Novel 1,2,4-Oxadiazoles and 1,2,4-Thiadiazoles as Dual 5-Lipoxygenase and Cyclooxygenase Inhibitors[1]

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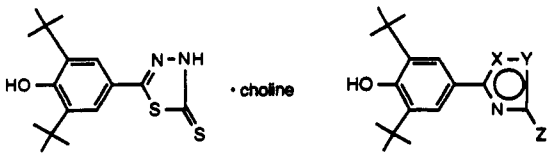
A series of 1,2,4-oxadiazoles and 1,2,4-thiadiazoles containing a 2,6-di-*tert*-butylphenol substituent were prepared and evaluated as dual inhibitors of 5-lipoxygenase and cyclooxygenase in rat basophilic leukemia (RBL-1) cells. Several of these compounds show oral efficacy in the rat carageenan footpad edema (CFE) and mycobacterium footpad edema (MFE) antiinflammatory models, without concomitant gastric ulceration. Structure–activity relationships are discussed. The best compounds (ID_{40} values in MFE of 3–8 mg/kg po) contain guanidine-derived substituents on the heterocyclic ring.

Many currently marketed nonsteroidal antiinflammatory drugs (NSAIDs) are inhibitors of the cyclooxygenase enzyme associated with metabolism of cellular arachidonic acid.[2] This mechanism is thought to be primarily responsible for the analgesic and antiinflammatory properties of NSAIDs, through the inhibition of prostaglandin biosynthesis.[3] However, cyclooxygenase inhibition has also been associated with the nephrotoxicity and gasotrintestinal side effects characteristic of NSAIDs.[4] Recent evidence suggests that increased production of leukotriene products through the 5-lipoxygenase enzyme pathway can also contribute to NSAID-induced side effects.[5] In addition, certain 5-lipoxygenase products exhibit proinflammatory and chemotactic properties.[6] Thus, a novel dual inhibitor of the cyclooxygenase (CO)/5-lipoxygenase (5-LO) enzyme pathways holds promise as an antiinflammatory agent with an improved efficacy and safety profile.

One type of dual CO/5-LO inhibitor that has been extensively studied includes derivatives of 2,6-di-*tert*-butylphenol. These phenolic compounds frequently contain a heterocyclic ring linked by a carbon or heteroatom chain to the 4-position of the phenol ring.[7] In some cases,[8] a heterocyclic ring is directly attached to the phenol 4-position.

We previously reported[9] the antiinflammatory activity of Cl-986 (1), a dual inhibitor comprised of the di-*tert*-butylphenol moiety linked to a 1,3,4-thiadiazole ring. We now report the synthesis and pharmacological properties of a series of 1,2,4-oxadiazoles and 1,2,4-thiadiazoles 2. Many of these compounds are potent inhibitors of both 5-LO and CO activity as measured in rat basophilic leukemia (RBL-1) cells. Several compounds are also orally active in animal models of inflammation.



1 Cl-986 2 X = N, O; Y = N,O,S

Chemistry

A series of 3-aryl-1,2,4-oxadiazoles were prepared from nitrile 3[10] (Scheme I), after conversion to a phenol-protected amidoxime 5. Acylation of 5 and cyclization of the resulting intermediates yielded analogs 6–8. Oxadiazolone 8 was converted to thione 9 through the imino

† Department of Chemistry.
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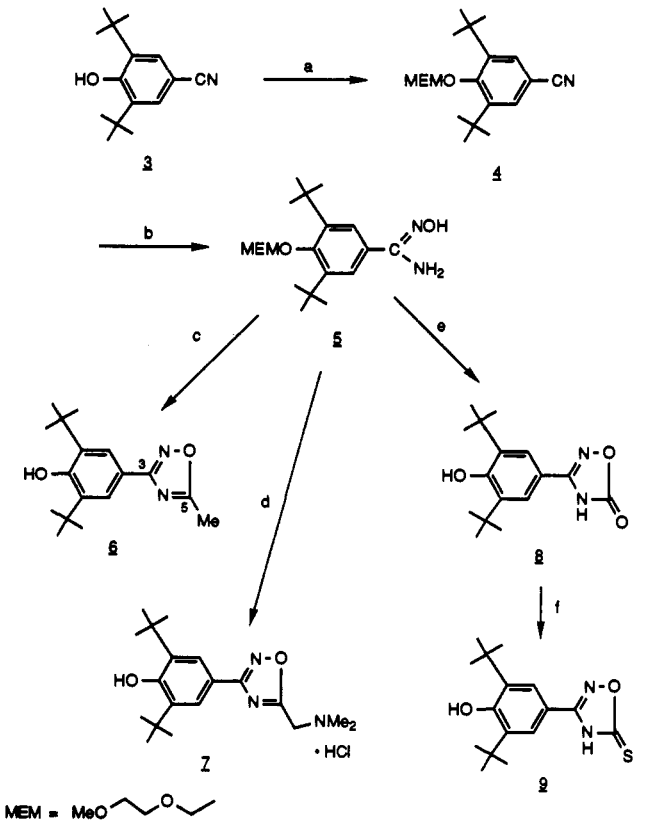
Scheme I [a]

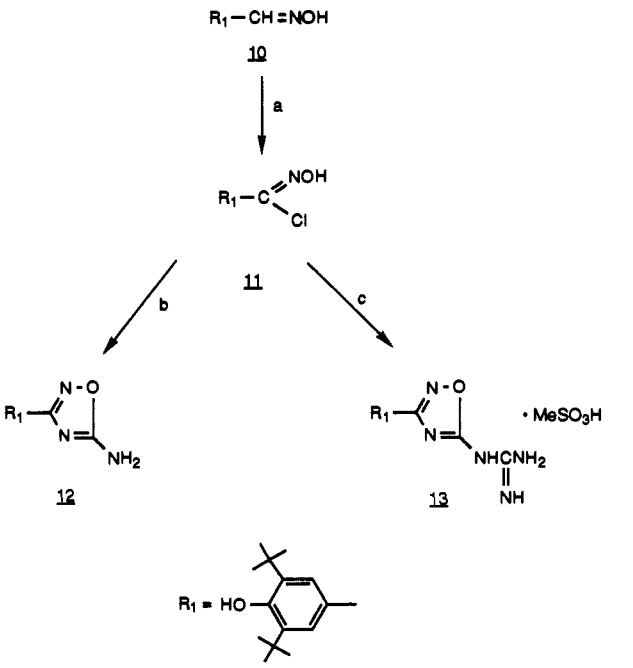


[a] Reagents: (a) MEMCl, Et_3N; (b) H_2NOH; (c) (1) AcCl, Et_3N; (2) xylene, 125 °C; (3) $ZnBr_2$; (d) (1) $ClCH_2COCl$, Et_3N; (2) Me_2NH; (3) HCl; (e) (1) $ClCO_2Et$, Et_3N; (2) toluene, 110 °C; (3) $ZnBr_2$; (f) (1) $POCl_3$, pyridine; (2) thiourea.

chloride. Additional 3-aryl derivatives were also prepared from oxime **10**[10] (Scheme II). Chlorination of **10** formed imidoyl chloride **11**, and cyclization[11,12] of **11** with guanidine and dicyanodiamide yielded oxadiazoles **12** and **13**, respectively.

