Novel Antiarthritic Agents with 1,2-Isothiazolidine-1,1-dioxide (γ-Sultam) Skeleton: Cytokine Suppressive Dual Inhibitors of Cyclooxygenase-2 and 5-Lipoxygenase

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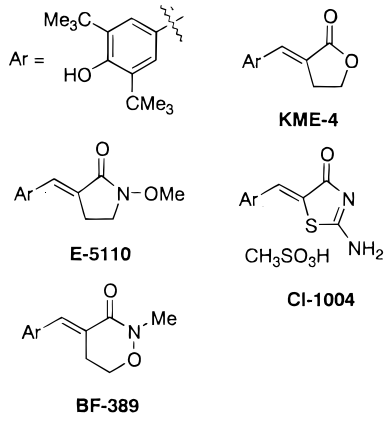
Various 1,2-isothiazolidine-1,1-dioxide (γ-sultam) derivatives containing an antioxidant moiety, 2,6-di-*tert*-butylphenol substituent, were prepared. Some compounds, which have a lower alkyl group at the 2-position of the γ-sultam skeleton, showed potent inhibitory effects on both cyclooxygenase (COX)-2 and 5-lipoxygenase (5-LO), as well as production of interleukin (IL)-1 in in vitro assays. They also proved to be effective in several animal arthritic models without any ulcerogenic activities. Among these compounds, (*E*)-(5)-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-2-ethyl-1,2-isothiazolidine-1,1-dioxide (S-2474) was selected as an antiarthritic drug candidate and is now under clinical trials. The structure−activity relationships (SAR) examined and some pharmacological evaluations are described.

Introduction

NSAIDs (nonsteroidal antiinflammatory drugs) are commonly prescribed in drug therapy for RA (rheumatoid arthritis). Currently available NSAIDs are thought to inhibit both isoforms of a constitutive form of COXs (cyclooxygenases), COX-1, and an inducible form, COX-2, to offer antiinflammatory therapeutic effects.[1] However, NSAIDs generally cause adverse effects, especially gastrointestinal ulceration,[2] because COX-1 is believed to be responsible for the synthesis of cytoprotective prostaglandins (PGs) in the gastrointestinal tract.[3] COX-2, on the other hand, is induced by many kinds of inflammatory mediators and plays an important role in prostaglandin biosynthesis associated with inflammatory responses.[4] Therefore, NSAIDs having selective COX-2 inhibitory activity should be more useful for the treatment of inflammatory diseases.[5] NSAIDs which have dual inhibitory activities against COX and 5-lipoxygenase (5-LO) such as KME-4,[6] E-5110,[7] BF-389,[8] and Cl-1004[9] (Chart 1) are hypothesized to be superior and are being studied as potential antiinflammatory agents with an improved safety profile in comparison with simple COX inhibitors because leukotrienes (LTs), produced through the 5-LO enzyme pathway, may contribute to both inflammation and NSAID-induced side effects.[10]

Methotrexate, the clinical efficacy of which is thought to be related to its inhibitory activities on the production of inflammatory cytokines such as IL-1, IL-6, and TNF-α[11] has been used as the first choice in drug therapy for RA. However, the possibility of drug side effects and difficulties in determining the adequate dosage demand particular caution and careful monitoring of patients.

Chart 1



From the pharmaco-economic viewpoint and the need for better compliance of patients with RA, an antiarthritic drug which can act as both NSAID and DMARD (disease modifying antirheumatic drug) with lesser adverse effects is desirable. The first promising agent was tenidap,[12] but its development had to be suspended due to some adverse effects.[13] A drug which has multiple inhibitory effects on both COX, especially COX-2, and 5-LO, as well as on cytokine production may display therapeutic advantages over NSAIDs in treating RA.

To develop an alternative antiarthritic agent of tenidap-like profile, we conducted exploratory research focusing on antioxidant-based lead compounds having di-*tert*-butylphenol functionality, such as KME-4, E-5110, BF-389, and Cl-1004. These antioxidants were chosen because they were known to inhibit both COX and 5-LO. Also, antioxidant drugs as exemplified by the antiatherosclerotic drug probucol display the possibility of suppressing cytokine production under inflammatory stimuli.[14] These compounds have the common structure of a 3,5-di-*tert*-butyl-4-hydroxy benzylidene moiety and

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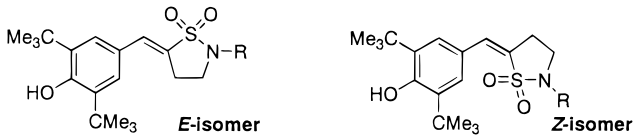
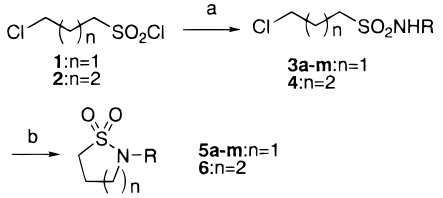
Table 1. In Vitro Inhibitory Effect on Production of PGE_2 by 3,5-Di-*tert*-butyl-4-hydroxybenzylidene-γ-sultam Derivatives and Reference Compounds



compd	R	*E*/*Z*	IC_{50} (μM)[a]	compd	R	*E*/*Z*	IC_{50} (μM)[a]
13	H	*E*	0.030	**15e**	CONHOH	*E*	0.024
10a	Me	*E*	0.0091	**15f**	CONMeOMe	*E*	0.10
10b	Et	*E*	0.0095	**16**	OH	*E*	0.010
10c	Pr	*E*	0.013	**14**	H	*Z*	<0.0010
10d	*i*-Pr	*E*	0.32	**12a**	Me	*Z*	<0.0010
10e	cyclo-Pr	*E*	0.0025	**12b**	Et	*Z*	0.0021
10f	*i*-Bu	*E*	0.41	**12c**	Pr	*Z*	0.012
10g	4-Cl-Ph	*E*	<0.0010	**12g**	4-Cl-Ph	*Z*	<0.0010
10h	2-pyridyl	*E*	1.0	**12h**	2-pyridyl	*Z*	4.4
10i	3-pyridyl	*E*	0.32	**12k**	OMe	*Z*	0.0025
10j	4-pyridyl	*E*	0.41	**17**	OH	*Z*	0.70
10k	OMe	*E*	<0.0010	**11**		*E*	0.0062
15a	CH_2CH_2OH	*E*	0.24	**19**		*E*	0.20
15b	$CH_2CH_2NMe_2$	*E*	0.34	tenidap[b]			0.053
15c	CH_2COOH	*E*	3.4	indomethacin			0.0029
15d	Ac	*E*	<0.0010				

[a] Concentration (μM) required for 50% inhibition of PGE_2 formation in rat synovial cells. [b] Tenidap was synthesized at our laboratories by the reported method.[32]

Scheme 1[a]



[a] Reagents (a) RNH_2, aq K_2CO_3 or $NaHCO_3$; (b) NaH or DBU.

five- or six-membered lactone or lactam rings. To develop antiinflammatory drugs in this area, we selected the sulfonyl group as an isostere of carbonyl function. Five- or six-membered sulfonamide ring systems are very unique and had not yet been prepared in this class of compounds.

