2'-Substituted Chalcone Derivatives as Inhibitors of Interleukin-1 Biosynthesis

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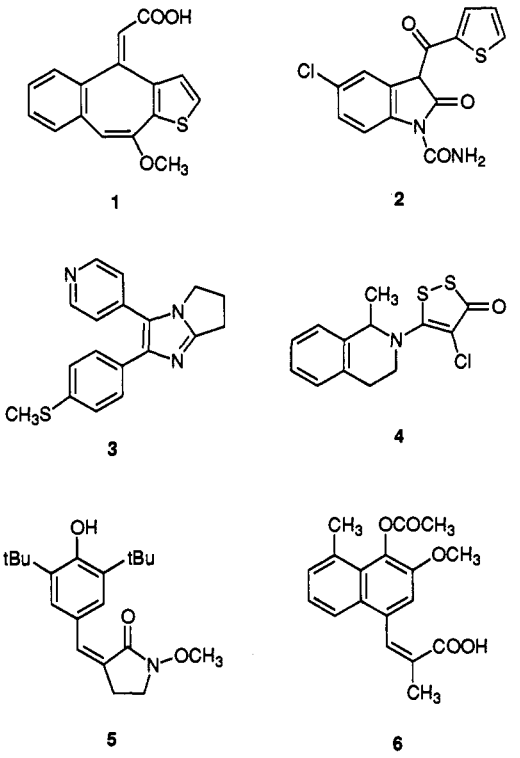
A series of 2'-substituted chalcone derivatives has been found to show potent inhibition of the production of IL-1β from human peripheral blood monocytes stimulated with lipopolysaccharide (LPS), with IC_{50} values in the 0.2–5.0-μM range. Some members of the series have also shown inhibition of septic shock induced in mice by injection of LPS, although with low potency. Qualitative structure–activity relationships have shown that the enone is required for activity, which may be mediated by conjugate addition of a biological nucleophile to the chalcone. Electron-poor aromatic rings β to the ketone give enhanced potency. Although electronic effects in the other ring (directly attached to the ketone) are minimal, this ring must possess an ortho substituent for good activity without cytotoxicity, suggesting a degree of selectivity which would not be expected for simple, nonspecific alkylating agents.

Interleukin-1 (IL-1) is a protein synthesized and released by white blood cells, most notably monocytes, in response to various injurious stimuli such as microorganisms, antigens, immune complexes, and various lymphokines. IL-1 affects many different target tissues, eliciting and amplifying the inflammatory response. An early name for IL-1, "endogenous pyrogen", was given because of its fever-inducing effects on the hypothalamus. Among other effects related to inflammation are the induction of acute phase protein release in the liver, stimulation of bone marrow resulting in neutrophilia, and enhanced expression of adhesion proteins in vascular endothelium. Fibroblasts, lymphocytes, and neutrophils are activated to release other inflammatory mediators such as prostaglandins, and chondrocytes release connective tissue degrading proteases. Synovial proliferation and bone resorption induced by IL-1 lead to joint damage and dysfunction. Because of these many effects, IL-1 is thought to be of central importance in chronic inflammatory diseases such as rheumatoid arthritis, as well as other diseases such as septic shock.[1]

Inhibition of the production or effects of such a multifunctional inflammatory mediator should produce beneficial effects in pathological conditions.[2] Several naturally occurring IL-1 receptor antagonists are known;[3] in particular, one from human monocytes has been cloned and expressed.[4,5] This material is effective in septic shock induced by bacteria or bacterial lipopolysaccharide (LPS) in animals, and in other models in which IL-1 is implicated.[6-9] Clinical trials of recombinant human IL-1 receptor antagonist are currently underway for the treatment of septic shock, rheumatoid arthritis, and several other diseases.[10]

A number of synthetic compounds have been reported to inhibit the biosynthesis or release of IL-1 from monocytes or macrophages; these have been reviewed.[11,12] Some examples are IX 207-887 (1),[13] Tenidap (2),[14] SK&F 105561 (3), and related compounds[15] RP 54745 (4),[16] E-5110 (5),[17] and E-5090 (6).[18] These compounds do not appear to be selective inhibitors of IL-1 synthesis or release, since many of them also inhibit the release of tumor necrosis factor, another proinflammatory cytokine.[19] Several of these drugs (2–6) are also mixed cyclooxygenase/5-lipoxygenase inhibitors. Other antioxidant-type compounds have been



reported to inhibit IL-1 generation,[11,20] supporting a hypothesis that 5-lipoxygenase activity is important to IL-1 biosynthesis. However, this hypothesis is challenged by recent reports showing that not all inhibitors of 5-lipoxygenase (and 5-lipoxygenase activating protein) affect IL-1 production at concentrations where leukotriene production is inhibited.[21-23]

In the course of screening for compounds which interfere with IL-1 production, we discovered that certain substituted chalcone derivatives, represented by 2',4',6'-trimethoxychalcone (7), showed potent IL-1 biosynthesis inhibitory (IBI) activity in LPS-stimulated human monocytes. We embarked on a program to explore the structure–activity relationships for this activity and sought oral efficacy in an IL-1 dependent animal model, LPS-induced septic shock in mice.

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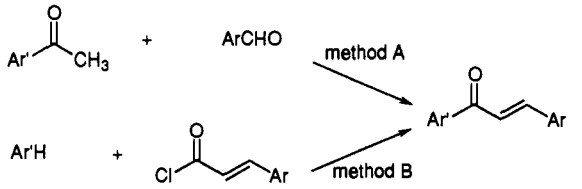
Scheme I. General Syntheses of Chalcone Derivatives



Chemistry

Chalcones are very well-known in the chemical literature.[24] We prepared a number of known and novel chalcone derivatives using two general synthetic routes (Scheme I). Most commonly, condensation of a benzaldehyde derivative with an acetophenone derivative was done in ethanol with sodium hydroxide catalyst (method A). Another general route involved the Friedel–Crafts acylation of a substituted aromatic compound with a substituted cinnamoyl chloride derivative (method B). In a number of cases, it was advantageous to modify functional groups on one of the rings after the synthesis of the chalcone derivative, for instance by esterification of the corresponding acid (method C) or conversion of an acid to an amide (method D). These methods provided access to a wide variety of compounds substituted on one or both rings, which are listed in Tables I–III. Many of these compounds had previously been reported in the literature; melting points were generally consistent with reported values.[25–49] A couple of the known compounds (**19**, **39**, **49**, and **61**) gave melting points significantly different from those reported in the literature, but spectral data and elemental analysis indicated purity and supported the structures assigned (see the supplementary material).

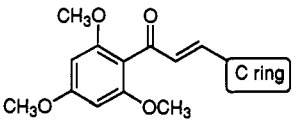
To evaluate the requirements for the enone for activity, several compounds with this moiety substituted or replaced by various reduced forms or other isosteres were prepared, as shown in Table IV.