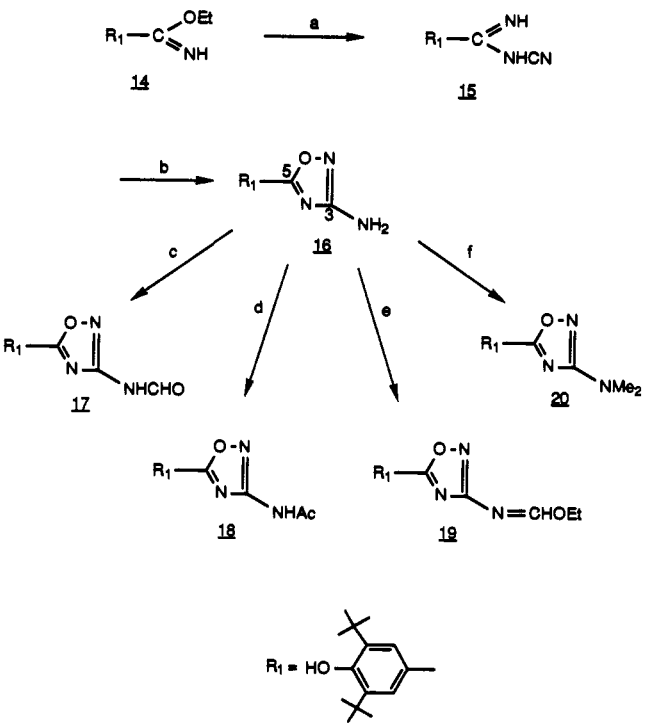
Several 5-aryloxadiazoles were prepared from imidate **14**[10,13] (Scheme III) after conversion to an *N*-cyanoamidine **15**.[14] Cyclization of **15** with hydroxylamine yielded aminooxadiazole **16**, and elaboration of the 3-amino function of **16** provided analogs **17–20**. Additional 5-aryloxadiazoles **22–26** were obtained (Scheme IV) by condensation of amidoximes with acid **21**, followed by thermal cyclization and reaction at the oxadiazole 3-position substituent.

Scheme II [a]



[a] Reagents: (a) NCS; (b) guanidine; (c) (1) dicyanodiamide; (2) $MeSO_3H$.

Scheme III [a]



[a] Reagents: (a) H_2NCN; (b) H_2NOH, pyridine; (c) acetic formic anhydride; (d) Ac_2O; (e) $HC(OEt)_3$; (f) HCHO, $NaBH_3CN$.

Cyclization of an in situ-generated nitrile sulfide[15,16] with various dipolarophiles was used to prepare a series of 3-aryl-1,2,4-thiadiazoles. An intermediate oxathiazole **28**[17] was prepared (Scheme V) from amide **27**. Thermolysis of **28** and trapping of the generated nitrile sulfide with tosyl

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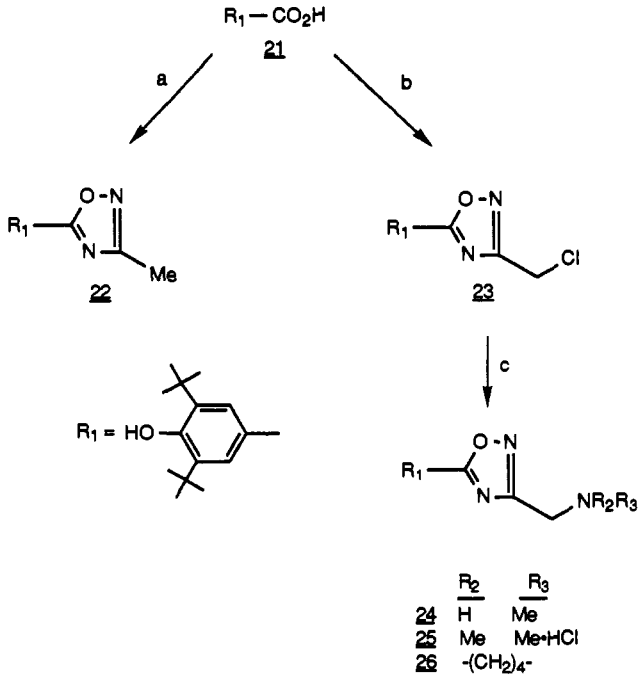
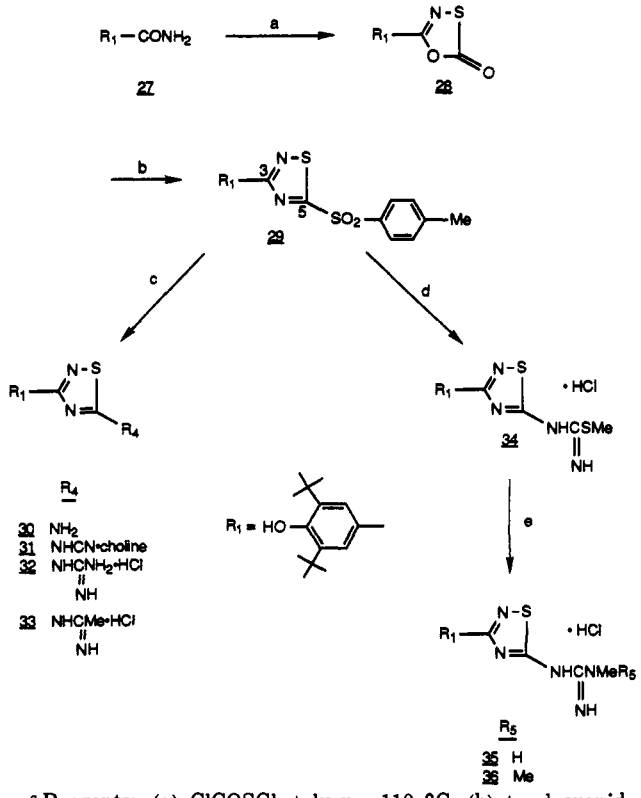
Scheme IV [a]



[a] Reagents: (a) (1) acetamidoxime, DCC; (2) xylene, 125 °C; (b) (1) 2-chloroacetamidoxime, DCC; (2) toluene, 110 °C (c) (1) R_2R_3NH; (2) HCl.

Scheme V [a]

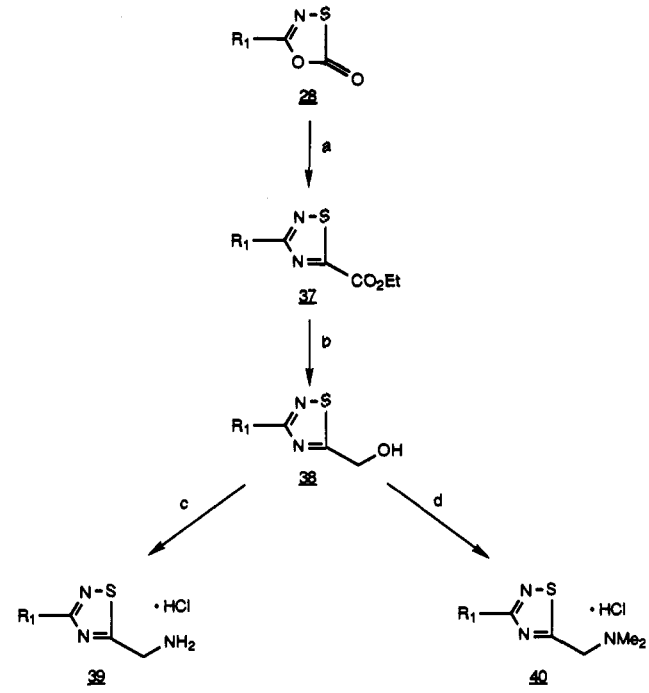


[a] Reagents: (a) ClCOSCl, toluene, 110 °C; (b) tosyl cyanide, dichlorobenzene, 165 °C; (c) (1) R_4H; (2) HCl or choline; (d) (1) $H_2NC(=NH)SMe$; (2) HCl; (e) (1) R_5MeNH; (2) HCl.

cyanide yielded **29**, a key intermediate with a labile tosyl substituent in the thiadiazole 5-position. Displacement of the tosyl group with various amines provided compounds **30–34**. Guanidino derivatives **35** and **36** were obtained by amine displacement of thiourea **34**.