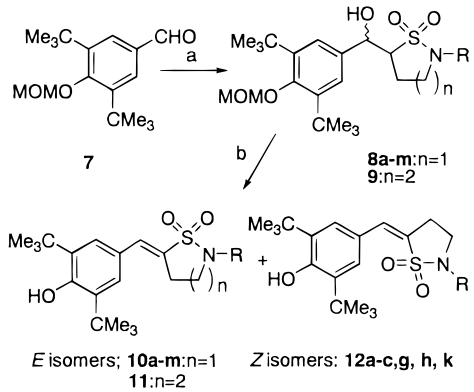
Our work led us to the discovery of novel antiarthritic agents having the unique γ-sultam skeleton with the di-*tert*-butylphenol substituent as an antioxidant moiety. We conducted an SAR study on the substituents at the 2-position of the γ-sultam skeleton and the antioxidant moieties. We report here on the synthesis and SAR study of the γ-sultam derivatives and the development of a new type of antiarthritic drug candidate, S-2474.

Chemistry

The compounds listed in Table 1 were synthesized by procedure A or B. Procedure A consists of construction of an *N*-substituted-γ-sultam ring[15] by cyclization of 3-chloropropanesulfonamide, followed by coupling with antioxidant aldehydes by the aldol-like reaction and dehydration to the benzylidene derivatives.

N-Alkyl-γ-sultam derivatives **5a**−**m** (**a**−**m**, for structures see Table 1 and Scheme 3) were prepared in good yields from commercially available 3-chloropropanesulfonyl chloride (**1**) by reacting with various primary amines in the presence of a base, followed by treatment with sodium hydride (NaH) or 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) as shown in Scheme 1. The aldol-like condensation reaction of 3,5-di-*tert*-butyl-4-methoxymethyloxybenzaldehyde (**7**) with *N*-alkyl-γ-sultams **5a**−**m** in the presence of lithium diisopropylamide (LDA) in THF smoothly proceeded to give the corresponding adducts **8a**−**m** as diastereoisomeric mixtures. Treatment of the crude aldol adducts with a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) resulted in dehydration and removal of the MOM (methoxymethyl) group yielding a mixture of *E*- and *Z*-isomers of 5-benzylidene-γ-sultam derivatives **10** and **12** as shown in Scheme 2. Their geometry of the double bond could be determined by nuclear Overhauser effect experiments[16] or X-ray crystallography.[17] In most cases, *E*-isomers **10a**−**m** were the major products and both isomers could be separated by column chromatography on silica gel.

Scheme 2[a]

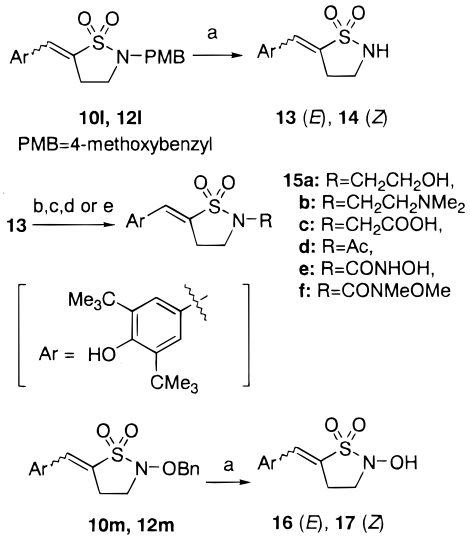


[a] Reagents: (a) **5a**−**m**, or **6**, LDA; (b) cat. *p*-TsOH.

Compounds **20**−**24**, having other antioxidant moieties, with the structures shown in Table 3, were also prepared using procedure A. The corresponding protected aldehydes were coupled with *N*-ethyl-γ-sultam **5b**, followed by the reaction with *p*-TsOH to give **20**−**24**.

Procedure B consists of construction of *N*-protected-5-benzylidene-γ-sultam derivative **13**, followed by depro-

Scheme 3[a]



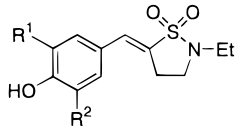
[a] Reagents: (a) $TiCl_4$; (b) alkyl halide, aq NaOH; (c) ethyl iodoacetate, K_2CO_3 then aq NaOH in MeOH; (d) Ac_2O, pyridine; (e) LHMDS, phenyl carbamate.

Table 2. In Vitro Inhibitory Effects on Productions of LTB_4 and IL-1 by γ-Sultam Derivatives and Reference Compounds

	IC_{50} (μM)[a]			IC_{50} (μM)[a]	
compd	LTB_4	IL-1	compd	LTB_4	IL-1
13	>100	>100	**16**	9.5	90
10a	2.8	28	**14**	1.8	27
10b	2.5	10	**12a**	1.8	29
10c	1.9	19	**12b**	2.5	10
10e	3.2	9.0	**12c**	0.8	23
10g	21	>100	**12k**	1.5	20
10k	3.0	>100	**11**	74	56
15d	4.9	28	tenidap	45	16
15e	6.0	20	ind.[b]	>100	>100
15f	28	>100			

[a] Concentration (μM) required for 50% inhibition of LTB_4 formation in rat peritoneal cells and IL-1 formation in human THP-1 cells. [b] ind.: indomethacin.

Table 3. In Vitro Inhibitory Effects on Productions of PGE_2, LTB_4, and IL-1 by γ-Sultam Derivatives with Various Antioxidant Moieties

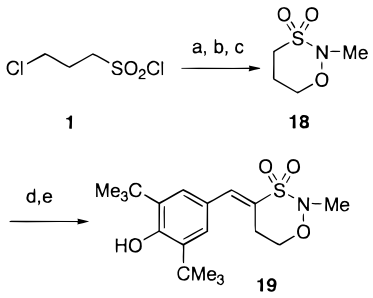


			IC_{50} (μM)[a]		
compd	R^1	R^2	PGE_2	LTB_4	IL-1
10b	*t*-Bu	*t*-Bu	0.0095	2.5	10
20	*i*-Pr	*i*-Pr	>1.0	>100	>100
21	Me	Me	>1.0	21	>100
22	OMe	OMe	>1.0	2.6	>100
23	OMe	H	>1.0	>100	>100
24	H	H	>1.0	>100	>100

[a] Concentration (μM) required for 50% inhibition of production of PGE_2, LTB_4, and IL-1.

tection and introduction of an alkyl or acyl substituent at the nitrogen atom as shown in Scheme 3. An isomeric mixture of 4-methoxybenzyl derivative **10l** and **12l** was prepared according to procedure A, and then the 4-methoxybenzyl group was removed with titanium tetrachloride to give **13** and **14** in good yield. *N*-Alkylated derivatives **15a** and **15b** were synthesized from the

Scheme 4[a]



[a] Reagents: (a) MeNHOH, aq K_2CO_3; (b) NaI; (c) DBU; (d) **7**; LDA; (e) *p*-TsOH.

corresponding alkyl halides and **13** in the presence of sodium hydroxide. Carboxymethyl derivative **15c** was synthesized by hydrolysis of the corresponding ethyl ester, obtained by the coupling reaction of **13** and ethyl iodoacetate, using aqueous sodium hydroxide in methanol. Acetyl derivative **15d** was prepared by using Ac_2O in pyridine. Carbamoyl derivatives **15e** and **15f** were prepared by treatment of **13** with lithium hexamethyldisilazane (LHMDS) and the corresponding phenyl carbamates.[18] *N*-Hydroxy-γ-sultam derivatives **16** and **17** were prepared by removal of the benzyl group of an isomeric mixture of **10m** and **12m** with titanium tetrachloride.