Biology

The IBI activities of the chalcones were evaluated in human monocytes, isolated by elutriation from whole blood, stimulated to produce IL-1 by the addition of LPS.[50] After incubation with LPS and the drugs for 20 h, the supernate was removed and assayed for IL-1β by ELISA. The treated cells were also checked for viability using the MTT assay.[51] Tests were run in duplicate, and each IC_{50} value determination was performed at least three times in separate experiments, using at least five concentrations of drug. To rule out apparent inhibition due to nonspecific toxic effects, cell viability in excess of 80% was required at drug concentrations giving 50% inhibition. In vitro results are shown in Tables I–IV. In general, the standard errors were less than 25% of the IC_{50} values. Some compounds gave less reproducible results; however, this may be attributable to poor solubility in the culture medium.

Selected compounds were evaluated in vivo in the mouse septic shock model.[8] In this model, intraperitoneal injection of LPS induces the release of cytokines, including

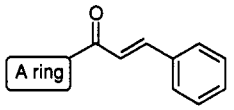
Table I. C-Ring Variation of Substituted Chalcone Derivatives



no.	C ring	method	yield, %	mp, °C	analysis[a]	IBI IC_{50}, μM[b]
7	C_6H_5	A	82	79–80.5[c]	$C_{18}H_{18}O_4$	1.25 ± 0.32
8	2-$CH_3OC_6H_4$	A	82	124–126	$C_{19}H_{20}O_5$	2.23 ± 0.85
9	3-$CH_3OC_6H_4$	A	58	76–78	$C_{19}H_{20}O_5$	1.80 ± 0.62
10	4-$CH_3OC_6H_4$	A	96	116–118[d]	$C_{19}H_{20}O_5$	4.60 ± 1.2
11	4-$N(CH_3)_2C_6H_4$	A	77	153–155[e]	$C_{20}H_{23}NO_4$	(15 ± 18%)
12	4-ClC_6H_4	A	89	132–133	$C_{18}H_{17}ClO_4$	0.970 ± 0.47
13	2,6-$Cl_2C_6H_3$	A	82	129–131	$C_{18}H_{16}Cl_2O_4$	1.09 ± 0.04
14	2,6-$F_2C_6H_3$	A	84	124–126	$C_{18}H_{16}F_2O_4$	1.29 ± 0.55
15	4-$NO_2C_6H_4$	A	87	176–178	$C_{18}H_{17}NO_6$	0.407 ± 0.047
16	3-$NO_2C_6H_4$	A	83	146–148	$C_{18}H_{17}NO_6$	0.220 ± 0.10
17	1-naphthyl	A	83	176–178	$C_{22}H_{20}O_4$	1.35 ± 0.68
18	2-naphthyl	A	87	145–146	$C_{22}H_{20}O_4$	2.15 ± 0.19
19	4-pyridyl	A	35	101–103[f]	$C_{17}H_{17}NO_4$	0.592 ± 0.12
20	3-pyridyl	A	43	123–125	$C_{17}H_{17}NO_4$	1.33 ± 0.25
21	2-pyridyl	A	57	100–102	$C_{17}H_{17}NO_4$	0.596 ± 0.16
22	pyrazyl	A	36	139–141	$C_{16}H_{16}N_2O_4$	0.693 ± 0.28
23	N-CH_3-2-imidazolyl	A	95	141–143	$C_{16}H_{18}N_2O_4$	3.80 ± 1.5
24	4-quinolyl	A	62	139–141	$C_{21}H_{19}NO_4$	0.311 ± 0.14
25	4-COOH-C_6H_4	A	50	224–226	$C_{19}H_{18}O_6$	6.23 ± 1.7
26	4-$COOCH_3$-C_6H_4	C	96	175–177	$C_{20}H_{20}O_6$	1.93 ± 0.85
27	4-$CONH_2$-C_6H_4	D	88	227.5–229.5	$C_{19}H_{19}NO_5$	1.79 ± 0.56
28	4-$CON(CH_3)_2$-C_6H_4	D	75	(amorphous)	$C_{21}H_{23}NO_5$	2.80 ± 0.42
29	4-$CONH(C_6H_5)$-C_6H_4	D	78	213–215	$C_{25}H_{23}NO_5$	0.803 ± 0.24
30	4-CONH(3-pyridyl)-C_6H_4	D	80	240 dec	$C_{24}H_{22}N_2O_5$	0.793 ± 0.11
31	4-CONH(2-thiazolyl)-C_6H_4	D	62	238.5–240	$C_{22}H_{20}N_2O_5S$	0.355 ± 0.21
32	4-$CON(CH_3)$(2-thiazolyl)-C_6H_4	(g)	95	228–231	$C_{23}H_{22}N_2O_5S$	1.01 ± 0.75
33	3-COOH-C_6H_4	A	97	177.8–180	$C_{19}H_{18}O_6$	6.76 ± 0.86
34	3-$COOCH_3$-C_6H_4	C	96	157–158	$C_{20}H_{20}O_6$	1.65 ± 0.071
35	3-$CONH(C_6H_5)$-C_6H_4	D	48	(amorphous)	$C_{25}H_{23}NO_5$	0.577 ± 0.43
36	3-CONH(3-pyridyl)-C_6H_4	D	77	(amorphous)	$C_{24}H_{22}N_2O_5$	1.63 ± 0.45
37	3-CONH(2-thiazolyl)-C_6H_4	D	75	126–129	$C_{22}H_{20}N_2O_5S$	0.503 ± 0.21

[a] Within 0.4% of calculated values. [b] Average ± SEM of at least three determinations. Numbers in parentheses are percent inhibition at 10 μM (the highest concentration assayed). [c] Literature 84 (ref 25). [d] Literature 119–121 (ref 26). [e] Literature 154 (ref 27). [f] Literature 80 (ref 28). [g] See Experimental Section for details.

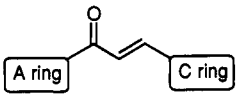
Table II. A-Ring Variation of Substituted Chalcone Derivatives



