 30 ^c
E-5110	8.1	<1.0	50	N	4.9	10% at 10 ^c

^a Percent inhibition of LTB₄ biosynthesis at the indicated dose po (*n* = 5 or 6 animals per experimental group). ^b Carrageenan footpad edema. ^c Gastric ulcerogenicity data are presented as percent rats with ulcers at the indicated dose po (*n* = 10 animals per experimental group).

butyllithium in hexane (2 mL, 1.6 M, 3.2 mmol) was added dropwise to a solution of 20 (0.5 g, 1.6 mmol) in dry THF (15 mL) at -78 °C under an argon atmosphere. The reaction mixture was stirred at -78 °C for 20 min and was poured over an excess of dry ice. The reaction mixture was allowed to warm to room temperature and was partitioned between water (100 mL) and ethyl acetate (200 mL). The organic layer was washed with saturated aqueous ammonium chloride and water. The organic layer was dried (Na₂SO₄) and evaporated. Flash chromatography of the residue (chloroform/methanol 95:5 containing 0.1% acetic acid) afforded 22 (0.4 g, 70%): mp 205–210 °C; ¹H NMR (100 MHz, DMSO-*d*₆) 7.34 (s, 2 H, Ar), 7.33 (d, *J* = 16.8 Hz, 1 H, vinyl), 6.90 (d, *J* = 16.8 Hz, 1 H, vinyl), 6.75 (s, 1 H), 3.88 (s, 2 H, CH₂), 1.41 (s, 18 H, *t*-Bu); EIMS *m/z* 357 (M⁺). Anal. (C₂₁H₂₇NO₄) C, H, N.

2,6-Bis(1,1-dimethylethyl)-4-[2-(1,5-dimethyl-1H-pyrazol-3-yl)ethenyl]phenol (34) and **2,6-Bis(1,1-dimethylethyl)-4-[2-(1,3-dimethyl-1H-5-yl)ethenyl]phenol (35)**. A mixture of 33 (4.00 g, 12.8 mmol), sodium acetate (11.55 g), and methyl iodide (18.17 g, 128 mmol) in DMF (200 mL) was stirred at room temperature for 12 h. The reaction mixture was poured into water (50 mL) and extracted with dichloromethane. The organic layer was washed with brine and then dried (MgSO₄). The solvent was evaporated and the two isomers were separated by flash chromatography (CHCl₃, silica).

Isomer 34 (1.04 g, 25%): TLC *R_f* = 0.24 (CHCl₃, silica); mp 155–157 °C; IR (KBr) 3510, 2960, 1547, 1436 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 7.38 (s, 2 H, ArH), 7.04 (m, 2 H, vinyl), 6.27 (s, 1 H, pyrazole H-4), 5.30 (s, 1 H, OH), 3.82 (s, 3 H, NMe), 2.32 (s, 3 H, Me), 1.51 (s, 18 H, *t*-Bu); EIMS *m/z* 326 (M⁺), 327 (M + 1). Anal. (C₂₁H₃₀N₂O) C, H, N.

Isomer 35 (0.29 g, 7%): TLC *R_f* = 0.12 (CHCl₃, silica); mp 120–122 °C; IR (KBr) 2960, 1634, 1541, 1437 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 7.34 (s, 2 H, ArH), 7.00 (d, 1 H, vinyl), 6.74 (d, 1 H, vinyl), 6.27 (s, 1 H, pyrazole H-4), 5.39 (s, 1 H, OH), 3.91 (s, 3 H, NMe), 2.31 (s, 3 H, Me), 1.52 (s, 18 H, *t*-Bu); EIMS *m/z* 326 (M⁺). Anal. (C₂₁H₃₀N₂O) C, H, N.

Pharmacology. A. Whole Cell 5-Lipoxygenase (5-LO) and Cyclooxygenase (CO) Assays. Materials. The rat basophilic leukemia cell line (RBL-1) was obtained from the American Type Culture Collection (Rockville, MD). Radioimmunoassay (RIA) kits of LTB₄ and PGF_{2α} were obtained from Amersham (Arlington Heights, IL) and Seragen (Boston, MA), respectively. All tissue culture media were obtained from GIBCO (Grand Island, NY).

Method. RBL-1 cells were grown in suspension culture in Eagle's minimum essential medium supplemented with 12% fetal bovine serum at 37 °C in an incubator supplied with air–5% carbon dioxide. Cells were harvested by centrifugation. They were washed with cold phosphate-buffered saline, pH 7.4 (PBS; NaCl, 7.1 g; Na₂HPO₄, 1.15 g; KH₂PO₄, 0.2 g; and KCl, 0.2 g/L). Cells were finally suspended in PBS containing 1.0 mM calcium at a density of 2 × 10⁶ cells/mL. Cells were incubated with and without test agent (in DMSO) (1% DMSO is without effect on arachidonic acid metabolism) for 10 min at room temperature. Calcium ionophore A23187 (5 μM) was added and cells were incubated for 7 min at 37 °C. The reaction was topped by chilling the tubes on ice for 10 min. Cells were separated by centrifugation and the supernatant was stored at -20 °C. Aliquots (100 μL) were analyzed for LTB₄ and PGF_{2α} by using radioimmunoassay kits as provided by the supplier.