As a ring-expanded six-membered analogue of the γ-sultam derivative, the δ-sultam derivative **11** was obtained starting from 4-chlorobutylsulfonyl chloride (**2**),[19] similarly to the method used for the synthesis of γ-sultam derivatives in Schemes 1 and 2. As a sulfonamide isostere of BF-389, 1,3,2-oxathiazine derivative **19** was prepared as a major isomer from **1** by the procedure shown in Scheme 4 in moderate yield.

Biological Results and Discussion

For the primary screening, we used tenidap and indomethacin as reference compounds in the following in vitro assays: inhibitory activity of IL-1β-induced PGE_2 production in rat synovial cells,[20] calcium ionophore (A23187)-induced LTB_4 productions in rat peritoneal cells,[20,21] and lipopolysaccharide (LPS)-induced IL-1β production in human THP-1 cells.[22] The compounds which showed sufficient in vitro potency were advanced to the secondary screening in the rat carrageenin-induced foot-pad edema model,[23] and some of the effective compounds were further evaluated by the rat adjuvant arthritic model.[24] Furthermore, the ulcerogenic activity[23b] of compounds which showed oral activity comparable to that of tenidap in these inflammatory model animals was also assessed in rats. For the selected compound **10b** and reference compounds, the ratio of IC_{50} against COX-2/IC_{50} against COX-1 was measured in in vitro assays[25] and their inhibitory activities of COX-1 and COX-2 were also assessed by in vivo assay.[26]

In Vitro Primary Screening Assays. The results of inhibitory effects on PGE_2 production from in vitro assay by the synthesized compounds, containing di-*tert*-butylphenol substituent, are summarized in Table 1. For the substituents at the 2-position of γ-sultam, good inhibitory activity was obtained in the case of sterically small groups, such as hydrogen, hydroxy, methyl, ethyl,

propyl, cyclopropyl methoxy, and acetyl, as illustrated by compounds **13**, **16**, **10a**−**c**, **10e, 10k,** and **15d**. On the other hand, polar groups and large alkyl groups were not suitable for the 2-position as illustrated by compounds **10d**, **10f**, and **15a**−**c**. Carbamoyl derivatives **15e** and **15f** showed moderate effects. In the case of aromatic groups, 4-chlorophenyl derivative **10g** was also effective, but pyridyl derivatives **10h**−**j** were not. For the geometry of the double bond of benzylidene groups, *E*- and *Z*-isomers showed almost equal potency to the corresponding compounds. For six-membered analogues, δ-sultam derivative **11** showed almost equal inhibitory potency to its corresponding five-membered γ-sultam derivative **10k**, but 1,3,2-oxathiazine derivative **19**, the sulfonamide isostere of BF-389, showed relatively weak activity.

The results of inhibitory effects on LTB_4 and IL-1 production by some selected compounds which showed good inhibitory effects on PGE_2 productions are summarized in Table 2. For these inhibitory effects, compounds having the di-*tert*-butylphenol substituent and the γ-sultam skeleton with sterically small alkyl groups at the 2-position showed better or nearly equal activities compared with those of tenidap. However, the hydroxy derivative **16**, 4-chlorophenyl derivative **10g**, and δ-sultam derivative **11** showed lesser inhibitory activities on both LTB_4 and IL-1 production. For the geometry of the double bond of benzylidene groups, *Z*-isomers showed slightly stronger activities than those of the corresponding *E*-isomers.

For the antioxidant phenol moieties, the di-*tert*-butyl phenol group was found to be the most effective in each of the in vitro assays as shown in Table 3, suggesting that the activities might be related to their free radical scavenging potency.

From the viewpoint of combined inhibition of PGE_2, LTB_4, and IL-1 production, compounds having the di-*tert*-butylphenol moiety and small alkyl groups on the 2-position of the γ-sultam skeleton were found to show good biological activities.

In Vivo Screening Assays. The in vivo screening results are summarized in Table 4. The oral antiinflammatory activities were found to be largely influenced by the substituent at the 2-position of the γ-sultam skeleton, being limited to small alkyl or alkoxy groups such as methyl, ethyl, cyclopropyl, and methoxy. They exhibited antiinflammatory effects on rat carrageenin-induced foot-pad edema and the rat adjuvant arthritic model at 30 mg/kg and 10 mg/kg po, respectively. *Z*-Isomers, showing more potent activities than the corresponding *E*-isomers in the in vitro assays, were found to be less effective than the *E*-isomers in the in vivo assays. We think these tendencies result from the low oral bioavailability of *Z*-isomers, because in the case of *N*-ethyl derivatives, no **12b** could be detected in the plasma after oral administration.[27]

Next, three selected compounds, **10a**, **10b**, and **10e**, and reference compounds were tested for their ulcerogenic activity as listed in Table 5. Most notably, despite the strong inhibitory effects on PGE_2 production, **10b** and **10e** showed little ulcerogenic activity compared with the reference compounds. Among the three compounds, *N*-ethyl derivative **10b** was selected as a candidate for further pharmacological evaluations because it has well balanced suppressive effects on PGE_2, LTB_4, and IL-1 productions without any ulcerogenic activities. To clarify this mechanism of lesser side effects despite its remarkable inhibitory effect on PGE_2 production, the inhibitory actions against COX-1 and COX-2 by **10b** were compared with selective COX-2 inhibitors, NS-398[28] and celecoxib,[29] and a COX-1 and COX-2 inhibitor, indomethacin, in an in vitro system previously reported by Kawai et al.[25] As shown in Table 6, **10b**, NS-398, and celecoxib were over 2000 times more potent against COX-2 than COX-1, but indomethacin was

Table 4. In Vivo Antiinflammatory Effects of γ-Sultam Derivatives and Reference Compounds

	anti-edema (carrageenin)		antiarthritis (adjuvant. proph.)	
compd	% inhib. 30 mg/kg[a]	ED_{30} mg/kg[b]	% inhib. 10 mg/kg[c]	ED_{30} mg/kg[d]
13	17.2		8.8	
10a	61.4**	5.4	39.4**	0.64
10b	49.4**	3.5	37.8**	0.76
10c	0.3		11.4	
10e	21.4*	11.9	32.9**	1.7
10g	6.3			
10k	26.5**	34.7	35.2**	2.4
15d	21.0**			
15e	14.9			
15f	11.2			
16	15.8*		3.6	
14	10.8			
12a	25.2**			
12b	7.3		11.4	
12c	11.2			
12k	6.0		5.0	
11	14.7			
tenidap		4.0		1.0
indomethacin		0.8		0.06

[a] Percent inhibition of carrageenin-induced footpad edema at 30 mg/kg po. [b] The dose (mg/kg) required for 30% inhibition of carrageenin-induced edema. [c] Percent Inhibition of adjuvant-induced arthritis at 10 mg/kg po. [d] The dose (mg/kg) required for 30% inhibition of adjuvant-induced arthritis. *$p < 0.05$, ** $p < 0.01$: significant difference vs vehicle control.

