Cyclooxygenase-2 Inhibitors. Synthesis and Pharmacological Activities of 5-Methanesulfonamido-1-indanone Derivatives[1]

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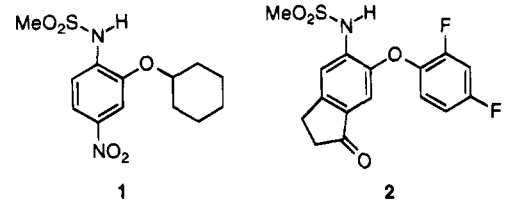
The recent discovery of an alternative form cyclooxygenase (cyclooxygenase-2, COX-2), which has been proposed to play a significant role in inflammatory conditions, may provide an opportunity to develop anti-inflammatory drugs with fewer side effects than existing nonsteroidal anti-inflammatory drugs (NSAIDs). We have now identified 6-[(2,4-difluorophenyl)thio]-5-methanesulfonamido-1-indanone (**20**) (L-745,337) as a potent, selective, and orally active COX-2 inhibitor. The structure–activity relationships in this series have been extensively studied. *Ortho*- and *para*-substituted 6-phenyl substitutents are optimal for *in vitro* potency. Replacement of this phenyl ring by a variety of heterocycles gave compounds that were less active. The methanesulfonamido group seems to be the optimal group at the 5-position of the indanone system. Compound **20** has an efficacy profile that is superior or comparable to that of the nonselective COX inhibitor indomethacin in animal models of inflammation, pain, and fever and appears to be nonulcerogenic within the dosage ranges required for functional efficacy. Although **20** and its oxygen linkage analog **2** (flosulide) are equipotent in the *in vitro* assays, compound **20** is more potent in the rat paw edema assay, has a longer $t_{1/2}$ in squirrel monkeys, and seems less ulcergenic than **2** in rats.

Introduction

Prostaglandins, particularly prostaglandin E_2 (PGE_2), are involved in many diverse physiological and pathological functions. These eicosanoids are produced by the action of cyclooxygenase on arachidonic acid. Recently, it has been shown that cyclooxygenase (COX) exists in two isoforms, termed cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 has been well-characterized, and the sheep, mouse, and human forms have been cloned.[2–4] This enzyme is believed to be involved in the maintenance of physiological functions such as platelet aggregation, cytoprotection in the stomach, and maintenance of normal kidney function. COX-2 has recently been described[5–7] to have a molecular mass similar to that of COX-1 (~70 kDa) and to share significant sequence homology with COX-1 at the amino acid level (~60%). Expression of COX-2 is induced by a variety of agents *in vitro* including endotoxin, cytokines, and mitogens.[8–10] COX-2 has also been shown to be induced significantly *in vivo* under inflammatory conditions.[11–14] This has led to the concept that COX-1 and COX-2 serve different physiological and pathological functions. Therefore, selective inhibitors of COX-2 may be effective anti-inflammatory agents without the ulcerogenic effects associated with current NSAIDs, all of which inhibit both COX-1 and COX-2.[15]

We[16] and others[16–18] have found that the arylsulfonamides **1** (NS-398) and **2** (flosulide, CGP 28238) are potent and selective COX-2 inhibitors. Both **1**[19] and **2**[20–22] (Chart 1) have demonstrated good anti-inflammatory and/or analgesic activities in various animal models with gastrointestinal tolerability superior to that of traditional NSAIDs such as indomethacin. Bioavailability studies performed by us have indicated that **2** has good plasma levels after oral dosing in both rat and squirrel monkey while **1** has poor plasma levels in squirrel monkey. The low bioavailability of **1** in primate and the potential for toxicity due to the nitro group led us to focus on **2** as a lead structure.

Chart 1



1 2

Ether-linked phenyl analogs of **2** have been previously synthesized, but the structure–activity relationship (SAR) of these compounds was not reported in the context of their COX-2 activity and selectivity.[20] The corresponding thioether-linked analogs of **2** have not been reported. Our discovery that the thioether analog of **2** is a potent and selective COX-2 inhibitor with superior pharmacokinetic and *in vivo* activities led us to initiate extensive SAR studies on this series. Preliminary studies suggested that both the acidic hydrogen of the sulfonamide group and the carbonyl group of the indanone are essential for COX-2 activity in this series.[23] Our attention was therefore focused on the modification of the 2,4-difluorophenyl ring. Some sulfonamide modifications and various linkage units have also been investigated. In this report, we describe our SAR studies on the thioether analogs of **2** and the identification of 6-[(2,4-difluorophenyl)thio]-5-methanesulfonamido-1-indanone (**20**) (L-745,337) as the optimal compound.[24] The pharmacology of compound **20** is also discussed.