An additional thiadiazole intermediate **37** was prepared (Scheme VI) by thermolysis of **28** in the presence of ethyl

Scheme VI [a]



[a] Reagents: (a) EtO_2CCN, dichlorobenzene, 165 °C; (b) $NaBH_4$; (c) (1) phthalimide, DEAD; (2) hydrazine; (3) HCl; (d) (1) $MeSO_2Cl$; (2) Me_2NH; (3) HCl.

cyanoformate. Ester reduction of **37** provided carbinol **38**, which was converted to the primary amine **39** under Mitsunobu[18] conditions, or the tertiary amine **40** by mesylate displacement.

Results and Discussion

Pharmacology and metabolism studies with **1** indicated (1) low bioavailability due to rapid metabolism, (2) generally good dual inhibitor activity when the sulfur was replaced with amino substituents, and (3) improved in vivo activity with analogs capable of salt formation.[19] Earlier work in a number of related chemical series demonstrated the desirability of maintaining the 4-hydroxy-di-*tert*-butyl substructure.[7c,f,20] With this information as a guide, we prepared a series of 1,2,4-oxadiazoles and 1,2,4-thiadiazoles with emphasis on amino substituents in the 3- or 5-position. Our goal was to find a balanced dual inhibitor with good antiinflammatory activity and a low potential for ulcerogenicity.

Compounds were evaluated for dual inhibitor activity by the inhibition of formation of LTB_4 or $PGF_{2\alpha}$ in rat basophilic leukemia (RBL-1) cells. Those compounds found to be dual inhibitors (IC_{50} values ≤ 10 μM in both assays) were then evaluated in several animal models of inflammation. Compounds that were predominantly cyclooxygenase inhibitors (arbitrarily those with a selectivity of CO vs 5-LO inhibition ≥ 15) were not pursued further, since such a profile is characteristic of many NSAIDs.

Our initial efficacy model was the carrageenan footpad edema test (CFE). This acute model serves to demonstrate

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(19) Unpublished results.

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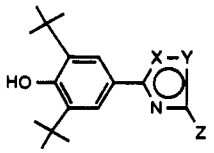
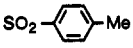
Table I. In Vitro Biochemistry of 1,2,4-Oxadiazoles and 1,2,4-Thiadiazoles



no.	X	Y	Z	IC_{50}, μM[a] 5-LO	IC_{50}, μM[a] CO	IC_{50} ratio: 5-LO/CO
6	N	O	Me	2.3	0.30	7.7
7	N	O	CH_2NMe_2·HCl	7.7	0.73	10.5
8	N	O	O(OH)	N[b]	4.7	
9	N	O	S(SH)	3.4	0.050	68
12	N	O	NH_2	2.5	3.0	0.83
13	N	O	$NHCNHNH_2$·$MeSO_3H$	1.7	0.67	2.5
16	O	N	NH_2	3.6	1.9	1.9
17	O	N	NHCHO	N	N	
18	O	N	NHCOMe	N	N	
19	O	N	NCHOEt	1.5	7.2	0.21
20	O	N	NMe_2	N	88[c]	
22	O	N	Me	3.3	0.46	7.2
23	O	N	CH_2Cl	0.78	0.10	7.8
24	O	N	CH_2NHMe	N	78[c]	
25	O	N	CH_2NMe_2·HCl	6.8	0.45	15
26	O	N		N	N	
29	N	S		N	N	
30	N	S	NH_2	0.33	2.2	0.15
31	N	S	NHCN·choline	1.9	0.011	173
32	N	S	$NHCNHNH_2$·HCl	0.14	1.6	0.088
33	N	S	NHCNHMe·HCl	N	N	
34	N	S	NHCNHSMe·HCl	1.2	0.17	7.1
35	N	S	NHCNHNHMe·HCl	0.37	4.4	0.084
36	N	S	$NHCNHNMe_2$·HCl	72[c]	N	
37	N	S	CO_2Et	91[c]	N	
39	N	S	CH_2NH_2·HCl	2.6	0.087	30
40	N	S	CH_2NMe_2·HCl	N	N	
1				2.8	0.80	3.5
			KME-4	2.5	0.15	17
			sodium meclofenamate	24.0	0.10	240

[a] Calculated as the concentration of test compound causing 50% inhibition of LTB_4 (5-LO) or $PGF_{2\alpha}$ (CO) formation. The standard errors average 11% of the values shown for 5-LO and 8% for CO. [b] Inactive (N) is defined as <50% inhibition at a screening concentration of 10 μM. [c] Percent inhibition at 10 μM screening concentration.

antiinflammatory activity as well as a reasonable level of oral bioavailability for the test compound. Those compounds showing ≥40% inhibition at a dose of 30 mg/kg po in CFE were then tested in the mycobacterium footpad edema test (MFE). The MFE test is a 3-day subacute model more demanding of antiinflammatory efficacy than CFE. Selected compounds active in CFE and MFE were then tested for gastric ulcerogenicity in a rat stress model.

An initial group of 3-aryl-1,2,4-oxadiazoles was prepared with various substituents in the oxadiazole 5-position (Table I). The thione **9**, in contrast to the oxadiazolone **8**, was a dual inhibitor in the enzyme assay, but with substantial selectivity as a CO inhibitor. The methyl **6**, dimethylaminomethyl **7**, and simple amino **12** derivatives were all dual inhibitors; however, their antiinflammatory activity in the CFE test was marginal (Table II). The inherent low basicity[21] of **12** precluded simple salt formation. The best compound in this initial series was the guanidino derivative **13**, a balanced dual inhibitor with excellent oral activity in the animal efficacy models.

Table II. Antiinflammatory Activity of 1,2,4-Oxadiazoles and 1,2,4-Thiadiazoles

no.	CFE:[a] % inhib ± SEM at dose in mg/kg po 30	10	MFE:[b] ID_{40}, mg/kg po
6	N[c]		
7	N		
12	39.0 ± 3.6	36.0 ± 4.6	
13	71.0 ± 4.0	51.2 ± 2.7	3.1 (1.2–5.5)
16	32.9 ± 3.2	43.6 ± 7.1	N[d]
17	54.3 ± 2.8	51.5 ± 4.4	N
18	N		
19	37.3 ± 4.7	21.0 ± 4.3	
22	N		
23	N		
25	50.3 ± 3.5	34.6 ± 3.9	N
32	61.3 ± 2.4	46.1 ± 2.7	5.1 (3.2–7.3)
34	41.4 ± 5.7	30.7 ± 4.8	7.3 (5.5–9.6)
35	38.9 ± 4.0	18.0 ± 4.7	6.0 (3.6–9.1)
1	47.9 ± 2.3[e]	49.9 ± 4.9[e]	7.7 (4.3–12.6)
KME-4	28.4 ± 5.1	16.6 ± 9.5	3.7 (2.0–7.2)
sodium meclofenamate	40.8 ± 5.3	34.4 ± 5.5	0.40 (0.02–1.0)

[a] Percent inhibition of edema in the carrageenan footpad edema test; n = 7–14 animals per experimental group. [b] ID_{40} of induced edema in the mycobacterium footpad edema test; n = 7–14 animals per experimental group; 95% confidence limits are in parentheses. [c] Inactive (N) is defined as <25% inhibition at a dose of 30 mg/kg po. [d] Inactive (N) is defined as <30% inhibition at a dose of 10 mg/kg po. [e] Data obtained from the sodium salt.