no.	A ring	method	yield, %	mp, °C	analysis[a]	IBI IC_{50}, μM[b]
7	2,4,6-$(CH_3O)_3C_6H_2$	A	82	79–80.5[a]	$C_{18}H_{18}O_4$	1.25 ± 0.32
38	3,4-$(CH_3O)_2C_6H_3$	A	72	85–87[d]	$C_{17}H_{16}O_3$	(44 ± 1%)
39	2,3-$(CH_3O)_2C_6H_3$	A	45	(oil)[e]	$C_{17}H_{16}O_3$	1.67 ± 0.31
40	3,4,5-$(CH_3O)_3C_6H_2$	A	70	79–80[f]	$C_{18}H_{18}O_4$	1.84 ± 0.35
41	2,3,4-$(CH_3O)_3C_6H_2$	A	63	70–71[g]	$C_{18}H_{18}O_4$	1.71 ± 0.53
42	2,6-$(CH_3O)_2C_6H_3$	A	90	123–124[h]	$C_{17}H_{16}O_3$	1.15 ± 0.43
43	2-$CH_3OC_6H_4$	A	16	(oil)[i]	$C_{16}H_{14}O_2$	2.23 ± 0.85
44	4-$CH_3OC_6H_4$	j				(29 ± 9%)
45	2-HOC_6H_4	A	12	89–90[k]	$C_{15}H_{12}O_2$	4.30 ± 1.9
46	4-HOC_6H_4	A	62	177–178.5[l]	$C_{15}H_{12}O_2$	6.13 ± 0.12
47	2-$C_2H_5OC_6H_4$	A	69	(oil)[m]	$C_{17}H_{16}O_2$	0.609 ± 0.18
48	4-$C_2H_5OC_6H_4$	A	58	74–76[n]	$C_{17}H_{16}O_2$	(15 ± 16%)
49	2,4,6-$(CH_3)_3C_6H_2$	A	81	(oil)[o]	$C_{18}H_{18}O$	6.50 ± 0.14
50	2,6-$Cl_2C_6H_3$	A	82	105–107	$C_{15}H_{10}Cl_2O$	1.08 ± 0.73
51	2-$C_6H_5CH_2OC_6H_4$	A	18	75–78[p]	$C_{22}H_{18}O_2$	2.23 ± 0.25
52	2-$NO_2C_6H_4$	A	59	125–126q	$C_{15}H_{11}NO_3$	1.52 ± 0.28
53	4-$NO_2C_6H_4$	A	63	145–147[r]	$C_{15}H_{11}NO_3$	(0%)
54	2-$CH_3OOCC_6H_4$	C	58	57–60	$C_{17}H_{14}O_3$	1.95 ± 0.59
55	4-$CH_3OOCC_6H_4$	A	68	132–133	$C_{17}H_{14}O_3$	(5 ± 5%)
56	2-$(CH_3)_2NCOC_6H_4$	A	75	(oil)	$C_{18}H_{17}NO_2$	5.50 ± 0.14
57	2-$HOOCC_6H_4$	A	83	145–147[s]	$C_{16}H_{12}O_3$	(37 ± 16%)
58	2-$CF_3C_6H_4$	A	39	(oil)	$C_{16}H_{11}F_3O$	0.748 ± 0.19
59	2-BrC_6H_4	A	47	(oil)	$C_{15}H_{11}BrO$	1.66 ± 0.45
60	2-$N(CH_3)_2C_6H_4$	A	43	(amorphous)	$C_{17}H_{17}NO$	1.77 ± 0.15
61	2-$CH_3CONHC_6H_4$	A	35	74–76[t]	$C_{17}H_{15}NO_2$	1.73 ± 0.21
62	2-$CH_3SO_2NHC_6H_4$	u	85	115–117	$C_{16}H_{15}NO_3S$	(0%)
63	2-$CF_3SO_2NHC_6H_4$	u	33	(amorphous)	$C_{16}H_{12}F_3NO_3S$	5.85 ± 1.8*
64	9-anthryl	B	27	201–204[v]	$C_{23}H_{16}O$	1.41 ± 0.35
65	2-thienyl	A	63	78–81[w]	$C_{13}H_{10}OS$	5.75 ± 1.0*
66	2-pyridyl	A	21	70–72[x]	$C_{14}H_{11}NO$	6.20 ± 0.85*
67	C_6H_5	j				6.28 ± 1.7*

[a] Within 0.4% of calculated values. [b] Average ± SEM of at least three determinations. Numbers in parentheses are percent inhibition at 10 μM (the highest concentration assayed); asterisk = cytotoxicity observed. [c] Literature 84 (ref 25). [d] Literature 86–87.5 (ref 29). [e] Literature 110–112 (ref 30). [f] Literature 78–78.5 (ref 31). [g] Literature 71–72 (ref 32). [h] Literature 124–125 (ref 33). [i] Literature 38–39 (ref 34). [j] Purchased from Aldrich Chemical Co., Milwaukee, WI. [k] Literature 88–89 (ref 35). [l] Literature 172–173 (ref 36). [m] Literature oil (ref 37). [n] Literature 74–75 (ref 38). [o] Literature 63 (ref 39). [p] Literature 84.5–85.5 (ref 40). [q] Literature 128–129 (ref 41). [r] Literature 149–150 (ref 42). [s] Literature 152.5–153.5 (ref 42). [t] Literature 92–93 (ref 43). [u] See Experimental Section for details. [v] Literature 201.5–202.5 (ref 44). [w] Literature 82 (ref 45). [x] Literature 71–72 (ref 46).

Table III. Miscellaneous Substituted Chalcone Derivatives



no.	A ring	C ring[a]	method	yield, %	mp, °C	analysis[b]	IBI IC_{50}, μM[c]
7	2,4,6-$(CH_3O)_3C_6H_2$	C_6H_5	A	82	79–80.5[d]	$C_{18}H_{18}O_4$	1.25 ± 0.32
68	C_6H_5	3,4-$(CH_3O)_2C_6H_3$	A	75	87–89[e]	$C_{17}H_{16}O_3$	2.33 ± 0.32*
69	C_6H_5	2,3-$(CH_3O)_2C_6H_3$	A	75	(oil)[f]	$C_{17}H_{16}O_3$	2.00 ± 0.20*
70	2-$C_2H_5OC_6H_4$	4-pyridyl	A	43	(oil)	$C_{16}H_{15}NO_2$	1.65 ± 0.07
71	3,4,5-$(CH_3O)_3C_6H_2$	4-$N(CH_3)_2C_6H_4$	A	59	149–151[g]	$C_{20}H_{23}NO_4$	(0%)
72	2-$COOHC_6H_4$	4-CONH(tz)C_6H_4	A	59	>250	$C_{20}H_{14}N_2O_4S$[h]	(22 ± 23%)
73	2-$COOCH_3C_6H_4$	4-CONH(tz)C_6H_4	C	82	194–196	$C_{21}H_{16}N_2O_4S$	2.03 ± 0.058

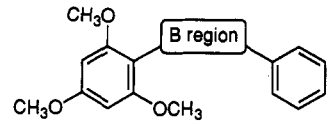
[a] tz = 2-thiazolyl. [b] Within 0.4% of calculated values. [c] Average ± SEM of at least three determinations. Numbers in parentheses are percent inhibition at 10 μM (the highest concentration assayed). Asterisk = cytotoxicity observed. [d] Literature 84 (ref 25). [e] Literature 88 (ref 47). [f] Literature oil (ref 48). [g] Literature 148–149 (ref 49). [h] C calcd 63.48, found 62.88.

IL-1, which causes fluid loss and death within 24-48 h. Human recombinant IL-1 receptor antagonist improves the survival rate from zero to about 50%, implicating IL-1 as a primary mediator of shock in this model. In vivo results, averaged from at least three experiments, are shown in Table V.