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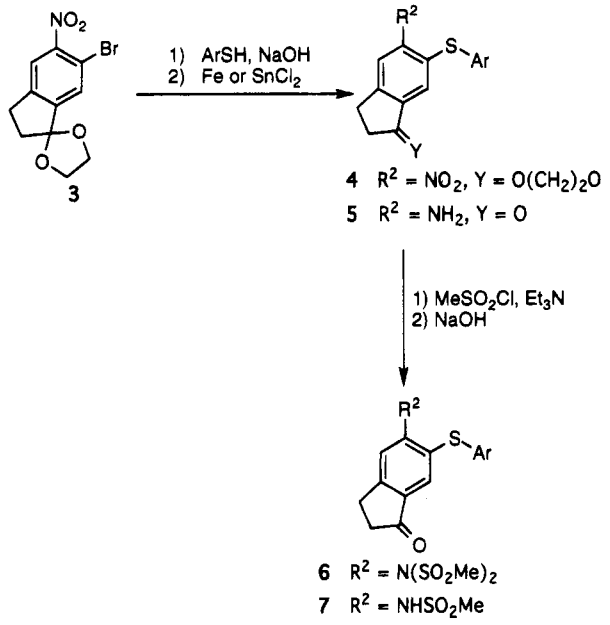
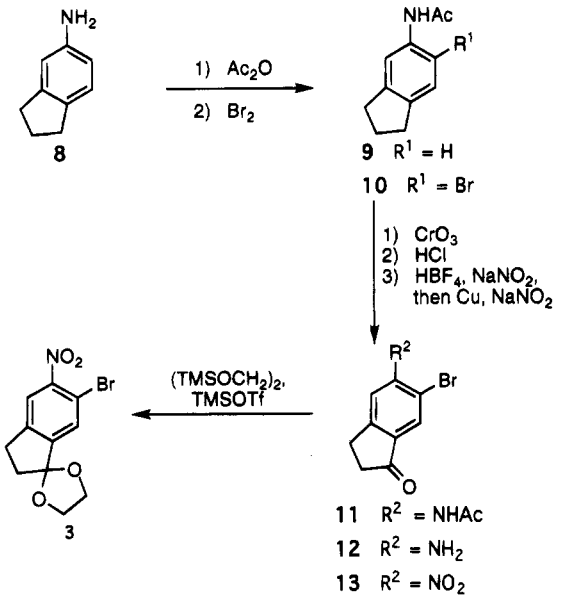
Scheme 1