In the 5-aryl-1,2,4-oxadiazole series, the amino **16** and methyl **22** derivatives exhibited good in vitro but poor in vivo activity, analogous to the corresponding 3-aryl compounds (compare **12** and **6**). A prodrug approach was attempted with **16** in order to improve activity in the CFE and MFE assays. Formyl **17**, acetyl **18**, and imidoyl **19** derivatives of **16** were prepared. Some improvement in CFE activity was observed with **17** as compared to the parent **16**, but activity in MFE was again lacking. The acetyl derivative **18** appeared to be too stable metabolically to serve as an adequate prodrug, while **19** may have been too labile. Several additional amine side chains were explored in this series. The dimethylamino **20**, (methylamino)methyl **24**, and pyrrolidine **26** analogs were found to lack dual inhibitor activity, while the synthetic intermediate **23** was surprisingly active in the enzyme assay, but not in the efficacy model. Once again, the (dimethylamino)methyl **25** analog was a dual inhibitor with selectivity toward CO inhibition (compare **7**). Unlike **7**, compound **25** exhibited reasonable CFE activity, but was inactive in MFE. Several attempts at preparing a guanidino derivative in the 5-aryl series analogous to **13** were unsuccessful.

In the 3-aryl-1,2,4-thiadiazole series, intermediates **29** and **37** were inactive, but the simple amine **30** was a potent dual inhibitor, as similarly observed in the oxadiazole series (compare **12** and **16**). The (dimethylamino)methyl derivative **40** in this series was unexpectedly inactive (compare **7** and **25**), while the cyanamide **31** and aminomethyl **39** analog were poorly balanced dual inhibitors. The thiadiazole guanidino derivative **32** again demonstrated excellent overall activity (compare **13**), with an ID_{40} of 5.1 mg/kg in the MFE test. Additional variations of the guanidino substituent were explored. A monomethyl analog **35** was roughly comparable to **32** in the enzyme assay and the MFE test, but considerably less potent in

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Table III. Comparison of 13, 32, 34, and 35 with Antiinflammatory Standards

compd	gastric ulcers[a]
13	0% at 200
32	0% at 200
34	0% at 200
35	0% at 200
1	0% at 200
KME-4	30% at 30
sodium meclofenamate	50% at 36

[a] Gastric ulcerogenicity data are percent rats with ulcers at the indicated dose in mg/kg; n = 10 animals per experimental group.

the CFE test. A related dimethyl derivative 36 was not a dual inhibitor.

Replacement of the guanidino function of 32 with an amidine (compare 33) also resulted in loss of activity, however, the *S*-methylisothiourea compound 34, originally prepared as a synthetic intermediate, was found to have reasonable activity in the enzyme assay and both animal models.

An oxadiazole 13, and three thiadiazoles 32, 34, and 35, with the best overall profile in the enzyme assay and animal efficacy models, were evaluated for gastric ulcerogenic potential in a rat stress model (Table III). All four compounds chosen were balanced dual inhibitors with in vivo activity comparable to that of 1 and KME-4, a reported[7d] dual inhibitor having selectivity for CO inhibition. In contrast to KME-4, none of the four compounds caused gastric ulceration in this model at oral doses up to 200 mg/kg. Sodium meclofenamate, a marketed antiinflammatory drug, is predominantly a cyclooxygenase inhibitor, with a 5-LO/CO ratio of 240 in our assay (Table I). Although more potent in the MFE test than the four test compounds, sodium meclofenamate also exhibits a greater incidence of gastric ulceration.

We have described the preparation of novel 1,2,4-oxadiazoles and 1,2,4-thiadiazoles linked to a di-*tert*-butylphenol. These compounds represent a new series of dual inhibitors of the cyclooxygenase/5-lipoxygenase enzyme pathways. Several compounds are being evaluated as potential antiinflammatory agents devoid of ulcerogenic potential.

Experimental Section

Melting points were determined on a Thomas-Hoover or Electrothermal capillary apparatus and are uncorrected. Elemental analyses were performed by the Analytical Chemistry staff of Parke-Davis (Ann Arbor, MI) and were within ±0.4% of the theoretical values, unless indicated otherwise. Infrared spectra were recorded as KBr disks on a Nicolet MX-1 FTIR spectrometer. Proton NMR spectra were recorded on a Brüker AM 250 spectrometer, with chemical shifts reported in δ units relative to internal TMS. Reactions were generally run under a nitrogen atmosphere. Organic solutions were concentrated at aspirator vacuum on a rotary evaporator. Flash chromatography was performed with E. Merck silica gel 60, 230–400 mesh ASTM, according to the method of Still.[22] The preparation of 4, 29, 30, and 37 will be described elsewhere.[23]

3,5-Bis(1,1-dimethylethyl)-4-[(2-methoxyethoxy)methoxy]benzamide Oxime (5). A mixture of 4[23] (19.0 g, 60 mmol) and hydroxylamine (3.0 g, 91 mmol) in absolute EtOH (600 mL) was heated at 80–90 °C for 18 h. Evaporation of the solvent and recrystallization of the residue from EtOAc/hexane yielded 13.0 g (62%) of 5, mp 134–135 °C. Anal. ($C_{19}H_{32}N_2O_4$) C, H, N.

General Procedure for the Preparation of 1,2,4-Oxadiazoles 6 and 8. 2,6-Bis(1,1-dimethylethyl)-4-(5-methyl-1,2,4-oxadiazol-3-yl)phenol (6). A solution of 5 (6.0 g, 17 mmol) and Et_3N (3.0 mL, 2.2 g, 22 mmol) in $CHCl_3$ (40 mL) was treated dropwise with acetyl chloride (1.2 mL, 1.3 g, 17 mmol). The solution was stirred at room temperature for 1 h and then washed with brine. The organic layer was dried ($MgSO_4$) and evaporated to give 4.5 g (67%) of intermediate ester 3,5-bis(1,1-dimethylethyl)-4-[(2-methoxyethoxy)methoxy]benzamide *O*-acetyloxime, mp 134–137 °C. Anal. ($C_{21}H_{34}N_2O_5$) C, H, N.

A solution of the above ester (4.0 g, 10 mmol) in xylene (125 mL) was stirred at 120–130 °C for 18 h. Evaporation of the solvent and recrystallization of the residue from EtOAc/hexane gave 3.0 g (79%) of MEM-protected intermediate 3-[3,5-bis(1,1-dimethylethyl)-4-[(2-methoxyethoxy)methoxy]phenyl]-5-methyl-1,2,4-oxadiazole, mp 75–77 °C. Anal. ($C_{21}H_{32}N_2O_4$) C, H, N.

The above intermediate (2.6 g, 6.9 mmol) in CH_2Cl_2 (20 mL) was treated with anhydrous $ZnBr_2$ (7.7 g, 34 mmol), and the mixture was stirred vigorously at room temperature for 3 h. The solvent was decanted and the solid residue washed with fresh CH_2Cl_2. The combined organic layers were washed with 10% $NaHCO_3$ and brine, then dried ($MgSO_4$), and evaporated to yield 0.94 g (47%) of 6: mp 126–127 °C; IR 3604, 1602, 1239, 901 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (s, 18 H, *t*-Bu), 2.63 (s, 3 H, CH_3), 5.55 (s, 1 H, OH), 7.89 (s, 2 H, ArH). Anal. ($C_{17}H_{24}N_2O_2$) C, H, N.

3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-oxadiazol-5(4*H*)-one (8). In an analogous manner, 5 (2.5 g, 7.1 mmol) was reacted with ethyl chloroformate (7.0 mL, 7.9 g, 7.3 mmol) to afford 2.6 g (86%) of intermediate ester 3,5-bis(1,1-dimethylethyl)-*N*-[(ethoxycarbonyl)oxy]-4-[(2-methoxyethoxy)-methoxy]benzenecarboximidamide, mp 115–117 °C. Anal. ($C_{22}H_{36}N_2O_6$) C, H, N.

Thermal cyclization of the above ester (5.0 g, 12 mmol) in toluene gave 3.1 g (70%) of MEM-protected intermediate 3-[3,5-bis(1,1-dimethylethyl)-4-[(2-methoxyethoxy)methoxy]phenyl]-1,2,4-oxadiazol-5(4*H*)-one, mp 112–114 °C. Anal. ($C_{20}H_{30}N_2O_5$) C, H, N.