Table 5. Ulcerogenic Effects of Selected Compounds and Reference Compounds

compd	dose (mg/kg)	ulcer index (mm)[a]
10a	400	26.8 ± 4.92
10b	400	1.6 ± 0.41
10e	400	0.7 ± 0.28
ind.[b]	30	19.5 ± 2.10
tenidap	30	12.9 ± 2.38

[a] Lengths of bleeding ulcer of drug-administrated group, 6 animals/group. [b] ind.: indomethacin. Details are given in the Experimental Section.

Table 6. Comparison of IC_{50} Values of Various NSAIDs for COX-1 and COX-2 in Human Intact Cells

compd	COX-1 TXB_2 (μM)[a]	COX-2 PGE_2 (μM)[b]	COX-1/COX-2 ratio
10b	27	0.011	2500
NS-398[c]	6.8	0.0019	3600
celecoxib[d]	19	0.0079	2400
ind.[e]	0.0058	0.0046	1.3

[a] Concentration (μM) required for 50% inhibition of TXB_2 production in human platelet cells. [b] Concentration (μM) required for 50% inhibition of PGE_2 production in human IL-1β stimulated synovial cells. Details of these assays are described in the Experimental Section. [c,d] NS-398 and celecoxib were synthesized at our laboratories by the reported methods.[33,29] [e] ind.: indomethacin.

Table 7. Comparison of ED_{50} Values of Various NSAIDs for COX-1 and COX-2 Activity in Rats

	ED_{50} value (mg/kg)[a]	
compd	COX-1 (a)	COX-2 (b)
10b	>30	3.19
NS-398	>30	1.00
tenidap	0.025	0.05
ind.[b]	0.37	0.15

[a] ED_{50} values required for 50% inhibition of increase in plasma PGE_2 level after arachidonic acid (AA) injection into non-LPS-treated rats (a) and LPS-treated rats (b). Details of these assays are described in the Experimental Section. [b] ind.: indomethacin.

approximately equipotent as an inhibitor of COX-1 and COX-2. In addition, we assessed the inhibitory activity of **10b**, NS-398, tenidap and indomethacin against COX-1 and COX-2 in the in vivo system previously described by Futaki et al.[26] Compound **10b** and NS-398 selectively inhibited COX-2 activity, while tenidap and indomethacin suppressed both COX-1 and COX-2 activities (Table 7). These results indicate that **10b** is a highly selective COX-2 inhibitor.

Conclusion

We have described the synthesis of novel γ-sultam derivatives containing the di-*tert*-butylphenol antioxidant moiety. Several compounds with lower alkyl groups at the 2-position of the γ-sultam skeleton showed potent inhibitory activities against PGE_2 production via the COX pathway and LTB_4 production via the 5-LO pathway, as well as production of IL-1 in in vitro assays. Extensive pharmacological characterizations revealed that 2-ethyl-γ-sultam derivative **10b** displays multiple inhibition of COX, 5-LO, and IL-1 production similar to tenidap and also good selective COX-2 inhibition like NS-398 and celecoxib. It exerted excellent antiinflammatory activity without any ulcerogenic effects and was designated as S-2474 an agent having both NSAID and cytokine modulating properties. S-2474 is now being developed as a promising alternative antiarthritic drug candidate.

Experimental Section

Chemistry. Melting points were uncorrected. ^1H NMR spectra were taken with a Varian VXR-200 or Gemini-200 300 FT-NMR spectrometer using tetramethylsilane as an internal standard. IR spectra were recorded on a Nicolet 20SXB FT-IR spectrometer. Mass spectra were measured on a JEOL JMS-SX/S102A or a HITACHI M-90 mass spectrometer. Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere with commercial grade solvents that had been dried over type 4A molecular sieves. Drying of organic extracts over anhydrous sodium sulfate is simply indicated by the word "dried". Column chromatography using Merck Silica gel 60 (70−230 or 230−400 mesh) or a Merck Lobar column is referred to "chromatography on silica gel".

3,5-Di-*tert*-butyl-4-methoxymethyloxybenzaldehyde (7). 3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde (100 g, 0.43 mol), ethylene glycol (35 mL, 0.64 mol), benzene (400 mL), and *p*-TsOH (1.0 g, 5.3 mmol) were stirred and heated. The water formed during the reaction was removed azeotropically (Dean−Stark trap) during 48 h. The reaction mixture was poured into saturated $NaHCO_3$, and a product was extracted with AcOEt. The organic layer was separated and washed with water followed by brine and dried. Removal of the solvent gave (3,5-di-*tert*-butyl-4-hydroxyphenyl)-1,3-dioxolane as a colorless solid. It was dissolved with THF (100 mL) and DMF (100 mL), and this solution was slowly added dropwise to a stirred suspension of NaH (60% in mineral oil, 18.8 g, 0.47 mol) in THF (100 mL) with ice-cooling and stirred for 10 min under the same conditions. Chloromethyl methyl ether (*Caution! Cancer suspect agent.*) (39 mL, 0.51 mol) was added to the reaction and stirred for 1 h under the same conditions. The reaction mixture was poured into saturated $NaHCO_3$ and extracted with AcOEt. The organic layer was separated and washed with water, followed by brine. Drying and evaporation of the solvent gave a residual oil. (3,5-Di-*tert*-butyl-4-methoxymethyloxyphenyl)-1,3-dioxolane was obtained as a colorless solid (110 g, 80%) by crystallization from hexane. It was dissolved with acetone (400 mL) and treated with 1 N HCl (68 mL, 68 mmol) with ice-cooling. The reaction mixture was stirred for 1 h and poured into saturated $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with water followed by brine. After drying and evaporation of the solvents, the title compound was obtained as a colorless solid (95 g, quant) which was used in the next reaction without further purification. An analytically pure sample was obtained by recrystallization from MeOH−water as a colorless crystal: mp 63−65 °C; IR ($CHCl_3$) 3435, 2958, 1685, 1591, 1379, 1196, 1167, 1082, 1005 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (s, 18H), 3.66 (s, 3H), 4.94 (s, 2H), 7.81 (s, 2H), 9.92 (s, 1H). Anal. ($C_{17}H_{26}O_3$) C, H.