Scheme 2

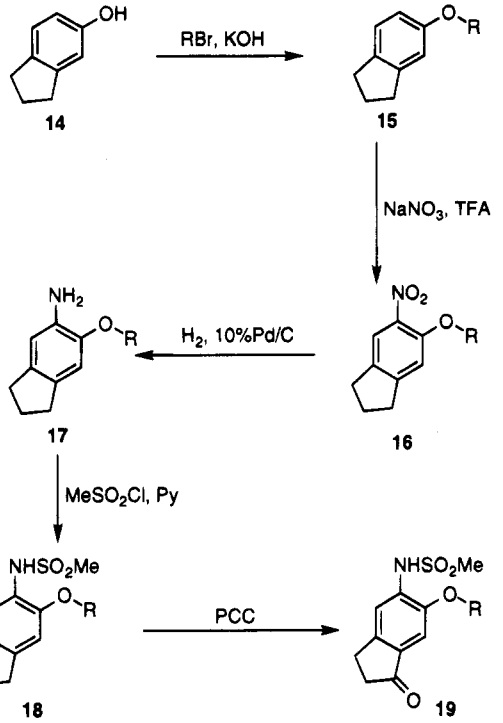


Chemistry

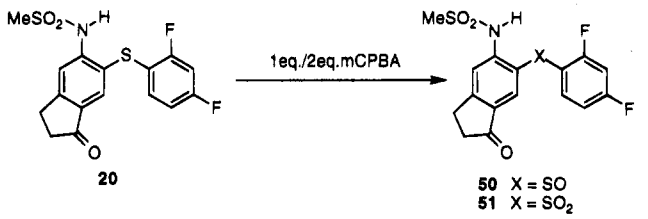
The thioether analogs of **2** were synthesized from a common intermediate, 5-nitro-6-bromo-1-indanone ethylene ketal (**3**), as shown in Scheme 1. Coupling with an appropriate nucleophile proceeded under basic conditions to give **4**. Reduction of the nitro group with iron powder or tin(II) chloride in aqueous ethanol with concomitant hydrolysis of the ketal group provided aminoindanone **5**. Sulfonylation with excess methanesulfonyl chloride in the presence of triethylamine yielded the corresponding bis-sulfonamide **6**, which upon hydrolysis with sodium hydroxide provided sulfonamide **7**. This approach was successfully used in the preparation of the substituted phenyl and heterocyclic analogs.

The key intermediate **3** was prepared from 5-aminoindane (**8**) (Scheme 2). Acetylation followed by bromination provided the 5-acetamido-6-bromoindane (**10**). Oxidation with chromium trioxide in aqueous acetic acid followed by hydrolysis gave 5-amino-6-bromo-1-indanone (**12**). The amino group of **12** was then subjected to diazotization followed by treatment of the diazonium salt with sodium nitrite in the presence of copper powder to provide nitro compound **13**. Attempts at displacing the bromine atom with nucleophiles instead resulted in *ipso* substitution of the nitro group. We reasoned that this was due to activation by the carbonyl group at the *para* position. Therefore, this carbonyl group was protected as a ketal using the conditions of Noyori[25] to provide **3**, which underwent substitution reactions with the desired regioselectivity.

Scheme 3



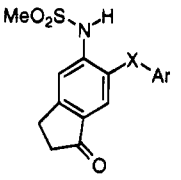
Scheme 4



The cyclohexyl thioether analog **49** was also synthesized according to Scheme 1. In the case of the cycloalkyl ether analogs, however, the compounds were more conveniently synthesized from indane **14** as shown in Scheme 3. Coupling of an appropriate cycloalkyl halide under basic conditions yielded ether **15**. Nitration with aqueous sodium nitrate in trifluoroacetic acid provided nitro compound **16** which could be reduced to provide aniline **17**. Sulfonylation then yielded **18**, which could be selectively oxidized with PCC to give indanone **19**.

With regard to the linkage unit, the nitrogen-linked analog **52** was prepared from **3** using the method described for the sulfur-linked compounds, although the reaction proceeded in lower yield. The sulfoxide-linked analog **50** and the sulfone-linked analog **51** were prepared in a straightforward manner by the selective oxidation of **20** (Scheme 4). The methylene-linked analog **53** was prepared from **12** in a multistep sequence (see the Experimental Section). Analogs with a modi-

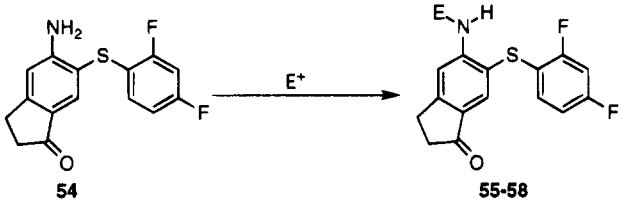
Table 1. Phenyl Analogs of Methanesulfonamido-1-indanone



compd no.	X	Ar	COX-2[a] IC_{50} (μM)	COX-1 IC_{50} (μM)	rat paw edema (ED_{30}, mpk)
2	O	2,4-F_2Ph	0.021 ± 0.006 (n = 3)	>50	0.60
20	S	2,4-F_2Ph	0.023 ± 0.008 (n = 7)	>50	0.21
21	S	2-FPh	0.1	>50*	
22	S	3-FPh	>10*		
23	S	4-FPh	~1	>50*	
24	S	2,6-F_2Ph	0.1−1	>50*	
25	S	4-BrPh	0.01	>10	~3.0
26	S	2,4-Cl_2Ph	0.01	~40	~0.1
27	S	2-MePh	0.1−1	>50*	
28	S	3-MePh	>10*		
29	S	4-MePh	0.05	≫1	~10
30	S	4-EtPh	0.1		
31	S	4-*i*-PrPh	>10*		
32	S	4-*t*-BuPh	>10		
33	S	2-MeOPh	>10		
34	S	4-MeOPh	0.1−1		
35	O	4-MeSPh	0.1		
36	O	4-$MeSO_2$Ph	>10		
37	S	4-CO_2HPh	>10		

[a] Each IC_{50} value is an average of at least two independent determinations. Those identified with an asterisk are the result of a single titration.