Results and Discussion

Chalcones have been reported to display a wide variety of biological activities.[52] For example, various derivatives have shown antibacterial,[53] antiviral,[54–56] gastric protectant,[57,58] antimutagenic,[59] retinoid,[60,61] antimitotic,[49,62] and antiinflammatory[63–66] activities. Interestingly, many of these compounds, particularly those with antiinflammatory activity, contain one or more phenolic hydroxyl groups. A wide assortment of phenolic natural products of various structural classes, such as flavonoids, also display many biological properties including antiinflammatory effects and 5-lipoxygenase inhibition,[67] suggesting that these effects may be related to activity as antioxidants. In view of this, it is interesting that the chalcone derivatives discussed herein do not, in general, contain phenolic

Table IV. Variation in Enone Moiety (B Region)



no.	B region	method	yield, %	mp, °C	analysis[a]	IBI IC_{50}, μM[b]
7	C(=O)CH=CH	A	82	79–80.5[c]	$C_{18}H_{18}O_4$	1.25 ± 0.32
74	C(=O)CH_2CH_2	B	70	(oil)	$C_{18}H_{20}O_4$	(0%)
75	CH(OH)CH=CH	d	99	87–89	$C_{18}H_{20}O_4$	5.40 ± 0.81
76	CH_2CH=CH	d	25	60.5–62.5	$C_{18}H_{20}O_3$	(0%)
77	C(=NOH)CH=CH	d	29	220–222	$C_{18}H_{19}NO_4$	(34 ± 1%)
78	NHC(=O)CH=CH	d	75	174–175	$C_{18}H_{19}NO_4$	(0%)
79	C(=O)NHCH_2	d	17	111–113	$C_{17}H_{19}NO_4$	(0%)
80	C(=O)-p-C_6H_4	B	63	138–140	$C_{22}H_{20}O_4$	(26 ± 5%)
81	C(=O)CH=C(Ph)	B	74	128–129	$C_{24}H_{22}O_4$	(0%)
82	C(=O)CH=C(CH_3)	B	67	124–126	$C_{19}H_{20}O_4$	(0%)
83	C(=O)C(CH_3)=CH	A	28	154–155	$C_{19}H_{20}O_4$	5.17 ± 1.2

[a] Within 0.4% of calculated values. [b] Average ± SEM of at least three determinations. Numbers in parentheses are percent inhibition at 10 μM. [c] Literature 84 (ref 25). [d] See Experimental Section for details.

Table V. In Vivo Activity for Selected Substituted Chalcones

no.	IBI IC_{50}, μM	septic shock % survival at 600 mg/kg
30	0.793 ± 0.11	50
31	0.355 ± 0.21	55
54	1.95 ± 0.59	20
73	2.03 ± 0.058	30

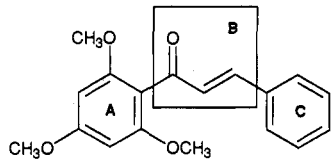


Figure 1.

hydroxyl groups. However, because of this extensive literature, we wanted to explore the structure–activity relationships for these compounds to rule out nonspecific effects. In particular, since chalcones contain an electrophilic enone moiety, we wanted to determine if these compounds were simply acting to alkylate biological nucleophiles by conjugate addition to the enone. Early evidence against a nonspecific toxic effect was provided by the lack of cytotoxicity of 7 at concentrations 10-fold higher than the IBI IC_{50}. In contrast, unsubstituted chalcone (67) did appear to be toxic to cells; although 50% inhibition of IL-1 release was seen at 6.28 μM, this inhibition was accompanied by severe loss of cell viability as measured by the MTT assay, indicating that the observed inhibition of 67 was probably due to cell morbidity.

To evaluate the structure–activity relationships, the effects of structural changes in three regions of the molecule were considered (Figure 1). The enone linkage (B) was of particular interest, since this moiety is capable of acting as an electrophilic alkylating agent. As shown in Table IV, the enone appears to be required for activity. Reduction to the dihydrochalcone 74 or replacement of the ketone by a methylene (76) abolished activity completely. Reduction to the allylic alcohol 75 reduced potency, but activity was still seen; in this case, oxidation back to the chalcone 7 in the cell culture may account for the activity. The oxime 77 and the cinnamide 78 were also inactive, as was the benzylamide 79 and the biphenyl 80. Substitution at the α position of the olefin (83) was consistent with activity (although with reduced potency), while β-substitution (81, 82) obliterated activity. These findings provided further evidence for the necessity of alkylation, via conjugate addition, of a biological nucleophile for IBI activity, since substitution at the β position would be expected to provide steric hinderance to such alkylation. Variation in the C ring (Table I) gave additional support to the possibility of conjugate addition as an integral part of the mechanism of action of these compounds. Electron-donating groups tended to weaken the IBI activity (compare 10 and 11 with 7), while electron-withdrawing groups increased the potency (12, 15, and 16). The same trend was seen with heterocyclic C rings (compare 23 with 19, 21, 22, and 24). This was consistent with conjugate addition, since electron-poor rings increase the electrophilicity of the chalcone.[68-70] There appeared to be little steric or positional specificity (compare 8, 9, and 10; 15 and 16; 17 and 18).

In attempts to gain better water solubility and in vivo activity, polar functionality was introduced into the C ring to decrease the considerable lipophilicity of these molecules. (Compound 7, for example, has a calculated[71] log P value of 4.13.) Carboxylic esters and amides (26–28, 34) were generally about as active as 7, but the corresponding carboxylic acids were weaker (25, 33). N-Aryl amides (29–32, 35–37) generally showed better potency than simple amides. These results suggest an inverse relationship between potency and polarity in the C ring: the absence of increased potency in the esters and simple amides (expected on electronic grounds) may be due to the increased hydrophilicity of these groups.

Variation in the A ring (see Table II) showed an interesting structure–activity correlation. Examination of the methoxy derivatives 38–44 revealed that, with the exception of 40, only those compounds possessing an ortho substituent showed good activity; isomers lacking at least one o-methoxy showed IBI IC_{50} values greater than 10 μM. This requirement for ortho substitution was particularly striking for monosubstituted cases, such as ethoxy (47 and 48), nitro (52 and 53), and carbomethoxy (54 and 55). The hydroxy analogs 45 and 46 were an exception to this rule, since both the ortho and para isomers appeared equally active (although less potent). This might be due to some additional less specific effect such as antioxidant activity, since several redox-active 5-lipoxygenase inhibitors have been shown to display IBI activity as discussed above.[11,20]

One possible reason for this specificity was a requirement for chelation of some metal ion by the ortho substituent and the ketone. However, this was ruled out by the fact

that three compounds incapable of forming a chelate (49, and particularly 50 and 64) retained IBI activity. Also, heterocyclic analogs which should be capable of chelation (65 and 66) showed no IBI activity not attributable to cytotoxicity.