***N*-Methyl-1,2-isothiazolidine-1,1-dioxide (5a).** To a stirred suspension of 3-chloropropanesulfonyl chloride (16.8 g, 94.9 mmol) and methylamine hydrochloride (13.5 g, 200 mmol) in AcOEt (500 mL) were added K_2CO_3 (27.6 g, 200 mmol) and benzyltrimethylammonium chloride (200 mg), and the reaction mixture was stirred for 2 h at room temperature. The mixture was passed through a pad of silica gel and then concentrated in vacuo to afford 12.0 g (74%) of crude **3a** as a pale yellow oil. To a stirred solution of **3a** (11.8 g, 68.7 mmol) in benzene (300 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (10.8 mL, 72.1 mmol), and the reaction mixture was refluxed for 24 h. After cooling to room temperature, the mixture was passed through a pad of silica gel and then concentrated in vacuo to give **5a** (7.0 g, 75%) as a colorless solid: mp 36−40 °C; IR ($CHCl_3$) 3016, 1451, 1307, 1218, 1187, 1127 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27−2.42 (m, 2H), 2.69 (s, 3H), 3.11−3.20 (m, 2H), 3.22 (t, J = 6.8 Hz, 2H).

***N*-Ethyl-1,2-isothiazolidine-1,1-dioxide (5b).** To a stirred solution of 3-chloropropanesulfonyl chloride (6.10 g, 34.5 mmol) in Et_2O (25 mL) was added ethylamine (a 70% aqueous solution, 4.40 g, 68.3 mmol) dropwise over a period of 15 min under ice-cooling. The reaction mixture was stirred for 1 h at room temperature and then concentrated in vacuo. To the residue, benzene (100 mL) was added and evaporated to remove the remaining water as a benzene azeotrope. Next, Et_2O was added (150 mL) to the residue, and the suspension was filtered off with Hyflo Super-Cel to remove the insoluble materials. The filtrate was concentrated in vacuo to give 6.96 g (quant) of crude **3b** (mp 30−32 °C). To a stirred solution of **3b** (6.96 g, 34.5 mmol) in THF (50 mL) was slowly added NaH (60%, 1.52 g, 38.0 mmol) under ice-cooling, and the mixture was refluxed for 30 min. After addition of Et_2O (50 mL), the mixture was filtered off with Hyflo Super-Cel to remove insoluble materials, and the organic solvent was removed with an evaporator. The residue was purified by distillation under reduced pressure to afford 4.93 g (93%) of **5b** as a pale yellow oil: bp 150−160 °C/25 mmHg; IR ($CHCl_3$) 3018, 2976, 2868, 1452, 1306, 1220, 1179, 1129, 1015 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (t, J = 7.4 Hz, 3H), 2.28−2.42 (m, 2H), 3.10 (q, J = 7.4 Hz, 2H), 3.15 (t, J = 7.6 Hz, 2H), 3.22−3.29 (m, 2H). Anal. ($C_5H_{11}NO_2S$) H, N, S; C: calcd, 40.25; found, 39.83.

Compounds **5c**−**f**, **5k**−**m**, and **6** were prepared from **1** or **2** and the corresponding primary amines using a procedure similar to that described for the preparation of **5a** or **5b**.

2-Methyl-[1,3,2]thiaoxazine-1,1-dioxide (18). *N*-Hydroxy-*N*-methyl-3-chloropropanesulfonamide (85% yield) prepared by the method described above was converted to the 3-iodide derivative (91%) using excess sodium iodide in methyl ethyl ketone, and this was treated with DBU in Et_2O at room temperarure to give the title compound **18** as a colorless crystal (27%): mp 79−81 °C; IR ($CHCl_3$) 3020, 2930, 1436, 1347, 1333,

1222, 1146 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.34−2.46 (m, 2H), 2.92 (s, 3H), 3.24−3.30 (m, 2H), 4.11−4.16 (m, 2H), 3.79 (s, 3H). Anal. ($C_4H_9NO_3S$) C, H, N, S.

***N*-(4-Chlorophenyl)-1,2-isothiazolidine-1,1-dioxide (5g).** To a stirred solution of 4-chloroaniline (5.24 g, 41.1 mmol) and pyridine (25 mL) was added 3-chloropropanesulfonyl chloride (7.28 g, 41.1 mmol) dropwise at −20 to −30 °C over a period of 5 min. The reaction mixture was allowed to warm to room temperature and stirred for additional 45 min. The mixture was concentrated in vacuo, and the residue was purified by chromatography on silica gel eluting with hexane−AcOEt (2:1) to give 10.2 g (93%) of **3g**. Compound **5g** (68% yield) was prepared in a similar manner described above as a colorless crystal: mp 110.5−111.5 °C; IR ($CHCl_3$) 3010, 2960, 1595, 1493, 1300, 1267, 1131 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.47−2.61 (m, 2H), 3.35−3.43 (m, 2H), 3.76 (t, J = 6.4 Hz, 2H), 7.16−7.36 (m, 4H). Anal. ($C_9H_{10}NO_2SCl$) C, H, N, S, Cl.

Compounds **5h**−**j** were also prepared from **1** and the corresponding arylamines using a procedure similar to that described for the preparation of **5g**.

(*E*)-2-Ethyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-1,2-isothiazolidine-1,1-dioxide (10b) and Its (*Z*)-Isomer (12b). To a stirred solution of diisopropylamine (15.5 mL, 111 mmol), BuLi in hexane (1.60 M, 69.5 mL, 111 mmol) was added dropwise under ice-cooling over a period of 20 min. After completion of the addition, stirring was continued for another 15 min and the reaction mixture was cooled to −78 °C, followed by the addition of THF (100 mL). To this mixture were added **5b** (15 g, 101 mmol), 3,5-di-*tert*-butyl-4-methoxymethyloxybenzaldehyde (**7**) (25 g, 90.5 mmol), and hexamethylphosphamide (HMPA) (*Caution! Cancer suspect agent.*) (30 mL) in THF (70 mL) dropwise over a period of 20 min. The reaction mixture was stirred for 30 min at the same temperature and then warmed to room temperature. The mixture was poured into 2 N HCl (100 mL) under ice-cooling and extracted with AcOEt (250 mL). The organic layer was washed with saturated $NaHCO_3$ (300 mL) and brine (300 mL), then dried, and evaporated. The residue was purified by chromatography on silica gel eluting with hexane−AcOEt (4:1 to 1:1) to give 21.3 g (55%) of **8b** as a colorless solid. To a solution of **8b** (8.5 g, 19.9 mmol) in toluene (150 mL) was added *p*-TsOH (2.49 g, 13 mmol), and the mixture was refluxed for 30 min. After cooling to room temperature, the mixture was poured into saturated $NaHCO_3$ (150 mL) and extracted with AcOEt (150 mL). The organic layer was washed with water (150 mL) and brine (150 mL) and then dried and evaporated. The residue was subjected to column chromatography on silica gel, eluting with hexane−AcOEt (3:1); *E*-isomer **10b** (2.59 g, 36%) and *Z*-isomer **12b** (376 mg, 7%) were obtained as crystals from the less polar and polar fractions, respectively. **10b**: mp 135−137 °C; IR (KBr) 3610, 3440, 2970, 2880, 1645, 1597, 1430, 1290, 1173, 1151, 1139 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (t, J = 7.2 Hz, 3H), 1.45 (s, 18H), 3.07−3.19 (m, 4H), 3.28 (q, J = 7.2 Hz, 2H), 5.50 (s, 1H), 7.24−7.26 (m, 3H). Anal. ($C_{20}H_{31}NO_3S$) C, H, N, S. **12b**: mp 160.5 °C; IR (KBr) 3560, 2975, 1637, 1600, 1431, 1289, 1275, 1168, 1150, 1111 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (t, J = 7.2 Hz, 3H), 1.45 (s, 18H), 3.00 (dt, J = 2.0, 6.0 Hz, 2H), 3.15 (q, J = 7.2 Hz, 2H), 3.25 (t, J = 6.0 Hz, 2H), 5.47 (s, 1H), 6.73 (t, J = 2.0 Hz, 1H), 7.52 (s, 2H). Anal. ($C_{20}H_{31}NO_3S$) C, H, N, S.