Scheme 5



54 **55-58**

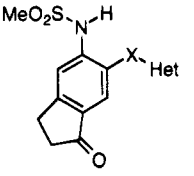
fied sulfonamide group were prepared from amine **54** using the appropriate electrophiles (Scheme 5).

Results And Discussion

Compounds prepared were initially tested for cyclooxygenase inhibition in a whole cell cyclooxygenase-2 assay. Subsequently, compounds found to have COX-2 IC_{50} < 0.1 μM were also tested in a whole cell cyclooxygenase-1 assay. These assays measured prostaglandin E_2 synthesis in response to arachidonic acid, using a radioimmunoassay. Cells used for these assays were human osteosarcoma 143 cells (which express COX-2 when cultured to confluency)[26] and human U-937 cells (which express COX-1).[27] In these assays, 100% activity is defined as the difference between prostaglandin E_2 synthesis in the absence and presence of arachidonate addition. Potent and COX-2 selective compounds were studied further in the rat paw edema assay.[28]

For the phenyl analogs (Table 1), 2,4-dihalogen substitution seemed to provide the most potent *in vitro* and *in vivo* activities. Compounds with monosubstitution at the 2- or 4-positions were less active. Alkyl groups larger than methyl were not tolerated at either the 2- or 4-position of the phenyl rings. Surprisingly, also, no substitution (neither halogen nor alkyl groups) at the 3-position of the phenyl ring was tolerated. The 2,4-difluorophenyl analog **20**, a thioether analog of **2**, was comparable to **2** in *in vitro* COX-2/COX-1 activity and

Table 2. Heterocyclic Analogs of Methanesulfonamido-1-indanone



compd no.	heterocycles	COX-2[a] IC_{50} (μM)	COX-1 IC_{50} (μM)	rat paw edema (ED_{30}, mpk)
38	2-thiazolyl	0.1	>10	0.85
39	4-thiazolyl	>10*		
40	5-thiazolyl	>10*		
41	2-thienyl	1		
42	3-thienyl	46% at 1*		
43	2-pyridyl	0.1−1	>10	
44	3-pyridyl	30% at 1		
45	4-pyridyl	1		

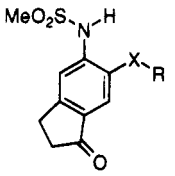
[a] Each IC_{50} value is an average of at least two independent determinations. Those identified with an asterisk are the result of a single titration.

selectivity. In the rat paw edema assay, however, compound **20** was more potent. The 4-bromophenyl analog **25** was very potent in the *in vitro* assay, but this activity did not translate to *in vivo* assays such as the rat paw edema assay. In contrast, the 2,4-dichlorophenyl analog **26** was very active both *in vitro* and *in vivo* but had lower selectivity for COX-2 over COX-1.

For the heterocyclic analogs (Table 2), only the 2-thiazolyl analog **38** showed reasonable potency; the 2-thienyl analogs **41** and the 2-pyridyl analog **43** were moderately active. Other heterocyclic analogs investigated were found to be significantly less active. Further investigation of other thiazole analogs indicated that the 4- and 5-thiazolyl analogs **39** and **40** were not potent.

In the case of cycloalkyl substituents (Table 3), the cyclohexyl analogs **46** and **49** were potent *in vitro* but

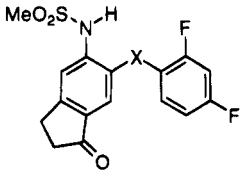
Table 3. Cycloalkyl Analogs of Methanesulfonamido-1-indanone



compd no.	X	R	COX-2[a] IC_{50} (μM)	COX-1 IC_{50} (μM)	rat paw edema (ED_{30}, mpk)
46	O	cyclohexyl	0.05	≫1	~10
47	O	cyclopentyl	≫1	>10	
48	O	4-tetrahydropyranyl	>10		
49	S	cyclohexyl	0.05	>50*	>3

[a] Each IC_{50} value is an average of at least two independent determinations. Those identified with an asterisk are the result of a single titration.