Another explanation of the requirement for an ortho substituent is a steric one, since ortho substitution should induce orthogonality between the A ring and the enone. This is supported by UV spectral data (in $CHCl_3$ solution) for the pair of ethoxy-substituted derivatives: 47 (which is active) had λ_{max} = 304 nm, log ϵ_{max} = 4.31, while 48 (inactive) had λ_{max} = 319 nm, log ϵ_{max} = 4.49. Clearly the ortho-substituted analog (47) shows reduced conjugation with respect to the para-substituted analog (48). Further support was gained from AM1 calculations[72] (performed using MOPAC on a Tektronix CAChe modelling system) on dimethyl-substituted analogs. 2',6'-Dimethylchalcone yielded an optimized geometry with the dihedral angle between the enone and the A ring of 70° (ΔH_f = 21.0 kcal/mol), while the 3',5'-isomer showed a nearly planar system (dihedral angle 2°; ΔH_f = 18.6 kcal/mol). It is not known what effect this conformational change would have on the ability of the chalcone to act as a conjugate addition acceptor; competition studies are in progress to address this point.

The electronic nature of the ortho substituent on the A ring seemed to have a relatively small effect on the IBI activity in general (47 and 52, 58–61). Highly polar or acidic groups in the ortho position appear to be detrimental to activity, as demonstrated by 45, 57, and 62, which were weak or inactive, and 63 which was cytotoxic. Literature evidence indicates that electronic effects in the A ring should affect susceptibility to conjugate addition in the same manner as seen in the C ring,[68] but since the ortho substituent reduced the transmission of electronic influences through alteration of the A-ring conformation, such effects should be minimal. It is of interest that both the electron-rich thiophene (65) and the electron-poor pyridine (66) were toxic/inactive, indicating that the electronic effects were overridden by the lack of an ortho substituent in these cases.

A few compounds were prepared in which both the A and B rings of 7 were altered (see Table III). Compounds 68 and 69 showed that the ortho substituent effect on activity is selective for the A ring, since both these analogs are toxic. No additivity in potency was seen when the A ring of 47 and the C ring of 19, both having submicromolar potency, were incorporated into the same molecule (70).

The mechanism of IL-1 biosynthesis inhibition by these compounds is currently unknown. A number of the analogs were evaluated for inhibition of 5-lipoxygenase (up to 25 μM) and cyclooxygenase (up to 750 μM), using methods published elsewhere.[73] Unlike some of the other reported compounds with IBI activity (such as 2, 3, 5, and 6), none of the chalcones evaluated showed significant inhibition of either enzyme at concentrations where IL-1 release was affected. In common with other reported IBI compounds, the release of TNF from monocytes stimulated with LPS was also inhibited, generally with a potency 3–10-fold less than that seen for IBI activity. This finding implies that the point of inhibition must be prior to processing and exporting of mature IL-1, possibly in a cell activation step or the regulation of transcription of the IL-1 and TNF genes. (5-Lipoxygenase, cyclooxygenase, and TNF data for selected compounds are listed in the supplementary material.) Interestingly, 71, which is an antimitotic agent in HeLa cells, was completely inactive, suggesting that the chalcones were not acting by inhibiting microtubule function.[49]

Selected compounds were evaluated in the mouse LPS-induced septic shock model, which is thought to be IL-1 dependent based on inhibition by the naturally-occurring IL-1 receptor antagonist (see Table V). Although significant inhibition of the LPS-induced mortality was seen with these compounds, the doses required for this effect (600 mg/kg po) were very high. The low potency observed in vivo may reflect poor bioavailability due to metabolism. Chalcones have been reported to undergo extensive metabolism by many routes, such as reversible conjugate addition of glutathione, demethylation of methoxy groups, oxidation of aromatic rings, and reduction of the enone moiety.[74–77]

Conclusion

A series of 2'-substituted chalcone derivatives has shown potent inhibition of IL-1 release from LPS-stimulated human monocytes. The enone moiety was required for activity, suggesting that alkylation of a biological nucleophile via conjugate addition is involved in the mechanism of action. The C ring tolerates a wide variety of substituents, but for best activity this ring must be electron poor, further supporting conjugate addition as integral to the mechanism of action. Substitution by acidic or highly polar groups on either ring is disfavored. Electronic effects on activity were much less in the A ring, but an ortho substituent appeared to be almost absolutely required for activity. This substitution presumably induces a conformational change in the molecule, forcing the A ring to be orthogonal to the enone. This apparent shape specificity, along with the lack of cell toxicity at IBI active concentrations, implies that inhibition of IL-1 release by these active analogs is not due to a simple nonspecific alkylation. Perhaps the shape is required for a fit into a receptor or enzyme, which is then alkylated by the electrophilic enone. Selected analogs showed significant activity in LPS-induced septic shock, but the potency was poor, possibly due to extensive metabolism. While this series is not of interest for further development, it represents a novel class of inhibitors of IL-1 production from inflammatory cells.

Experimental Section

^{1}H NMR spectra were recorded on a Varian Gemini 300 spectrometer (300 mHz), using Me_4Si as an internal standard, in the solvents shown. Infrared spectra were recorded as neat films, mineral oil mulls, or KBr pellets on a Perkin-Elmer 1710 FT spectrometer. Mass spectral data was recorded on a Finnigan-MAT 8239 instrument, using chemical ionization with ammonia as reagent gas. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ, and were within 0.4% of the calculated values. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Other commercial reagents and solvents were used as received without further purification. All reactions were carried out under a nitrogen atmosphere. Organic phases were dried over anhydrous $MgSO_4$ and concentrated on a Buchi rotary evaporator at aspirator pressure. Chromatography was done using the medium-pressure flash method.[78] LPS (*Salmonella typhemurium*) for the in vitro assay was purchased from Calbiochem, La Jolla, CA. LPS (*Escherichia coli*, serotype 0127;B8) for the in vivo assay was purchased from Sigma Chemical Co., St. Louis, MO. C57B1/6 mice were obtained from Charles River Laboratories, Wilmington, MA. The recombinant human

IL-1 receptor antagonist was produced in *E. coli*, purified by standard chromatographic methods[79] by the Biotechnology Group at The Du Pont Merck Pharmaceutical Co., and shown to contain less than 10 EU/mg of endotoxin.

Method A. 2,2',4',6'-Tetramethoxychalcone (8). A solution of 2',4',6'-trimethoxyacetophenone (5.25 g, 25.0 mmol), 2-anisaldehyde (3.50 g, 25.0 mmol), and NaOH (0.59 g, 14.8 mmol) in ethanol (100 mL) was stirred at room temperature for 24 h. The resulting precipitate was collected by filtration, washed with ethanol, and dried to provide 8 (6.70 g, 82%) as a yellow powder: mp 124–126 °C (ethanol); NMR ($CDCl_3$) δ 7.70 (d, 1 H), 7.53 (d, 1 H), 7.33 (t, 1 H), 7.04 (d, 1 H), 6.92 (m, 2 H), 6.16 (s, 2 H), 3.85 (s, 3 H), 3.83 (s, 3 H), 3.76 (s, 6 H); IR (KBr) 1640 cm^{-1}; MS *m/z* 329 (M + H^+, 100). Anal. ($C_{19}H_{20}O_5$) C, H.