Compounds **10a**, **10c**−**m**, **12a**, **12c**, **12g**, **12h**, **12k**−**m**, **11**, and **19** were prepared from **7** and the corresponding γ-sultams, **6**, or **18** using a procedure similar to that described for the preparation of **10b** and **12b**. Compounds **20**−**24** were also prepared from **5b** and the corresponding protected aldehydes using the procedure described above.

(*E*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-1,2-isothiazolidine-1,1-dioxide (13) and Its *Z*-Isomer (14). To a stirred solution of the mixture of **10l** and **12l** (8.83 g, 19.2 mmol) which were obtained (64% yield) from the reaction of **7** (9.00 g, 32.0 mmol) and **5l** (7.24 g, 30.0 mmol), in CH_2Cl_2 (150 mL), was added $TiCl_4$ (4.10 mL, 37.4 mmol) with ice-cooling, and the mixture was stirred for 30 min at 0 °C. The mixture was poured into ice water (200 mL) and extracted with AcOEt (200 mL), and the organic phase was washed with saturated $NaHCO_3$ (200 mL) and brine (200 mL), dried, and evaporated. The residue was purified by chromatography on silica gel eluting with hexane−AcOEt (1:1) to give 3.35 g (41%) of **13** and 0.12 g (1.5%) of **14**. **13**: mp 233−235 °C; IR (Nujol) 3556, 3244, 2914, 1632, 1594, 1430, 1319, 1240 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.40 (s, 18H), 3.02−3.13 (m, 2H), 3.22−3.34 (m, 2H), 6.92 (br t, J = 7.2 Hz, 1H), 7.08 (t, J = 2.4 Hz, 1H), 7.29 (s, 2H), 7.45 (s, 1H). Anal. ($C_{18}H_{27}NO_3S\cdot 0.3H_2O$) C, H, N, S. **14**; mp 161−164 °C; IR ($CHCl_3$) 3620, 2954, 1432, 1371, 1312, 1241, 1157 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 18H), 3.11 (dt, J = 2.1, 6.7 Hz, 2H), 3.39−3.51 (m, 2H), 4.26−4.40 (br, 1H), 5.49 (s, 1H), 6.80 (t, J = 2.1 Hz, 1H), 7.55 (s, 2H). Anal. ($C_{18}H_{27}NO_3S$) C, H, N, S.

(*E*)-2-(2-Hydroxyethyl)-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-1,2-isothiazolidine-1,1-dioxide (15a). To a stirred suspension of **13** (675 mg, 2.0 mmol), a catalytic amount of *N*-benzyltrimethylammonium chloride, 2-iodoethanol (0.624 mL, 8.0 mmol) in CH_2Cl_2 (20 mL), and water (10 mL) was added 2 N NaOH (1.5 mL) at room temperature. The reaction mixture was refluxed for 3 days and diluted with water. The organic layer was separated and washed with brine, dried, and evaporated. The crude residue was purified by chromatography on silica gel eluting with hexane−AcOEt (7:3) to give **15a** (190 mg, 25%) as a colorless crystal: mp 156−157 °C; IR ($CHCl_3$) 3620, 2956, 1434, 1290, 1240, 1151, 1066 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 18H), 3.16 (dt, J = 2.4, 6.5 Hz, 2H), 3.30 (m, 2H), 3.41 (t, J = 6.5 Hz, 2H), 3.87 (t, J = 5.2 Hz, 2H), 5.53 (s, 1H), 7.23−7.29 (m, 3H). Anal. ($C_{20}H_{31}NO_4S$) C, H, N, S.

Compound **15b** was prepared using a procedure similar to that described for the preparation of **15a**.

(*E*)-2-Carboxymethyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-1,2-isothiazolidine-1,1-dioxide (15c). A mixture of **13** (14.0 g, 41.6 mmol), ethyl iodoacetate (7.38 mL, 62.4 mmol), and K_2CO_3 (8.60 g, 62.2 mmol) in DMF (80 mL) was stirred for 3 h at room temperature. The reaction mixture was poured into AcOEt (500 mL) and water (400 mL), and the organic phase was washed with water (400 mL) and brine (400 mL). The solvent was removed, and the residue was purified by chromatography on silica gel eluting with hexane−AcOEt to give an ethyl ester derivative as a pale red solid (7.43 g, 72%). To a stirred solution of this compound (15.9 g, 37.5 mmol) in THF (200 mL) and MeOH (50 mL) was added 2 N NaOH (37.5 mL) with ice-cooling, and the mixture was stirred for 30 min at the same temperature. To this mixture were added 1 N HCl (250 mL) and AcOEt (450 mL), and the organic phase was washed with water (300 mL) and brine (300 mL), dried, and concentrated to give a colorless oil. Crystallization from isopropyl ether gave pure **15c** (9.6 g, 65%) as a colorless crystal: mp 175−177 °C; IR (Nujol) 3596, 2914, 1733, 1645, 1594, 1460, 1430, 1290, 1243, 1213 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 18H), 3.21 (dt, J = 2.4, 6.6 Hz, 2H), 3.51 (t, J = 6.6 Hz, 2H), 3.95 (s, 2H), 5.54 (s, 1H), 7.25−7.30 (m, 3H). Anal. ($C_{20}H_{29}NO_5S$) C, H, N, S.