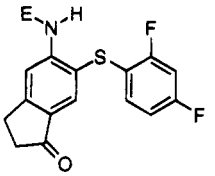
Table 4. Effect of the Linkage Unit on *in Vitro* Activities



compd no.	X	COX-2[a] IC_{50} (μM)	COX-1 IC_{50} (μM)
50	SO	27% at 1	
51	SO_2	>10*	
52	NH	1	
53	CH_2	>0.2	<1

[a] Each IC_{50} value is an average of at least two independent determinations. The value identified with an asterisk is the result of a single titration.

Table 5. Sulfonamide Modifications on *in Vitro* Activities



compd no.	E	COX-2[a] IC_{50} (μM)	COX-1 IC_{50} (μM)
55	SO_2Et	>10	≫10
56	SO_2CF_3	0.04	
57	Ac	>10	
58	$COCF_3$	>10	

[a] Each IC_{50} value is an average of at least two independent determinations.

showed low activity in the rat paw edema assay. The cyclopentyl analog **47** and the tetrahydropyranyl analog **48** were not potent inhibitors.

Various linkages between the indanone and the aryl ring have been studied (Table 4). Compounds with either an oxygen linkage or a sulfur linkage showed good activity, while other linkages were less potent and/or selective.

Modifications of the sulfonamide substituent have also been investigated (Table 5). Replacement of the methyl group with an ethyl group to give **55** resulted in the loss of COX-2 activity. The trifluoromethylsulfonyl group of **56** was tolerated, but the compound was somewhat unstable. The acetamide **57** and the trifluoroacetamide **58** were not potent.

Overall, the methanesulfonamido-1-indanone class of compounds exhibits very tight SAR. Only oxygen and sulfur links are well-tolerated, and only a limited number of substituents may be placed on the phenyl rings, with *ortho* and *para* substitution appearing to be optimal. Heterocyclic analogs in general are less active. The methanesulfonylamido group appears to tolerate very few changes. Considering the COX-1/COX-2 selectivity and *in vivo* potency, compound **20** (now designated as L-745,337) appears to be the optimal compound in this series. The pharmacology of **20** was therefore studied in detail.[29] A summary of the pharmacological results of **20** is discussed below.

Compound **20** is active in the *in vivo* models of inflammation, pain, pyrexia, and biochemical efficacy, and the potency of **20** is comparable to or greater than that of indomethacin.[29] In the carrageenan-induced rat paw edema, **20** (ED_{50} = 2.0 mg/kg) and indomethacin (ED_{50} = 2.0 mg/kg) were equipotent, but **20** was approximately 4 times more potent than indomethacin against carrageenan-induced rat paw hyperalgesia (ID_{50} values of 0.37 and 1.47 mg/kg, respectively)[30] and 2 times more potent than indomethacin in the endotoxin-induced rat pyresis (ID_{50} values of 3.8 and 7 mg/kg, respectively).[29] Compound **20** significantly inhibited the production of PGE_2 (80% at 3 mg/kg) in the inflammatory exudate in carrageenan-induced pleurisy in rats.[29] The tissue content of PGE_2 in the stomach was unaffected at the same dose. In both the rat paw hyperalgesia assay and the endotoxin-induced pyresis assay, **20** is able to completely reverse both the nociceptive[30] and pyretic responses.[29] Induction of COX-2 message and protein have been found in the rat paw after local injection of carrageenan,[11] in the rat brain after systematic administration of endotoxin,[11] and in the rat pleural exudate after intrapleural injection of carrageenan.[14] This would suggest that, at least in these models, COX-2-derived prostaglandins are largely responsible for both the acute nociception and the endotoxin-induced pyresis.

A major side effect of conventional NSAIDs is gastropathy manifested as gastric bleeding, ulceration, alterations in gut motility, emesis, and diarrhea and is thought to be caused by the inhibition of COX-1 in the gastrointestinal tract. It is conceivable that selective COX-2 inhibitors would possess a better safety profile and demonstrate a gastric sparing effect. We have measured gastric damage by both gross visual scoring and a fecal ^{51}Cr excretion assay in rats and squirrel monkeys. Compound **20** was without effect at doses up to 30 mg/kg in the visible gastric lesion study, while indomethacin produced visible, dose dependent hemorrhagic gastric lesions in rats 4 h after dosing.[29] In the fecal ^{51}Cr excretion assay[31-34] used to determine gastrointestinal integrity, compound **20** had no effect on fecal ^{51}Cr excretion at doses up to 100 mg/kg in rats or after chronic dosing at 10 mg/kg, bid, for 5 days in squirrel monkeys.[29] In contrast, indomethacin significantly induced ^{51}Cr excretion in rats (threshold dose between 3 and 10 mg/kg) and in squirrel monkeys (5 mg/kg, bid, 1 day).[29]