Removal of the MEM group from the above intermediate (1.9 g, 5.0 mmol) yielded 0.70 g (48%) of 8, mp 189–191 °C, after recrystallization from EtOAc/hexane: IR 3613, 1757, 1427, 974 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (s, 18 H, *t*-Bu), 5.72 (s, 1 H, OH), 7.63 (s, 2 H, ArH), 11.55 (br s, 1 H, NH). Anal. ($C_{16}H_{22}N_2O_3$) C, H, N.

4-[5-[(Dimethylamino)methyl]-1,2,4-oxadiazol-3-yl]-2,6-bis(1,1-dimethylethyl)phenol Hydrochloride (7). A solution of 5 (14.4 g, 41 mmol) and Et_3N (14.4 mL, 10.5 g, 103 mmol) in $CHCl_3$ (145 mL) was treated dropwise with a solution of chloroacetyl chloride (3.6 mL, 5.1 g, 45 mmol) in $CHCl_3$ (14 mL). The mixture was stirred at room temperature for 48 h and then evaporated, and the residue was redissolved in EtOAc. The solution was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (30% EtOAc/hexane) to provide 4.0 g of the 5-(chloromethyl)oxadiazole intermediate. This intermediate was dissolved in DMF (60 mL) and treated over 20 min with excess dimethylamine gas. The mixture was stirred for an additional 1 h, then poured into ice/H_2O, and extracted with EtOAc. The extracts were washed with H_2O, dried (Na_2SO_4), and evaporated. The residue was dissolved in Et_2O and treated with HCl gas, and the precipitated salt was filtered and washed with Et_2O. The salt was dissolved in a minimum of MeOH and treated with HCl gas for 20 min to complete removal of the MEM protecting group. The mixture was stirred for 1 h and then evaporated, and the residue was washed thoroughly with Et_2O to yield 3.3 g (22%) of 7: mp 238–240 °C; IR 3627, 1604, 1416, 1239 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (s, 18 H, *t*-Bu), 3.06 (s, 6 H, CH_3), 4.69 (s, 2 H, CH_2), 5.68 (s, 1 H, OH), 7.89 (s, 2 H, ArH). Anal. ($C_{19}H_{29}N_3O_2$·HCl) C, H, N.

2,6-Bis-(1,1-dimethylethyl)-4-(5-thioxo-1,2,4-oxadiazol-3-yl)phenol (9). A mixture of the MEM-protected precursor intermediate to 8 (1.8 g, 4.8 mmol), pyridine (0.18 mL, 0.18 g, 2.2 mmol), and $POCl_3$ (2.0 mL, 3.3 g, 21 mmol) was heated at 80 °C for 20 h. The cooled mixture was added to ice/H_2O and immediately extracted with Et_2O. The organic layer was dried

(22) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(23) Shrum, G. P.; Unangst, P. C.; Connor, D. T. *J. Heterocycl. Chem.*, submitted for publication.

($MgSO_4$) and evaporated. Purification of the residue by flash chromatography (30% EtOAc/hexane) gave 0.27 g (18%) of chloro intermediate 4-(5-chloro-1,2,4-oxadiazol-3-yl)-2,6-bis(1,1-dimethylethyl)phenol, mp 111–112 °C. Anal. ($C_{16}H_{21}ClN_2O_2$) C, H, N.

A mixture of the above chloro intermediate (0.18 g, 0.58 mmol) and thiourea (0.050 g, 0.66 mmol) in EtOH (15 mL) was stirred at reflux for 1 h. The mixture was concentrated and the residue taken up in EtOAc. The insoluble material was filtered and the filtrate evaporated. Trituration of the residual oil with hexane afforded 0.16 g (89%) of **9**. A sample was recrystallized from EtOAc/hexane: mp 183–186 °C; IR 3620, 1607, 1443, 1243 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (s, 18 H, *t*-Bu), 5.78 (s, 1 H, OH), 7.59 (s, 2 H, ArH). Anal. ($C_{16}H_{22}N_2O_2S$) C, H, N.

3,5-Bis(1,1-dimethylethyl)-*N*-4-dihydroxybenzenecarboximidoyl Chloride (11). A solution of 10[10] (9.5 g, 38 mmol) in CH_2Cl_2 (20 mL) was treated dropwise at room temperature with a solution of *N*-chlorosuccinimide (6.3 g, 47 mmol) in CH_2Cl_2 (150 mL). The mixture was stirred for 16 h, then washed several times with cold H_2O, and dried ($MgSO_4$). Evaporation of the CH_2Cl_2 solution gave 5.4 g (50%) of **11**, mp 168–170 °C. Anal. ($C_{15}H_{22}ClNO_2$) C, H, N.

4-(5-Amino-1,2,4-oxadiazol-3-yl)-2,6-bis(1,1-dimethylethyl)phenol (12). A solution of **11** (6.2 g, 22 mmol) in absolute EtOH (60 mL) was added dropwise to a solution of guanidine (2.6 g, 44 mmol) in EtOH (100 mL). The mixture was stirred at room temperature for 18 h and then evaporated. The residual oil was partitioned between EtOAc (125 mL) and H_2O (125 mL), and the two-phase mixture was acidified to pH 4–5 with 1.0 N HCl. The organic layer was separated, washed with H_2O, dried ($MgSO_4$), and evaporated to yield 5.0 g of crude product. Purification by flash chromatography (EtOAc/hexane) gave 1.0 g (16%) of **12**: mp 202–204 °C; IR 3613, 1653, 1390, 1241 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (s, 18 H, *t*-Bu), 5.53 (s, 1 H, OH), 5.77 (m, 2 H, NH_2), 7.78 (s, 2 H, ArH). Anal. ($C_{18}H_{23}N_3O_2$) C, H, N.

***N*-[3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-oxadiazol-5-yl]guanidinium Methanesulfonate (13).** A mixture of **11** (13.9 g, 49 mmol) and dicyanodiamide (6.2 g, 74 mmol) in EtOH (100 mL) was stirred at reflux for 16 h. The reaction mixture was evaporated and the residue triturated with a small amount of warm EtOH. The precipitated solid (9.0 g) was filtered and washed with hexane. This solid was suspended in Et_2O (225 mL), and MeOH was added until the solid dissolved. A solution of methanesulfonic acid (1.8 mL, 2.7 g, 28 mmol) in Et_2O (20 mL) was added dropwise, and the mixture was stirred for an additional 30 min. The precipitate was filtered and washed with Et_2O to give 10.3 g (49%) of **13**: mp 199–200 °C; IR 3617, 1715, 1357, 1046 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.43 (s, 18 H, *t*-Bu), 2.41 (s, 3 H, CH_3SO_3), 7.61 (br s, 1 H, OH), 7.74 (s, 2 H, ArH), 8.00 (br s, 5 H, NH, NH_2). Anal. ($C_{17}H_{25}N_5O_2 \cdot CH_4O_3S$) C, H, N.

N′-Cyano-3,5-bis(1,1-dimethylethyl)-4-hydroxybenzenecarboximidamide (15). A mixture of 14[10,13] (17.6 g, 63 mmol) and cyanamide (3.2 g, 76 mmol) in MeOH (250 mL) was stirred at reflux for 16 h, then cooled and filtered. The filtrate was evaporated and the residue digested briefly on the steam bath with 15% EtOAc/CH_2Cl_2 (150 mL) and refiltered. The final filtrate was condensed to 20 mL and chromatographed (15–25% EtOAc in CH_2Cl_2) to obtain 11.3 g (65%) of **15**. A sample recrystallized from EtOAc/hexane had mp 192–194 °C. Anal. ($C_{18}H_{23}N_3O$) C, H, N.