(*E*)-2-Acetyl-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-1,2-isothiazolidine-1,1-dioxide (15d). To a stirred solution of **13** (585 mg, 1.74 mmol) in pyridine (10 mL) were added a catalytic amount of 4-*N*,*N*-(dimethylamino)pyridine (DMAP) and acetic anhydride (6 mL) under ice-cooling. The reaction mixture was stirred for 1 h at room temperature and then concentrated in vacuo. The residue was purified by chromatography on silica gel to afford **15d** (360 mg, 55%) as a colorless crystal: mp 177−179 °C; IR ($CHCl_3$) 3618, 2958, 1695, 1435, 1379, 1297, 1153, 1117 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.46 (s, 18H), 2.53 (s, 3H), 3.20 (dt, J = 2.2, 7.0 Hz, 2H), 3.86 (t, J = 7.0 Hz, 2H), 5.60 (s, 1H), 7.52 (s, 2H), 7.39 (t, J = 2.2 Hz, 1H). Anal. ($C_{20}H_{29}NO_5S$) C, H, N, S.

(*E*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(*N*-methoxy-*N*-methylcarbamoyl)-1,2-isothiazolidine-1,1-dioxide (15f). To a solution of **13** (450 mg, 1.33 mmol) and *N*-methoxy-*N*-methyl phenyl carbamate (300 mg, 1.66 mmol) in THF (10 mL) and HMPA (*Caution! Cancer suspect agent.*) (10 mL) was slowly added LHMDS (1.0 M in THF) (3.2 mL, 3.2 mmol) at −40 °C. The reaction mixture was allowed to

warm to room temperature and then poured into 1 N HCl. The mixture was extracted with AcOEt, and the organic phase was washed with water and brine, dried, and evaporated to give a crude oil. It was then purified by chromatography on silica gel to afford 230 mg (41%) of **15f** as an amorphous powder: IR ($CHCl_3$) 3620, 2958, 1673, 1435, 1388, 1330, 1240, 1207, 1155, 1092 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 18H), 3.21 (dt, J = 2.2, 6.8 Hz, 3H), 3.31 (s, 3H), 3.78 (s, 3H), 3.89 (t, J = 6.8 Hz, 2H), 5.54 (s, 1H), 7.23 (s, 2H), 7.31 (t, J = 2.2 Hz, 1H); MS 424 (M^+).

(*E*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(*N*-hydroxycarbamoyl)-1,2-isothiazolidine-1,1-dioxide (15e). (*E*)-5-(3,5-Di-*tert*-butyl-4-hydroxybenzylidene)-2-(*N*-methoxymethyl-*N*-benzyloxylcarbamoyl)-1,2-isothiazolidine-1,1-dioxide (600 mg, 1.13 mmol), similarly prepared from *N*-benzyloxy-*N*-methoxymethyl phenyl carbamate and **13**, was treated with $TiCl_4$ (0.5 mL, 4.56 mmol) in CH_2Cl_2 (8 mL) with ice-cooling and stirred for 1.5 h at the same temperature. To the reaction mixture was added 2 N HCl solution, and stirring was done vigorously for 30 min. A product was extracted with CH_2Cl_2, and the organic phase was washed with brine, dried, and evaporated. The residue was purified by chromatography on silica gel to afford 150 mg (33%) of **15e** as an amorphous powder: IR ($CHCl_3$) 3618, 2956, 1707, 1434, 1320, 1151, 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 18H), 3.23 (dt, J = 2.2, 7.0 Hz, 3H), 3.94 (t, J = 7.0 Hz, 2H), 5.61 (s, 1H), 6.85−6.95 (br, 1H), 7.24 (s, 2H), 7.30 (t, J = 2.2 Hz, 1H), 8.61 (s, 1H); MS 396 (M^+).

(*E*)-2-Hydroxy-5-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-1,2-isothiazolidine-1,1-dioxide (16) and Its (*Z*)-Isomer (17). Compounds **16** and **17** were prepared from a mixture of **10m** and **12m** by treating with $TiCl_4$. **16**: 27% yield; mp 177−182 °C; IR (KBr) 3560, 3430, 1425, 1333, 1240, 1155, 1130, 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 18H), 3.18 (dt, J = 2.6, 6.8 Hz, 2H), 3.89 (t, J = 6.8 Hz, 2H), 5.56 (s, 1H), 6.18−6.30 (br, 1H), 7.26−7.35 (m, 3H). Anal. ($C_{18}H_{27}NO_4S$) C, H, N, S. **17**: 3.0% yield; mp 190−198 °C; IR ($CHCl_3$) 3622, 3540, 3020, 2954, 1632, 1431, 1340, 1241, 1157 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (s, 18H), 3.17 (dt, J = 2.2, 6.8 Hz, 2H), 3.62 (m, 2H), 5.51 (s, 1H), 6.22 (s, 1H), 7.04 (t, J = 2.2 Hz, 1H), 7.49 (s, 2H). Anal. ($C_{18}H_{27}NO_4S$) H, N, S; C: calcd, 61.16; found, 60.67.

Inhibitory Activity against the Production of PGE_2 in Rat Synovial Cells. Synovial cells were collected from synovium of knee joints of male Lewis rats (Charles River Japan, 300−350 g) and subcultured under a constant condition until the number of cells reached sufficient for the test. The cultured cells were placed into 96-well plates at 4×10^3 cells/160 μL/well and incubated in a CO_2 incubator for 72 h. To each well were added a solution (20 μL) containing various concentration of a drug to be tested and human IL-1β (20 μL) (final concentration: 30 U/mL) simultaneously, and the reaction was carried out in a CO_2 incubator for 15 h. The supernatant was stored at −80 °C until the measurement of PGE_2. The measurement of PGE_2 was conducted using a ^{125}I-PGE_2 radioimmunoassay (RIA) kit after thawing the stored samples.

Inhibitory Activity against the Production of LTB_4 in Rat Peritoneal Cells. Male SD rats (Clea Japan, 300−350 g) were injected intraperitoneally with Hanks' solution (10 mL) containing 0.1% bovine serum albumin (BSA) and 25 U/mL heparin. Ascites was collected and centrifuged for 5 min at 4 °C at 1500 rpm. The cell fraction (precipitates) was suspended in Hanks' solution containing 0.1% BSA and cell density was adjusted to 1×10^6 cells/mL. The adjusted suspension (800 μL; 8×10^5 cells) was transferred to polypropylene tubes and incubated at 37 °C for 10 min. After the addition of a solution (100 μL) containing a variable concentration of a drug to be tested, the tube was incubated for another 10 min, which was followed by addition of Ca-ionophore A23187 (100 μL; final concentration, 1 μM). The reaction was carried out for 15 min and then stopped by cooling with ice. The suspension was centrifuged at 4 °C at 3000 rpm for 5 min to collect the supernatant, which was stored at −80 °C until measurement. The measurement of LTB_4 was carried out using a ^{3}H-LTB_4 RIA kit after thawing stored sample.

Inhibitory Activity against the Production of IL-1 under LPS Stimulation in THP-1 Cells. THP-1 cells were dispersed in RPMI1640. To each well of a 24-well plate were added 800 μL of the dispersion (5×10^6 cells/mL), 100 μL of a solution containing a variable concentration of drug to be tested, and 100 μL of LPS (final concentration, 10 μg/mL), and the reaction was started. The reaction mixture was allowed to stand for 24 h at 37 °C. The supernatant was collected and centrifuged at 3000 rpm for 10 min. The measurement of IL-1 in the supernatant was conducted using a ^{125}I-IL-1β RIA kit.