The profile of **20** was also compared with that of **2** (Table 6). Compound **20** and **2** were equipotent *in vitro*,

Table 6. Comparsion of Compound **20** with Compound **2**

	20	**2**
COX-2 (IC_{50}, μM)	0.023 ± 0.008	0.021 ± 0.006
COX-1 (IC_{50}, μM)	>50	>50
rat paw edema (ED_{30} mg/kg)	0.21	0.6
bioavailability in rats	F = 90% C_L = 0.5 mL/min	F = 92% C_L = 1.2 mL/min
bioavailability in squirrel monkeys	F = 145% C_L = 0.5 mL/min	F = 63% C_L = 1.2 mL/min
$T_{1/2}$ in squirrel monkeys[a]	6.9 ± 0.4 h n = 7	4.9 ± 0.8 h n = 5

[a] $T_{1/2}$ is the terminal half-life calculated from an iv administration at 1 mg/kg.

but **20** was more potent in the rat paw edema assay and has a longer $t_{1/2}$ in primates. More importantly, during a chronic dosing fecal ^{51}Cr excretion assay in rats at 25 mg/kg, bid, for 5 days, no significant chromium leakage was observed for **20**. On the other hand, **2** showed signs of gastric damage in this assay.[35]

In conclusion, **20** has been identified as a potent, selective, and orally active COX-2 inhibitor that has a superior or comparable efficacy profile compared with the conventional NSAID indomethacin in animal models of inflammation, pain, and fever. It is superior to **2** in both pharmacokinetic properties and ulcerogenicity sparing activity in animal models. In contrast with indomethacin, **20** was non-ulcerogenic within the dosage ranges required for functional efficacy. Selective COX-2 inhibitors such as **20** therefore may represent a new generation of NSAIDs with dramatically improved margins of safety.

Experimental Section

Chemistry. Melting points were determined on a Buchi 510 melting point apparatus in open capillary tubes and are uncorrected. ^1H NMR spectra were obtained on Bruker AM300 or Bruker AMX400 spectrometers, and proton chemical shifts are relative to tetramethylsilane as internal standard. Elemental analyses were performed by Oneida Research Services Inc., Whitesboro,NY. Substituted thiophenols used for the coupling reaction as described in Scheme 1 were either obtained from commerical sources or prepared from the corresponding anilines according to the procedures of Klages and Bott[36] or from the Newman–Kwart rearrangement of the corresponding phenols.[37,38]

Preparation of the Key Intermediate, 6-Bromo-5-nitro-1-indanone Ethylene Ketal (3). 5-Acetamidoindane (9). To a solution of 5-aminoindane (**8**) (10.0 g, 7.5 mmol) in CH_2Cl_2 (100 mL) was added dropwise acetic anhydride (9.2 g, 90.1 mmol) over a period of 15 min. After the mixture was stirred for a further 30 min, it was quenched with 1 M aqueous NaOH (100 mL). The CH_2Cl_2 layer was separated, washed successively with 1 M aqueous HCl and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Chromatography on silica gel, eluting with EtOAc:hexanes (1:1), afforded 12.2 g (85%) of **9** as a light brown powder: ^1H NMR ($CDCl_3$) δ 7.44 (s, 1H), 7.12 (three overlapping s, 3H), 2.88 (m, 4H), 2.15 (s, 3H), 2.06 (m, 2H).

5-Acetamido-6-bromoindane (10). To a solution of **9** (53.0 g, 0.30 mol) in glacial HOAc (1 L) at 10 °C was added dropwise over a period of 1 h a solution of Br_2 (19.0 mL, 0.37 mol) in HOAc. The mixture was further stirred at 10 °C for 15 min and then diluted with H_2O until no more precipitate formed. The precipitate was collected, washed with H_2O, and dried under vacuum to give 61 g (80%) of **10**: ^1H NMR ($CDCl_3$) δ 8.14 (s, 1H), 7.50 (s, 1H), 7.38 (s, 1H), 2.88 (m, 4H), 2.20 (s, 3H), 2.08 (m, 2H).