According to method A, other analogs were prepared as summarized in Tables I and II. Starting materials were commercially available except for 2-formylpyrazine[80] for **22**, *N*-methylimidazole-2-carboxaldehyde[81] for **23**, 3-formylbenzoic acid[82] for **33**, 2',3'-dimethoxyacetophenone[83] for **39**, 2'-ethoxyacetophenone[84] for **47** and **70**, methyl 4-acetylbenzoate[85] for **55**, 2-acetyl-*N*,*N*-dimethylbenzamide[86] for **56**, *N*,*N*-dimethyl-2-acetylaniline[87] for **60**, and 2',4',6'-trimethoxypropiophenone[88] for **83**.

Method C. Methyl 2',4',6'-Trimethoxychalcone-4-carboxylate (26). A solution of **25** (1.71 g, 5.00 mmol) in methanol (50 mL) was treated with concentrated H_2SO_4 (15 drops) and heated at reflux for 16 h. The solution was cooled to room temperature, diluted with water, and made basic with saturated aqueous $NaHCO_3$. The resulting creamy yellow precipitate was collected by filtration, washed with water, and dried to provide **26** (1.72 g, 96%): mp 175–177 °C (1-chlorobutane); NMR ($CDCl_3$) δ 8.03 (d, 2 H), 7.58 (d, 2 H), 7.40 (d, 1 H), 7.03 (d, 1 H), 6.16 (s, 2 H), 3.93 (s 3 H), 3.87 (s, 3 H), 3.78 (s, 6 H); IR (KBr) 1710 cm^{-1}; MS *m/z* 357 (M + H^+, 100). Anal. ($C_{20}H_{20}O_6$) C, H.

Method D. 2',4',6'-Trimethoxychalcone-4-carboxamide (27). An ice-cold solution of **25** (1.71 g, 5.00 mmol) in CH_2Cl_2 (35 mL) was treated with oxalyl chloride (0.45 mL, 5.10 mmol) and then with *N*,*N*-dimethylformamide (4 drops). Gas evolution ensued, and the mixture became an orange solution. After 3 h, the solution was poured into ice-cold, rapidly-stirred aqueous ammonia (350 mL). After 30 min, the CH_2Cl_2 was removed on a rotary evaporator. The resulting solid was isolated by filtration, washed with water, and dried to provide **27** (1.51 g, 88%) as a bright yellow solid: mp 227.5–229.5 °C (CH_3CN); NMR ($CDCl_3$) δ 7.81 (d, 2 H), 7.60 (d, 2 H), 7.40 (d, 1 H), 7.02 (d, 1 H), 6.16 (s, 2 H), 6.07 (bs, 1 H), 5.60 (bs, 1 H), 3.87 (s, 3 H), 3.78 (s, 6 H); IR (KBr) 3388, 1689, 1640, 1607 cm^{-1}; MS *m/z* 342 (M + H^+, 100). Anal. ($C_{19}H_{19}NO_5$) C, H, N.

Method D. 2',4',6'Trimethoxy-*N*-(3-pyridyl)chalcone-4-carboxamide (30). The acid chloride of **25** (2.05 g, 6.00 mmol) was prepared as described for **27** and concentrated. It was redissolved in THF (30 mL) and added rapidly to a solution of 3-aminopyridine (0.57 g, 6.00 mmol) and triethylamine (0.92 mL, 6.60 mmol) in THF (30 mL). The mixture was stirred at room temperature for 16 h and filtered. The solid was boiled in aqueous $NaHCO_3$ for 15 min and collected by filtration. After washing with water and drying, the solid was boiled in methanol (200 mL) for 30 min. The slurry was filtered while warm, and the solid was dried to provide **30** (2.03 g, 80%) as a bright yellow powder: mp ca. 240 °C dec; NMR (DMSO-d_6) δ 10.52 (s, 1 H), 8.93 (d, 1 H), 8.32 (d, 1 H), 8.18 (m, 1 H), 8.00 (d, 2 H), 7.86 (d, 2 H), 7.40 (m, 1 H), 7.30 (d, 1 H), 7.11 (d, 1 H), 6.33 (s, 2 H), 3.85 (s, 3 H), 3.73 (s, 6 H); IR (KBr) 3343, 1677, 1657, 1606, 1592 cm^{-1}; MS *m/z* 419 (M + H^+, 100). Anal. ($C_{24}H_{22}N_2O_5$) C, H, N.

2',4',6'-Trimethoxy-*N*-(2-thiazolyl)-*N*-methylchalcone-4-carboxamide (32). Sodium hydride (60% in mineral oil; 40 mg, 0.98 mmol) was stirred with hexane and the solvent removed by pipet. It was resuspended in THF (5 mL). Compound **31** (350 mg, 0.820 mmol) was added as a solid, and the mixture was stirred at room temperature for 10 min, when an orange solution was obtained. CH_3I (0.3 mL, 4.9 mmol) was added, and the solution was warmed to reflux. After 22 h, the mixture (now a yellow slurry) was cooled to room temperature and diluted with water. The initially formed gummy solid formed a fine powder on continued stirring. The solid was isolated by filtration, washed with water, and dried to provide **32** (342 mg, 95%) as a bright yellow powder: mp 228–231 °C; NMR ($CDCl_3$) δ 8.33 (d, 2 H), 7.59 (d, 2 H), 7.42 (d, 1 H), 7.03 (m, 2 H), 6.72 (d, 1 H), 6.17 (s, 2 H), 3.86 (s, 6 H), 3.78 (s, 6 H); IR (KBr) 1599 cm^{-1}; MS *m/z* 439 (M + H^+, 100). Anal. ($C_{23}H_{22}N_2O_5S$) C, H, N, S.

2'-((Methylsulfonyl)amino)chalcone (62). A solution of 2'-aminochalcone[89] (1.47 g, 6.60 mmol) and methanesulfonyl chloride (0.75 g, 6.60 mmol) in pyridine (20 mL) was heated at 85 °C for 2 h. The cooled mixture was partitioned between ethyl acetate and 1 N HCl. The organic phase was washed with aqueous $CuSO_4$, dried, and concentrated. The residue was chromatographed (10% ethyl acetate/toluene) to provide **62** (1.68 g, 85%) as a yellow solid: mp 115–117 °C; NMR ($CDCl_3$) δ 11.23 (bs, 1 H), 8.03 (d, 1 H), 7.85 (d, 1 H), 7.80 (d, 1 H), 7.7–7.55 (m, 4 H), 7.44 (m, 3 H), 7.21 (t, 1 H), 3.08 (s, 3 H); IR (KBr) 1640, 1581, 1574 cm^{-1}; MS *m/z* 302 (M + H^+, 33), 319 (M + NH_4^+, 100). Anal. ($C_{16}H_{15}NO_3S$) C, H, N, S.