4-(3-Amino-1,2,4-oxadiazol-5-yl)-2,6-bis(1,1-dimethylethyl)phenol (16). A mixture of **15** (3.8 g, 14 mmol), hydroxylamine hydrochloride (1.0 g, 14 mmol), and pyridine (4.3 mL, 4.2 g, 53 mmol) in EtOH (50 mL) was stirred at reflux for 24 h. The cooled mixture was filtered and evaporated, and the residue was distributed between Et_2O (100 mL) and H_2O (250 mL). The aqueous layer was extracted several times with fresh Et_2O, and the combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated. Recrystallization of the residue from EtOAc/hexane provided 2.0 g (50%) of **16**: mp 167–170 °C; IR 3608, 1647, 1411, 1253 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (s, 18 H, *t*-Bu), 4.44 (br s, 2 H, NH_2), 5.72 (s, 1 H, OH), 7.89 (s, 2 H, ArH). Anal. ($C_{16}H_{23}N_3O_2$) C, H, N.

***N*-[5-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-oxadiazol-3-yl]formamide (17).** In order to generate acetic formic anhydride, Ac_2O (1.8 mL, 2.0 g, 19 mmol) was cooled in ice and treated over 20 min with 99% HCO_2H (0.9 mL, 1.1 g, 24 mmol). The mixture was heated at 50–60 °C for 2 h, then cooled, and THF (5.0 mL) was added, followed by **16** (2.0 g, 6.9 mmol) and additional THF (15 mL). The mixture was stirred at room temperature for 18 h, and the solvent was evaporated. Recrystallization of the residue from EtOAc/hexane yielded 1.6 g (75%) of **17**: mp 222 °C dec; IR 3549, 1718, 1395, 1113 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.43 (s, 18 H, *t*-Bu), 7.84 (s, 2 H, ArH), 8.07 (br s, 1 H, OH), 8.97 (br m, 1 H, CHO), 11.43 (br m, 1 H, NH). Anal. ($C_{17}H_{23}N_3O_3$) C, H, N.

***N*-[5-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-oxadiazol-3-yl]acetamide (18).** A mixture of Ac_2O (13.0 mL, 14.1 g, 138 mmol) and **16** (4.0 g, 14 mmol) in toluene (200 mL) was stirred at reflux for 27 h. The cooled solution was washed with H_2O, 5% $NaHCO_3$, and H_2O again, then dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (1–20% EtOAc/CH_2Cl_2) to provide 1.3 g (28%) of **18**. A sample recrystallized from MeCN/H_2O had mp 241–243 °C; IR 3621, 1695, 1530, 1164 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.43 (s, 18 H, *t*-Bu), 2.11 (s, 3 H, CH_3), 7.83 (s, 2 H, ArH), 8.01 (s, 1 H, OH), 11.12 (s, 1 H, NH). Anal. ($C_{18}H_{25}N_3O_3$) C, H, N.

Ethyl *N*-[5-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-oxadiazol-3-yl]methanimidoate (19). A mixture of **16** (0.87 g, 3.0 mmol) and anhydrous triethyl orthoformate (25.0 mL, 22.3 g, 150 mmol) was stirred at reflux for 24 h, then cooled, and evaporated. The residue was distributed between CH_2Cl_2 (75 mL) and saturated aqueous $NaHCO_3$ (100 mL). The aqueous layer was extracted several times with fresh CH_2Cl_2, and the combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated. Recrystallization of the residue from MeCN/H_2O gave 0.70 g (67%) of **19**: mp 159–161 °C; IR 3605, 1636, 1360, 1158 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 (t, 3 H, CH_2CH_3), 1.49 (s, 18 H, *t*-Bu), 4.48 (q, 2 H, CH_2CH_3), 5.74 (s, 1 H, OH), 7.96 (s, 2 H, ArH), 8.59 (s, 1 H, NCH). Anal. ($C_{18}H_{27}N_3O_3$) C, H, N.

4-[3-(Dimethylamino)-1,2,4-oxadiazol-5-yl]-2,6-bis(1,1-dimethylethyl)phenol (20). A mixture of **16** (2.0 g, 6.9 mmol) and paraformaldehyde (2.0 g, 67 mmol) in glacial HOAc (45 mL) was treated over 10 min with sodium cyanoborohydride (2.0 g, 32 mmol). The mixture was stirred at room temperature for 24 h, then cooled in ice, and treated cautiously with ice/H_2O (250 mL). Solid Na_2CO_3 was added until the mixture was slightly basic. The mixture was extracted with EtOAc, and the combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated. Recrystallization of the residue from MeOH/H_2O provided 1.0 g (46%) of **20**: mp 135–138 °C; IR 3479, 1598, 1392, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (s, 18 H, *t*-Bu), 3.07 (s, 6 H, CH_3), 5.67 (s, 1 H, OH), 7.89 (s, 2 H, ArH). Anal. ($C_{18}H_{27}N_3O_2$) C, H, N.

General Procedure for the Preparation of 1,2,4-Oxadiazoles 22 and 23. 2,6-Bis(1,1-dimethylethyl)-4-(3-methyl-1,2,4-oxadiazol-5-yl)phenol (22). A mixture of acid **21** (1.7 g, 6.8 mmol), acetamidoxime[24] (0.50 g, 6.8 mmol), and 1-hydroxybenzotriazole hydrate (0.90 g, 6.7 mmol) in DMF (35 mL) was cooled in an ice bath and treated with 1,3-dicyclohexylcarbodiimide (1.5 g, 7.3 mmol). The mixture was stirred at room temperature for 18 h, then poured into ice/H_2O (60 mL), and made basic by the addition of 10% $NaHCO_3$. The mixture was extracted with EtOAc, and the combined organic layers were washed with 10% $NaHCO_3$ and brine, then dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography (EtOAc/hexane) to yield 1.3 g (63%) of intermediate ester acetamide *O*-[3,5-bis(1,1-dimethylethyl)-4-hydroxybenzoyl]oxime, mp 175–176 °C. Anal. ($C_{17}H_{26}N_2O_3$) C, H, N.

A solution of the above ester (4.2 g, 14 mmol) in xylene (125 mL) was stirred at 120–130 °C for 18 h. Evaporation of the solvent and recrystallization of the residue from EtOAc/hexane gave 2.4 g (61%) of **22**: mp 126–127 °C; IR 3539, 1609, 1344, 1117 cm^{-1}; ^1H NMR δ 1.49 (s, 18 H, *t*-Bu), 2.45 (s, 3 H, CH_3), 5.72 (s, 1 H, OH), 7.94 (s, 2 H, ArH). Anal. ($C_{17}H_{24}N_2O_2$) C, H, N.

4-[3-(Chloromethyl)-1,2,4-oxadiazol-5-yl]-2,6-bis(1,1-dimethylethyl)phenol (23). In an analogous manner, **21** (13.2 g, 53 mmol) was reacted with 2-chloroacetamidoxime[24] (5.7 g, 53

(24) Flora, K. P.; van't Riet, B.; Wampler, G. L. Antitumor Activity of Amidoximes (Hydroxyurea Analogs) in Murine Tumor Systems. *Cancer Res.* **1978**, *38*, 1291–1295.

mmol) to afford after flash chromatography (EtOAc/hexane), 12.1 g (67%) of intermediate 2-chloroacetamide *O*-[3,5-bis(1,1-dimethylethyl)-4-hydroxybenzoyl]oxime, mp 173–175 °C. Anal. ($C_{17}H_{25}ClN_2O_3$) C, H, N.

Thermal cyclization of the above ester (3.7 g, 11 mmol) in toluene yielded 2.8 g (80%) of **23**, mp 93–94 °C, after recrystallization from EtOAc/hexane: IR 3544, 1609, 1377, 1247 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (s, 18 H, *t*-Bu), 4.65 (s, 2 H, CH_2), 5.77 (s, 1 H, OH), 7.97 (s, 2 H, ArH). Anal. ($C_{17}H_{23}ClN_2O_2$) C, H, N.