5-Acetamido-6-bromo-1-indanone (11). To a solution of **10** (43.0 g, 0.17 mol) in glacial HOAc (400 mL) at 50–55 °C was added dropwise a solution of CrO_3 (70.0 g, 0.7 mol) in 50% aqueous HOAc (400 mL) over a period of 30 min. After the mixture was stirred for a further 15 min, it was cooled to 0 °C and the reaction quenched with 2-propanol (100 mL). The solvent was removed *in vacuo*, and the residue was diluted with H_2O (1 L) and extracted with EtOAc (2 × 500 mL). The combined EtOAc layers were washed with 0.5 M aqueous NaOH (1 L) and brine, dried over anhydrous $MgSO_4$, and concentrated to give 36 g (80%) of **11** as a light brown solid which was contaminated with about 10% of 6-acetamido-5-bromo-1-indanone: ^1H NMR ($CDCl_3$) δ 8.60 (s, 1H), 7.98 (s, 1H), 7.90 (s, 1H), 3.10 (t, 2H, J = 6.0 Hz), 2.70 (t, 2H, J = 6.0 Hz), 2.30 (s, 3H).

5-Amino-6-bromo-1-indanone (12). A mixture of **11** (36.0 g, 0.13 mol) and 6 M aqueous HCl (800 mL) was refluxed for 1 h. The homogeneous solution was then cooled to 0 °C and adjusted to pH 8 with 10 M aqueous NaOH (~480 mL). The precipitate formed was collected, washed with H_2O, and dried under vacuum to afford 30.0 g (quantitative) of **12** as a light brown powder: ^1H NMR (acetone-d_6) δ 7.65 (s, 1H), 6.90 (s, 1H), 5.80 (brs, 2H), 2.95 (t, 2H, J = 6.0 Hz), 2.50 (t, 2H, J = 6.0 Hz).

6-Bromo-5-nitro-1-indanone (13). To a suspension of **12** (30.0 g, 0.13 mol) in 20% aqueous HBF_4 (120 mL) at 0 °C was added dropwise 4 M aqueous $NaNO_2$ (50 mL, 0.20 mol) over a period of 30 min. The mixture was stirred for 30 min after the addition was completed. The resulting foamy suspension was added portionwise to a vigorously stirred mixture of Cu powder (40 g, 0.62 mol) and $NaNO_2$ (120 g, 1.74 mol) in H_2O (240 mL) at room temperature over a period of 15 min. During the addition, excessive foaming was broken up by the addition of small amounts of Et_2O. After the mixture was stirred for a further 30 min, it was filtered through Celite and washed with EtOAc (5 × 300 mL). The EtOAc layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Chromatography on silica gel, eluting with hexanes: EtOAc (2:1), yielded 17.5 g (51%) of **13** as a pale yellow solid: ^1H NMR ($CDCl_3$) δ 8.10 (s, 1H), 7.85 (s, 1H), 3.20 (t, 2H, J = 6.0 Hz), 2.85 (t, 2H, J = 6.0 Hz); MS (DCI/CH_4) m/z 256 (M^+ + H).

6-Bromo-5-nitro-1-indanone Ethylene Ketal (3). To a suspension of **13** (11.0 g, 43 mmol) and bis[(trimethylsilyl)-oxy]ethane (22.0 mL, 90 mmol) in CH_2Cl_2 (90 mL) at room temperature was added trimethylsilyl trifluoromethanesulfonate (100 μL). The mixture was stirred for 2 h, and the reaction was quenched with saturated aqueous $NaHCO_3$ (100 mL). The CH_2Cl_2 layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Chromatography on silica gel, eluting with EtOAc:hexanes (2:5), furnished 10.2 g (79%) of **3** as a pale yellow solid: ^1H NMR ($CDCl_3$) δ 7.70 (s, 1H), 7.68 (s, 1H), 4.15 (m, 4H), 2.98 (t, 2H, J = 6.0 Hz), 2.38 (t, 2H, J = 6.0 Hz).