2'-(((Trifluoromethyl)sulfonyl)amino)chalcone (63). A solution of 2'-aminochalcone[89] (0.96 g, 4.30 mmol) and triethylamine (0.60 mL, 4.3 mmol) in CH_2Cl_2 (17 mL) at −78 °C was treated dropwise with trifluoromethanesulfonic anhydride (0.72 mL, 4.3 mmol). The mixture was stirred at −78 °C for 1.5 h and then at room temperature for 2.5 h. The reaction was partitioned between ethyl acetate and pH 7.0 buffer. The aqueous phase was extracted with ethyl acetate, and the combined organic phases were dried and concentrated. The residue was chromatographed (5% methanol/CH_2Cl_2) to provide **63** (0.50 g, 33%) as an amorphous solid: NMR ($CDCl_3$) δ 12.23 (bs, 1 H), 8.06 (d, 1 H), 7.91 (d, 1 H), 7.83 (d, 1 H), 7.7–7.6 (4 H), 7.46 (m, 3 H), 7.33 (t, 1 H); IR (KBr) 1642, 1587, 1576 cm^{-1}; MS *m/z* 356 (M + H^+, 27), 373 (M + NH_4^+, 54). Anal. ($C_{16}H_{12}F_3NO_3S$) C, H, N, S.

4-(2-Thiazolylcarbamoyl)chalcone-2'-carboxylic Acid (72). An ice-cold suspension of 4-carboxybenzaldehyde (12.00 g, 80.0 mmol) in CH_2Cl_2 (240 mL) and *N*,*N*-dimethylformamide (1 mL) was treated with oxalyl chloride (7.68 mL, 88.0 mmol) whereupon vigorous gas evolution began. The mixture was stirred while warming to room temperature. After 24 h, the mixture was concentrated, and the residue was dissolved in THF (80 mL) and added with stirring to an ice-cold solution of 2-aminothiazole (8.00 g, 80.0 mmol) and triethylamine (16.8 mL, 120 mmol) in THF (80 mL). After 6 h, the mixture was filtered. The filtrate was concentrated, and the residue was slurried with THF (40 mL) and filtered. The combined tan solids were stirred in water (350 mL), collected by filtration, and dried to provide **4-formyl-*N*-(2-thiazolyl)benzamide (84)** (13.46 g, 72%): mp 192–197 °C; NMR ($CDCl_3$) δ 10.15 (s, 1 H), 8.19 (d, 2 H), 8.05 (d, 2 H), 7.02 (m, 2 H), 1.60 (bs, 1 H); IR (mull) 1690, 1670 cm^{-1}; MS *m/z* 233 (M + H^+, 100). Anal. ($C_{11}H_8N_2O_2S$) C, H, N, S. This material (6.97 g, 30.0 mmol) and 2-acetylbenzoic acid (4.92 g, 30.0 mmol) were added to a solution of NaOH (3.00 g, 75.0 mmol) in methanol (75 mL). The mixture was heated at reflux for 7 h and then cooled to room temperature. Acidification with concentrated HCl (to pH 2) provided a dense precipitate which was collected by filtration, washed with water, and dried. The resulting solid was recrystallized from acetic acid to provide **72** (6.67 g, 59%) as tan crystals: mp >250 °C; NMR (DMSO-d_6) δ 8.11 (d, 2 H), 7.93 (d, 1 H), 7.85 (d, 2 H), 7.65 (dt, 2 H), 7.55 (d, 1 H), 7.51 (d, 1 H), 7.30 (m, 3 H); IR (KBr) 2476 (broad), 1662, 1562 cm^{-1}; MS *m/z* 379 (M + H^+, 100); HRMS calcd 379.0752, found 379.0747. Anal. ($C_{20}H_{14}N_2O_4S$) H, N, S; C: calcd 63.48; found: 62.88.

Method B. 2',4',6'-Trimethoxy-3-phenylpropiophenone (74). A solution of 1,3,5-trimethoxybenzene (1.68 g, 10.0 mmol) in CH_2Cl_2 (100 mL) was stirred at −5 °C and treated with $AlCl_3$ (1.33 g, 10.0 mmol). A solution of hydrocinnamoyl chloride (1.69 g, 10.0 mmol) in CH_2Cl_2 (50 mL) was added over 30 min. When addition was complete, water was added and the mixture was stirred for 5 min. The layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with 1 N NaOH, dried, and concentrated. The residue was chromatographed (20% ethyl acetate/hexane) to provide **74** (2.10 g, 70%) as an oil: NMR ($CDCl_3$) δ 7.3–7.1 (m, 5 H), 6.08 (s, 2 H), 3.82 (s, 3 H), 3.75 (s, 6 H), 3.04 (m, 4 H); IR ($CHCl_3$) 1694 cm^{-1}; MS *m/z* 301 (M + H^+, 100). Anal. ($C_{18}H_{20}O_4$) C, H.

According to method B, other analogs were prepared as summarized in Table II. Starting materials were commercially available except for 3-phenylcinnamoyl chloride[90] for **81** and 3-methylcinnamoyl chloride[91] for **82**.

1-(2,4,6-Trimethoxyphenyl)-3-phenylpropenol (75). A solution of phenylacetylene (2.55 g, 25.0 mmol) in THF (35 mL) at −78 °C was treated dropwise with *n*-butyllithium (1.6 M in hexane; 15.6 mL, 25.0 mmol). After stirring at −78 °C for 30 min, the heterogeneous mixture was treated with *N,N,N',N'*-tetramethylethylenediamine (7.6 mL, 50 mmol), whereupon it became homogeneous. After stirring for 15 min, 2,4,6-trimethoxybenzaldehyde (4.90 g, 25.0 mmol) was added. The mixture was stirred at −78 °C for 20 min, warmed to −50 °C, and stirred for 60 min. The reaction was quenched with 1 M phosphate buffer (pH 7.0) and extracted with ethyl acetate. The organic phase was dried and concentrated. The residue was triturated in ethyl acetate/hexane to provide **1-(2,4,6-trimethoxyphenyl)-3-phenylpropyn-1-ol (85)** (6.33 g, 85%) as tan needles: mp 98.8–99.9 °C (1-chlorobutane/hexane); NMR ($CDCl_3$) δ 7.40 (m, 2 H), 7.25 (m, 3 H), 6.16 (s, 2 H), 6.02 (d, 1 H), 3.90 (d, 1 H), 3.88 (s, 6 H), 3.81 (s, 3 H); IR (KBr) 3542 cm^{-1}; MS *m/z* 299 ($M + H^+$, 33). Anal. ($C_{18}H_{18}O_4$) C, H. This material (596 mg, 2.00 mmol) was dissolved in THF (10 mL), treated with $LiAlH_4$ (1.0 M in THF; 2.40 mL, 2.40 mmol), and heated at reflux for 4 h. The mixture was cooled to room temperature and treated sequentially with water (0.1 mL), 15% aqueous NaOH (0.1 mL), and water (0.3 mL). The suspension was diluted with ether, filtered, dried, and concentrated to give **75** (0.60 g, 99%) as a yellow oil which gradually crystallized: mp 87–89 °C (1-chlorobutane/hexane); NMR ($CDCl_3$) δ 7.4–7.1 (5 H), 6.52 (dd, 2 H), 6.16 (s, 2 H), 5.74 (dd, 1 H), 3.84 (s, 6 H), 3.82 (s, 3 H), 3.8 (bm, 1 H); IR (KBr) 3553, 1607 cm^{-1}; MS *m/z* 301 ($M + H^+$, 52), 283 ($M + H^+ - H_2O$, 100). Anal. ($C_{18}H_{20}O_4$) C, H.