Table I contains biochemical data obtained from this whole cell assay. IC₅₀s were calculated as the amount of test compound causing 50% inhibition of formation of LTB₄ or PGF_{2α}.

B. Carrageenan Footpad Edema.³⁴ Carrageenan solution (1% w/v) was prepared by dissolving 100 mg of carrageenan (Marine Colloidal Div., Springfield, NJ) in 10 mL of sterile saline (0.9%) solution (Travenol). Male Wistar rats were dosed with compound (in 10 mL/kg of 0.5% (hydroxypropyl)methylcellulose/0.2% Tween 80 or Labrafils) 1 h before carrageenan challenge. Foot paw edema was induced by injecting 0.05 mL of the carrageenan solution subcutaneously into the planter portion of the right hind paw of each rat under light anesthesia. Initial foot paw volume was measured immediately following carrageenan challenge by using mercury plethysmography (Buxco Electronics). Edema was measured 5 h after carrageenan administration. The swelling in each test group of animals was used to calculate the percent inhibition of edema achieved by the compound at the test dose compared with the vehicle control group.

C. Zymosan-Sponge Assay.³³ Zymosan-impregnated sponges were prepared by soaking polyurethane sponges in a 1% suspension of zymosan A in saline. Male Wistar rats were dosed with the test compounds (in 10 mL/kg of 0.5% (hydroxypropyl)methylcellulose and 0.2% Tween 80 in distilled water) 1 h before implantation of the sponges under the skin on the lateral side of the rat. The rats were anesthetized with ether during the implantation procedure. Five hours later, the rats were sacrificed by CO₂ asphyxiation, the sponge was removed, and the volume of fluid exudate in the sponge was determined. LTB₄ levels were measured by radioimmunoassay.

D. Gastric Ulcerogenicity (UD₅₀). Male outbred Wistar rats (100–250 g) were fasted for 24 h. After fasting, test compounds were administered orally (in 2 mL/kg of 0.5% (hydroxypropyl)methylcellulose) and the rats were denied access to food and water for six more hours. The rats were then sacrificed with CO₂ so that the stomachs could be removed, opened along the greater curvature, and evaluated for the presence of gastric ulcers. Results are expressed as the percent of rats with gastric ulcers at a given dose.

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Registry No. 1, 458-37-7; 5, 131068-23-0; 6, 113464-93-0; 14, 113465-15-9; E)-15, 42457-28-3; (Z)-15, 75624-83-8; 16, 113465-18-2; 17, 113465-35-3; 18, 113465-19-3; 19, 131068-24-1; 20, 113482-92-1; 21, 131068-25-2; 22, 113465-54-6; 23, 131068-26-3; 24, 131068-27-4; 25, 113465-25-1; 26, 113465-24-0; 27, 113465-36-4; 28, 113465-16-0; 29, 131068-28-5; 30, 113482-91-0; 31, 113482-93-2; 32, 113464-79-2; 33, 113465-55-7; 34, 113464-88-3; 35, 113465-03-5; 36, 131068-29-6; 37, 131068-30-9; 38, 131068-31-0; 39, 131068-32-1; 40, 113465-77-3; 3,5-dimethylisoxazole, 300-87-8; 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzaldehyde, 1620-98-0; 3,5-dichloro-4-hydroxybenzaldehyde, 2314-36-5; 3-methyl-5-isoxazoleacetic acid, 19668-85-0; syringaldehyde, 134-96-3; 5-methyl-3-isoxazoleacetic acid, 57612-87-0; 2-(1,1-dimethylethyl)-6-methoxyphenol, 57373-95-2; hexamethylenetetramine, 100-97-0; 3-methyl-5-pyrazoleacetic acid, 41669-06-1; 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, 1620-98-0; (4-acetoxy-3-methoxybenzyl)triphenylphosphonium chloride, 20361-59-5; 3-methylisothiazole-5-carboxaldehyde, 88511-32-4; 5-(chloromethyl)-3-methylisothiazole, 17265-68-8; [(3-methyl-5-isothiazolyl)methyl]triphenylphosphonium chloride, 131068-33-2;

4-acetoxy-3,5-dimethoxybenzaldehyde, 53669-33-3; 5-(chloromethyl)-3-(trifluoromethyl)isoxazole, 126572-12-1; 3-(trifluoromethyl)-5-(hydroxymethyl)isoxazole, 93498-41-0; [[3-(trifluoromethyl)-5-isoxazolyl]methyl]triphenylphosphonium chloride,

113465-75-1; 1,7-bis(4'-hydroxy-3'-methoxyphenyl)-3,5-heptanedione, 36062-04-1; 5-lipoxygenase, 80619-02-9; cyclooxygenase, 39391-18-9; α -(3,5-dichloro-4-hydroxyphenyl)-3-methyl-5-isoxazoleethanol, 20361-59-5; **42**, 113465-70-6; **43**, 131193-37-8.