General Procedure for the Preparation of 1,2,4-Oxadiazoles 24–26. 2,6-Bis(1,1-dimethylethyl)-4-[3-[(methylamino)methyl]-1,2,4-oxadiazol-5-yl]phenol (24). A solution of **23** (0.80 g, 2.5 mmol) in DMF (10 mL) was saturated with methylamine gas and stirred at room temperature for 3 h. The mixture was poured into ice/H_2O and extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated. Purification of the residue by flash chromatography (EtOAc/hexane) yielded 0.20 g (25%) of **24**: mp 120–122 °C; IR 3320, 1608, 1417, 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (s, 18 H, *t*-Bu), 2.50 (s, 3 H, CH_3), 3.93 (s, 2 H, CH_2), 5.75 (br s, 1 H, OH), 7.98 (s, 2 H, ArH). Anal. ($C_{18}H_{27}N_3O_2$) C, H, N.

4-[3-[(Dimethylamino)methyl]-1,2,4-oxadiazol-5-yl]-2,6-bis(1,1-dimethylethyl)phenol Hydrochloride (25). In an analogous manner, a solution of **23** (4.0 g, 12 mmol) was treated with excess dimethylamine. The free base product (2.4 g) was dissolved in EtOH (115 mL) and treated with HCl gas. The reaction mixture was evaporated to an oil. Trituration with Et_2O yielded a solid which was recrystallized from EtOH/Et_2O to give 2.3 g (50%) of **25**: mp 199–201 °C; IR 3227, 1608, 1420, 1330 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50 (s, 18 H, *t*-Bu), 2.95 (s, 6 H, CH_3), 4.38 (s, 2 H, CH_2), 5.87 (s, 1 H, OH), 7.97 (s, 2 H, ArH). Anal. ($C_{19}H_{29}N_3O_2$·HCl) C, H, N.

2,6-Bis(1,1-dimethylethyl)-4-[3-(1-pyrrolidinylmethyl)-1,2,4-oxadiazol-5-yl]phenol (26). In an analogous manner, a solution of **23** (0.50 g, 1.5 mmol) was reacted with pyrrolidine (0.47 mL, 0.40 g, 5.6 mmol) to yield 0.40 g (75%) of **26**: mp 100–102 °C; IR 3410, 1611, 1394, 1118 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (s, 18 H, *t*-Bu), 1.84 (m, 4 H, ring CH_2), 2.68 (m, 4 H, ring CH_2), 3.84 (s, 2 H, acyclic CH_2), 5.72 (s, 1 H, OH), 7.98 (s, 2 H, ArH). Anal. ($C_{21}H_{31}N_3O_2$) C, H, N.

[3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-thiadiazol-5-yl]cyanamide Choline Salt (31). A mixture of cyanamide (1.0 g, 24 mmol) and Et_3N (3.1 mL, 2.5 g, 24 mmol) in DMF (75 mL) was stirred for 20 min and treated with **29**[23] (2.0 g, 4.5 mmol). The new mixture was heated at 65 °C for 16 h, then cooled, and added to ice/H_2O. The pH of the solution was adjusted to 12.0 with 1.0 N NaOH, and the solution was washed twice with Et_2O and then acidified to pH 4.0 with concentrated HCl. The mixture was stored at 0–5 °C for 24 h, and the precipitated product (0.95 g) was filtered, dissolved in MeOH (35 mL), and treated dropwise with 45% aqueous choline bicarbonate (1.1 g, 3.0 mmol). The mixture was stirred for 1 h, heated on the steam bath for a few minutes, then cooled, and evaporated. Trituration of the residue with *t*-BuOMe and EtOAc yielded 1.2 g (62%) of **31**: mp 153–155 °C; IR 3630, 2148, 1504, 1336 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.41 (s, 18 H, *t*-Bu), 3.10 (s, 9 H, CH_3), 3.38 (m, 2 H, CH_2), 3.84 (m, 2 H, CH_2), 5.29 (t, 1 H, choline OH), 7.18 (s, 1 H, phenol OH), 7.87 (s, 2 H, ArH). Anal. ($C_{17}H_{21}N_4OS$·$C_5H_{14}NO$) C, H, N.

General Procedure for the Preparation of 1,2,4-Thiadiazoles 32 and 33. *N*-[3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-thiadiazol-5-yl]guanidine Hydrochloride (32). A suspension of guanidine hydrochloride (8.4 g, 88 mmol) in *t*-BuOH (600 mL) was treated with *t*-BuOK (9.9 g, 88 mmol) and stirred for 45 min. After the addition of **29** (13.0 g, 29 mmol), the mixture was stirred at reflux for 2 h, cooled, and evaporated. The residue was partitioned between EtOAc and H_2O. The organic layer was washed with H_2O, dried (Na_2SO_4), and evaporated to give 6.0 g of the free base product. This solid was dissolved in a minimum of Et_2O, and excess HCl gas was added. The precipitated solid was filtered, washed with Et_2O, and recrystallized from MeOH/Et_2O to yield 5.0 g (45%) of **32**: mp 268–270 °C; IR 3628, 1696, 1609, 1407 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.43 (s, 18 H, *t*-Bu) 7.54 (s, 1 H, OH), 7.93 (s, 2 H, ArH), 8.59 (s, 4 H, NH, NH_2). Anal. ($C_{17}H_{25}N_5OS$·HCl) C, H, N.

***N*-[3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-thiadiazol-5-yl]ethanimidamide Hydrochloride (33).** In an analogous manner, acetamidine hydrochloride (0.20 g, 2.1 mmol) was reacted with **29** (0.30 g, 0.67 mmol) to give 0.23 g (88%) of **33** mp 237–239 °C; IR 3616, 1685, 1596, 1408 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.45 (s, 18 H, *t*-Bu), 2.50 (s, 3 H, CH_3), 7.56 (s, 1 H, OH), 8.00 (s, 2 H, ArH). Anal. ($C_{18}H_{26}N_4OS$·HCl) C, H, N.

***N*-[3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-thiadiazol-5-yl]carbamimidothioic Acid Methyl Ester Hydrochloride (34).** A mixture of 5-methylisothiouronium sulfate (1.2 g, 4.3 mmol) and Na_2CO_3 (2.0 g, 19 mmol) in CH_2Cl_2 (8.0 mL) and H_2O (2.0 mL) was stirred for 15 min, and a solution of **29** (2.0 g, 4.5 mmol) in $CHCl_2$ (4.0 mL) was added in portions. The new mixture was stirred for 16 h, and the liquid was decanted and the solid residue washed with CH_2Cl_2. The combined organic layers were washed with 10% citric acid, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (30% EtOAc/hexane) to afford 0.93 g of the parent of the title compound. Conversion to the HCl salt as described in the preparation of **32** yielded 0.84 g (45%) of **34**: mp 221–223 °C; IR 1654, 1569, 1384, 1232 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 1.43 (s, 18 H, *t*-Bu), 2.60 (s, 3 H, CH_3), 6.57 (br s, 1 H, OH), 7.84 (s, 2 H, ArH), 10.68 (br s, 1 H, NH), 11.05 (br s, 1 H, NH). Anal. ($C_{18}H_{26}N_4OS_2$·HCl) C, H, N.