1-Phenyl-3-(2,4,6-trimethoxyphenyl)propene (76). A solution of 1,3,5-trimethoxybenzene (1.68 g, 10.0 mmol) in CH_2Cl_2 (10 mL) was stirred at 0 °C and treated sequentially with $AlCl_3$ (1.33 g, 10.0 mmol) and a solution of cinnamoyl chloride (1.52 g, 10.0 mmol) in CH_2Cl_2 (5 mL). After 60 min, the mixture was poured into 1 N HCl, and the phases were separated. The aqueous phase was extracted with CH_2Cl_2, and the combined organic phases were dried and concentrated. The residue was chromatographed (50% 1-chlorobutane/hexane) to provide **76** (720 mg, 25%) as a colorless oil which gradually crystallized: mp 60.5–62.5 °C (hexane); NMR ($CDCl_3$) δ 7.30 (d, 2 H), 7.22 (d, 2 H), 7.13 (t, 1 H), 6.32 (m, 2 H), 6.15 (s, 2 H), 3.81 (s, 9 H), 3.47 (d, 2 H); IR (KBr) 1596 cm^{-1}; MS *m/z* 285 ($M + H^+$, 67). Anal. ($C_{18}H_{20}O_3$) C, H.

2',4',6'-Trimethoxychalcone Oxime (77). A mixture of **7** (1.49 g, 5.00 mmol), hydroxylamine hydrochloride (0.35 g, 5.00 mmol), ethanol (25 mL), and 0.5 N phosphate buffer (pH 7.0; 25 mL) was heated at 60 °C for 48 h. The mixture was cooled and diluted with 1 N NaOH, and extracted with CH_2Cl_2. The organic phase was dried and concentrated, and the residue recrystallized from CH_2Cl_2/hexane to provide **77** (0.46 g, 29%): mp 220–222 °C; NMR ($CDCl_3$) δ 7.74 (d, 1 H), 7.46 (m, 2 H), 7.29 (m, 3 H), 6.47 (d, 1 H), 6.20 (s, 2 H), 3.87 (s, 3 H), 3.76 (s, 6 H); IR (KBr) 3200, 1608 cm^{-1}; MS *m/z* 314 ($M + H^+$, 100). Anal. ($C_{18}H_{19}NO_4$) C, H, N.

2,4,6-Trimethoxy-*N*-cinnamoylaniline (78). A solution of 2,4,6-trimethoxyaniline (2.75 g, 15.0 mmol) and triethylamine (2.90 mL, 20.0 mmol) in THF (50 mL) was cooled to 0 °C and treated dropwise with a solution of cinnamoyl chloride (2.50 g, 15.0 mmol) in THF (25 mL). The solution was stirred at room temperature for 60 min and then was partitioned between water and ethyl acetate. The aqueous phase was extracted with ethyl acetate, and the combined organic layers were dried and concentrated. The residue was chromatographed (40% ethyl acetate/hexane; then ethyl acetate), and the isolated crude product was triturated with hexane/ethyl acetate to provide **78** (3.52 g, 75%) as a solid: mp 174–175 °C; NMR ($CDCl_3$) δ 7.71 (d, 1 H), 7.50 (m, 2 H), 7.34 (m, 3 H), 6.73 (bs, 1 H), 6.63 (d, 1 H), 6.17 (s, 2 H), 3.82 (s, 9 H); IR (KBr) 1666 cm^{-1}; MS *m/z* 314 ($M + H^+$, 100). Anal. ($C_{18}H_{19}NO_4$) C, H, N.

***N*-Benzyl-2,4,6-trimethoxybenzamide (79).** 2,4,6-Trimethoxybenzoic acid (1.06 g, 5.00 mmol) was converted to the acid chloride as described for method D. This was dissolved in CH_2Cl_2 (10 mL) and added dropwise at 0 °C to a solution of benzylamine (0.55 mL, 5.00 mmol) in CH_2Cl_2 (10 mL). After stirring at room temperature for 16 h, the mixture was poured into water, the layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The combined extracts were dried and concentrated. The residue was chromatographed (50% ethyl acetate/toluene) to provide **79** (250 mg, 17%) as a white solid: mp 111–113 °C (1-chlorobutane); NMR ($CDCl_3$) δ 7.4–7.2 (5 H), 6.11 (s, 2 H), 6.02 (bs, 1 H), 4.66 (d, 2 H), 3.82 (s, 3 H), 3.81 (s, 6 H); IR (KBr) 3360, 1643, 1521 cm^{-1}; MS *m/z* 302 ($M + H^+$, 100). Anal. ($C_{17}H_{19}NO_4$) C, H, N.

LPS-Induced IL-1 Release From Monocytes. Normal human blood was layered over Ficoll-Hypaque and centrifuged to isolate the leukocytes.[92] These cells were further separated by elutriation,[93] and the monocyte population was used. Monocytes were plated in 1 mL of RPMI 1640 with 5% fetal bovine serum in a 12-well tissue culture plate at 2×10^6 cells/well. Test compounds were diluted in DMSO to the appropriate concentration, and the drug solution or DMSO alone (1.0 μL) was added to the monocytes. The cells were incubated for 60 min, and then LPS (*Salmonella typhemurium*) in RPMI 1640 buffer (1.0 μg/mL stock; 20 μL/well) was added to stimulate IL-1 production.[50] After 20 h, the supernates were removed from the cells and assayed for IL-1β by ELISA.[94] The cells remaining in the wells were tested for viability using the MTT assay.[51] Each drug concentration was tested in duplicate, and the ELISA assay was run in duplicate for each drug concentration. IC_{50} values were calculated from percent inhibition of control levels of IL-1 using at least five drug concentrations. The IC_{50} values reported are the means of at least three separate experiments.

LPS-Induced Septic Shock in Mice. This model is based on data reported by Alexander et al.[8] Test compounds were formulated in 0.25% methylcellulose and beadmilled for 60 min. Groups of 10 female C57B1/6 mice, weighing 18–20 g, were dosed intraperitoneally with LPS (reconstituted in 0.9% saline; 40 mg/kg). After 20 min, the animals were dosed with the test compound or with formulating solvent alone by oral gavage (0.1 mL/g body weight). Recombinant human IL-1 receptor antagonist was dosed by tail vein injection. Dosing was continued every 4 h for a total of 24 h. Animal deaths were recorded every 6–8 h after the last administered dose of test compound for 1 day, and then every day for 2 additional days. Lethality in the solvent control animals occurred at 24–48 h, at which time surviving animals were stable and recovering. Percent survival was calculated and the significance determined by χ^2 analysis at the $p < 0.05$ level. Reported values are averaged of three separate experiments.

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Supplementary Material Available: Characterization data for compounds **19**, **39**, **49**, and **61**, as well data for selected compounds for inhibition of 5-lipoxygenase, cyclooxygenase, and the release of TNF (2 pages). Ordering information is given on any current masthead page.

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