Suppressive Activity against Carrageenin-Induced Edema in Rats. The experiment was carried out in accordance with the Winter's method[23a] with a modification. Namely, male Lewis rats (Charles River Japan, 6 weeks of age, 140−170 g) which had been fasted for 24 h were divided into groups each consisting of seven to eight rats. One hour after the administration of a drug, each animal was injected subcutaneously with 0.1 mL of 1% λ-carrageenin (PICININ-A, Zushikagaku) solution at plantar of right hind paw to cause edema. The volume of the right hind paw was measured with a plethysmometer by the water displacement method before the injection and every 1 h for 5 h after the injection. The effect of a drug was evaluated by calculating the edema suppression rate in drug-administered group relative to vehicle-administered group. The anti-edema effect of a drug was expressed as ED_{30} (a dose required for 30% of inhibition) which was obtained by regression analysis on the basis of the suppression rate at 3 or 4 h after carrageenin administration.

Effect on Adjuvant-Induced Arthritis (Prophylactic Test) in Rats. A modification of the method described by Winder, C. V., et al.[24a] was used. First, 0.05 mL of 1% *M. butyticum* suspension in liquid parafin was injected into the left hind paw of female Leweis rats (Charles River Japan, 8 weeks of age, 140−160 g). The test drugs were orally administered once daily from day-1 to day-21 after the adjuvant injection. The volumes of adjuvant-treated and untreated paws were measured plethysmographically at intervals of 2 to 4 days. Severity of arthritis was determined by quantitating the change in paw volume by subtracting normal paw volume (1.2 mL). ED_{30} (a dose required for 30% of inhibition) and statistical significance were determined by comparing AUCs (area under curves of paw volume) from vehicle-treated animals with those from drug-treated animals.

Inhibitory Effect of Lesion Formation of Gastric Mucosa in Rats. Male Lewis rats (Charles River Japan, 6 weeks of age, 140−160 g) were divided in groups of 6 rats on the basis of dose of a drug to be tested. A drug was administered to animals which had been fasted for 24 h prior to the test, and 6 h later, animals were anesthetized with ether and killed by exsanguination. The stomach was extracted and physiological saline (about 6 mL) was infused in it. Subsequently, the stomach was dipped in 1% formaldehyde solution for about 15 min and cut out along its greater curvature. The state of the lesion of the stomach was observed with a stereoscopic microscope, and the number of rats showing the gastric lesion and the length of bleeding plaques were determined. The extent of lesion was expressed by Lesion Index (mm) which is a cumulative value of length of bleeding plaques of each group of drug administrated groups.

Inhibitory Effect on Thromboxane B_2 (TXB_2) Production in Human Platelets. Platelets were prepared by a previously described method.[30] Peripheral venous blood was obtained from healthy volunteers and mixed with 7.5% (v/v) 77 mM ethylenediaminetetraacetic acid (EDTA) by gentle inversion. The mixture was centrifuged at 150*g* for 10 min to obtain platelet-rich plasma, which was then centrifuged at 800*g* for 10 min to obtain a platelet-rich pellet. This was suspended in Hanks' balanced salt solution (HBSS) with 10 mM HEPES buffer solution (HEPES) and 1.54 mM EDTA. The washed platelets were resuspended in HBSS with 10 mM HEPES and 0.5 mM $MgCl_2$ and used for the following study.

The platelet count was adjusted to 2×10^8 cells/mL, and the suspension was placed into tubes that were incubated at 37 °C for 3 min. Then drugs (the final concentration of ethanol

was 0.1%) were added to the suspensions, and incubation was done at 37 °C for 15 min. After incubation, arachidonic acid (AA, final concentration: 3 μM) was added to each platelet suspension to evaluate the cyclooxygenase enzyme activity by inducing TXB_2 production. After 15 min, ice-cold HBSS was added to stop the reaction. Each reaction solution was then centrifuged at 10000*g* for 3 min at 4 °C, and the TXB_2 concentration was measured using an ELISA kit.

Inhibitory Effect on PGE_2 Production Induced by IL-1β in Human Synovial Cells. Human synovial cells were obtained from patients with RA. Synovial tissues were digested with 0.2% collagenase for 90 min and cultured in RPMI-1640 (RPMI) with 100 U/mL penicillin and 100 μg/mL streptomycin and 10% fetal calf serum (FCS) at 37 °C under 5% CO_2. Synovial cells that adhered to the plastic flasks were cultured as described previously.[31] The human synovial cells were placed in 48-well plastic plates (0.7 to 3 × 10^4 cells/well) and cultured for 24 to 48 h. After washing with RPMI, the cells were incubated with IL-1β 1 ng/mL (RPMI with 2% FCS) for an additional 24 h. Then cells were washed with RPMI and then treated with RPMI containing drugs (the final concentration of ethanol was 0.1%) and incubated at 37 °C for 30 min. After incubation, AA (final concentration: 3 μM) was added to each well and the plate was incubated for an additional 30 min. The culture medium was centrifuged at 1000*g* at 4 °C for 1 min, and the concentration of PGE_2 in supernatant was measured using an ELISA kit.

Effect on COX-1 and COX-2 Activity in Vivo. According to a slightly modified method described by Futaki et al.,[26] COX-1 and COX-2 activity was determined based on the increase in plasma PGE_2 level after AA using nonlipopolysaccharide (LPS)-treated or LPS-treated rats, respectively. Briefly, male SD rats (Charles River Japan, 200−350 g) were anesthetized with pentobarbital, and then the carotid artery was cannulated for sampling blood. After surgery, AA (3 mg/kg) was given iv into the vena bulbi vein of non-LPS-treated or LPS-treated rats, and 30 s later, 2.25 mL of blood sample was withdrawn via the catheter into a disposable plastic syringe containing 0.25 mL of EDTA (final concentration: 7.7 mM) and indomethacin (final concentration: 10 μg/mL) solution to prevent artificial generation of COX products. These samples were centrifuged at 3000 rpm for 15 min, and then the plasma was added into four volumes of 99.5% ethanol. The mixture was centrifuged at 3000 rpm, and the supernatant was stored at −20 °C. The supernatant was acidified to pH 3 and extracted by using a Sep-Pak C_{18} cartridge (Waters Associates), and the concentrations of PGE_2 in the extract were measured by RIA (NEN Research Products). LPS-treated rats were prepared by iv injection of LPS (055:B5, 1 mg/kg) into the tail vein 6 h before AA injection. Drugs were administered 2 h before injection of AA.

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Supporting Information Available: Elemental analysis, characteristic data for some of the synthetic intermediates and final products for some of the compounds, and X-ray crystal structures for **10b** and **12b**. This material is available free charge via the Internet at http://pubs.acs.org.

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