General Procedure for the Preparation of 1,2,4-Thiadiazoles 35 and 36. *N*-[3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-thiadiazol-5-yl]-*N'*-methylguanidine Hydrochloride (35). A solution of parent free base of **34** (0.20 g, 0.53 mmol) in DMF (10 mL) was treated with methylamine gas for 10 min. The solution was then heated at 60 °C for 2 h, the addition of methylamine was repeated, and heating was continued for 16 h. The reaction mixture was cooled and added to ice/H_2O (100 mL), and the precipitated solid was extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (30% EtOAc/hexane). The purified product was converted to the HCl salt as described in the preparation of **32** to yield 0.18 g (86%) of **35**: mp 271–273 °C; IR 3622, 1684, 1602, 1409 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.43 (s, 18 H, *t*-Bu), 2.96 (d, 3 H, CH_3), 7.54 (s, 1 H, OH), 7.94 (s, 2 H, ArH), 8.91 (br s, 2 H, NH), 9.02 (br s, 1 H, NH). Anal. ($C_{18}H_{27}N_5OS$·HCl) C, H, N.

***N'*-[3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-thiadiazol-5-yl]-*N,N*-dimethylguanidine Hydrochloride (36).** In an analogous manner, a solution of the parent free base of **34** (0.33 g, 0.87 mmol) was reacted with dimethylamine to give 0.25 g (69%) of **36**, mp 253–255 °C; IR 3630, 1663, 1626, 1409 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.43 (s, 18 H, *t*-Bu), 3.24 (s, 6 H, CH_3), 7.55 (br s, 1 H, OH), 7.93 (s, 2 H, ArH), 9.22 (br s, 2 H, NH). Anal. ($C_{19}H_{29}N_5OS$·HCl) C, H, N.

3-[3,5-Bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,2,4-thiadiazole-5-methanol (38). A solution of **37**[23] (12.4 g, 34 mmol) in MeOH (700 mL) was treated, in portions, with solid $NaBH_4$ (2.2 g, 58 mmol). The mixture was stirred for 16 h and then quenched by the cautious addition of 1.0 N HCl (125 mL). The reaction mixture was concentrated and extracted with EtOAc. The organic layers were washed with brine, dried ($MgSO_4$), and evaporated to an oil. Trituration with 20% EtOAc/hexane gave 8.3 g (78%) of **38**, mp 123–125 °C. Anal. ($C_{17}H_{24}N_2O_2S$) C, H, N.

4-[5-(Aminoethyl)-1,2,4-thiadiazol-3-yl]-2,6-bis(1,1-dimethylethyl)phenol Hydrochloride (39). A mixture of **38** (3.6 g, 12 mmol), triphenylphosphine (3.1 g, 12 mmol), and phthalimide (1.8 g, 12 mmol) in THF (20 mL) was treated dropwise with a solution of diethyl azodicarboxylate (1.9 mL, 2.1 g, 12 mmol) in THF (6.0 mL). The mixture was stirred for 16 h and then concentrated, and the residue was redissolved in EtOAc. The organic layer was washed with 1.0 N HCl, H_2O, saturated $NaHCO_3$, and H_2O again, then dried (Na_2SO_4), and evaporated. Purification of the residue by flash chromatography (20% EtOAc/hexane) yielded 3.5 g (67%) of intermediate 2-[[3-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenol]-1,2,4-thiadiazol-5-yl]methyl]-1*H*-isoindole-1,3(2*H*)-dione, mp 174–176 °C. Anal. ($C_{25}H_{27}N_3O_3S$) C, H, N.

A solution of the above intermediate (4.2 g, 9.3 mmol) in EtOH (20 mL) was treated dropwise with a solution of anhydrous hydrazine (0.54 mL, 0.55 g, 17 mmol) in EtOH (5.0 mL). The

reaction mixture was heated at 80 °C for 90 min, then cooled, and diluted with $CHCl_3$. The insoluble material was filtered and discarded. The filtrate was concentrated, the residue redissolved in $CHCl_3$, and the mixture filtered again. The filtrate was evaporated to a white solid, representing the parent free base of the title compound. This solid was converted to the HCl salt as described in the preparation of **32** to yield 1.2 g (36%) of **39**: mp 247–250 °C; IR 3634, 1602, 1404, 1159 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.44 (s, 18 H, *t*-Bu), 4.67 (s, 2 H, CH_2), 7.55 (s, 1 H, OH), 8.08 (s, 2 H, ArH), 8.94 (s, 3 H, NH_3^+). Anal. ($C_{17}H_{25}N_3OS$·HCl) C: calcd, 57.36; found, 56.95; H, N.

4-[5-[(Dimethylamino)methyl]-1,2,4-thiadiazol-3-yl]-2,6-bis(1,1-dimethylethyl)phenol Hydrochloride (40). A solution of **38** (1.0 g, 3.2 mmol) and Et_3N (0.92 mL, 0.67 g, 6.6 mmol) in CH_2Cl_2 (70 mL) was cooled to −60 °C and treated dropwise with a solution of methanesulfonyl chloride (0.25 mL, 0.37 g, 3.2 mmol) in CH_2Cl_2 (15 mL). The mixture was stirred at −60 °C for 1 h, diluted with Et_2O, and washed with H_2O and brine. The organic layer was dried (Na_2SO_4) and evaporated to provide 1.2 g of the crude sulfonate ester intermediate. This intermediate was dissolved in DMF (20 mL) and treated with excess dimethylamine gas for 30 min. After stirring for 1 h, the solution was poured into ice/H_2O and extracted with EtOAc. The organic layer was washed with H_2O, dried (Na_2SO_4), and evaporated to a solid, representing the parent free base of the title compound. This solid was converted to the HCl salt as described in the preparation of **32** to yield 1.2 g (97%) of **40**: mp 248–250 °C; IR 3630, 1653, 1400, 1238 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.45 (s, 18 H, *t*-Bu), 2.89 (s, 6 H, CH_3), 4.98 (s, 2 H, CH_2), 7.58 (s, 1 H, OH), 8.08 (s, 2 H, ArH), 11.75 (br s, 1 H, NH^+). Anal. ($C_{19}H_{29}N_3OS$·HCl) 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 LTB_4 and $PGF_{2\alpha}$ 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; Na_2HPO, 1.15 g; KH_2PO_4, 0.2 g; and KCl, 0.2 g/L). Cells were finally suspended in PBS containing 1.0 mM calcium at a density of 2×10^6 cell/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 stopped 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 LTB_4 and $PGF_{2\alpha}$ by using radioimmunoassay kits as provided by the supplier. Compounds were tested at concentrations of 0.10, 0.33, 1.0, 3.3, and 10.0 μM and the IC_{50} values determined by linear regression analysis.

B. Carrageenan Footpad Edema. 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 orally 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 0.10 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 ±SEM of edema achieved by the compound at the test dose compared with the vehicle control group. Compounds were tested at doses of 10.0 and 30.0 mg/kg po, with 7–14 animals at each test dose.

C. Mycobacterium Footpad Edema. *Mycobacterium butyricum* (5 mg/mL) was suspended in paraffin oil by sonication for 10 min in an ice bath. Footpad edema was induced on day 0 by injecting 0.1 mL of the *Mycobacterium* mixture into the left hindpaw of lightly anesthetized rats. Swelling in the injected hindpaw was determined by mercury plethsmography 72 h after injection. Groups of rats were orally dosed with test compounds (suspended in 0.5% (hydroxypropyl)methylcellulose with 0.2% Tween-80) or vehicle 1 h before *Mycobacterium* injection and on days 1 or 2. Inhibition of swelling was determined by comparing the change in hindpaw volume in compound- and vehicle-treated rats. An ID_{40} (the dose at which swelling is inhibited by 40%) was calculated by linear regression analysis. Compounds were tested at a dose of 2.0, 10.0, and 50.0 mg/kg po, with 7–14 animals at each test dose.

D. Gastric Ulcerogenicity. 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 6 more hours. The rats were then sacrificed with CO_2 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. Compounds were tested up to a maximum dose of 200 mg/kg po, with 10 animals per dose.

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Supplementary Material Available: IR and NMR data for compounds **5**, **11**, **15**, **38** and the intermediates for **6**, **8**, **9**, **22**, **23**, and **39** (3 pages). Ordering information is given on any current masthead page.