General Procedure for the Preparation of Phenyl and Heterocyclic Analogs of 5-Methanesulfonamido-1-indanone as Illustrated by 6-[(2,4-Difluorophenyl)thio]-5-methanesulfonamido-1-indanone (20).[39] **6-[(2,4-Difluorophenyl)thio]-5-nitro-1-indanone Ethylene Ketal (4a).** To a mixture of **3** (600 mg, 2.0 mmol) and 2,4-difluorothiophenol (440 mg, 3.0 mmol) in pyridine (4.0 mL) was added a solution of 8 M aqueous KOH (375 μL, 3.0 mmol) at room temperature. The mixture was stirred for 2 h, diluted with H_2O, and extracted with EtOAc. The EtOAc extract was washed successively with 1 M aqueous NaOH (2×), 0.5 M aqueous HCl, and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Chromatography on silica gel, eluting with toluene:EtOAc (10:1), afforded **4a** (590 mg, 81%) as a pale yellow solid: ^1H NMR ($CDCl_3$) δ 8.12 (s, 1H), 7.60 (m, 1H), 7.00 (m, 2H), 6.70 (s, 1H), 4.10–3.90 (m, 4H), 2.95 (t, 2H, J = 6.0 Hz), 2.30 (t, 2H, J = 6.0 Hz).

5-Amino-6-[(2,4-difluorophenyl)thio]-1-indanone (5a). A mixture of **4a** (580 mg, 1.59 mmol), iron powder (500 mg, 8.9 mmol), and NH_4Cl (50 mg, 0.93 mmol) in 30 mL of EtOH:H_2O (2:1) was refluxed for 1 h. The hot mixture was filtered through Celite and concentrated *in vacuo*. The residue was diluted with H_2O and extracted with EtOAc. The EtOAc extract was dried over anhydrous $MgSO_4$ and concentrated to give **5a** (410 mg, 81%) as a light brown solid: ^1H NMR ($CDCl_3$)

δ 7.95 (s, 1H), 7.00 (m, 1H), 6.80 (m, 2H), 6.72 (s, 1H), 4.95 (br s, 2H), 3.05 (t, 2H, J = 6.0 Hz), 2.65 (t, 2H, J = 6.0 Hz).

6-[(2,4-Difluorophenyl)thio]-5-methanesulfonamido-1-indanone (20). A mixture of **5a** (400 mg, 1.25 mmol), Et_3N (1.0 mL, 7.2 mmol), and MsCl (300 μL, 3.9 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 1 h. After the mixture was diluted with CH_2Cl_2 (20 mL), it was washed successively with saturated aqueous $NaHCO_3$, 1 M aqueous HCl, and brine. The CH_2Cl_2 layer was separated, dried over anhydrous $MgSO_4$, and concentrated to give the bis-sulfonamide as a dark solid.

To a solution of the crude bis-sulfonamide in MeOH:THF (2:1, 24 mL) at room temperature was added 10 M aqueous NaOH (375 μL, 3.75 mmol). The mixture was stirred at room temperature for 30 min and then acidified with 3 M aqueous HCl (1.5 mL). The solvent was evaporated *in vacuo*, and the residue was diluted with H_2O and extracted with EtOAc. The EtOAc layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated. Chromatography on silica gel, eluting with hexanes:EtOAc (1:1), yielded **20** (350 mg, 76%) as a light brown solid. Recrystallization from EtOH provided an analytically pure sample: mp 169–170 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (s, 1H), 7.98 (s, 1H), 7.78 (s, 1H), 7.30 (m, 1H), 6.88 (m, 2H), 3.16 (m, 2H), 3.06 (s, 3H), 2.70 (m, 2H). Anal. ($C_{16}H_{13}F_2NO_3S_2$) C, H, N.

Following the procedure described for compound **20**, the following compounds were obtained (overall yield, 20–50%) from the appropriate thiophenols, phenols, or heterocyclic thiols.

6-[(2-Fluorophenyl)thio]-5-methanesulfonamido-1-indanone (21): mp 153–154 °C; ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1H), 7.97 (brs, 1H), 7.28 (m, 1H), 7.15 (m, 1H), 7.08 (m, 2H), 3.13 (m, 2H), 2.97 (s, 3 H), 2.70 (m, 2H). Anal. ($C_{16}H_{14}FNO_3S_2$) C, H, N.

6-[(3-Fluorophenyl)thio]-5-methanesulfonamido-1-indanone (22): mp 153–154 °C; ^{1}H NMR ($CDCl_3$) δ 8.05 (s, 1H), 7.84 (s, 1H), 7.80 (s, 1H), 7.25 (m, 1H), 6.95–6.65 (m, 3H), 3.20 (m, 2H), 2.90 (s, 3H), 2.75 (m, 2H). Anal. ($C_{16}H_{14}FNO_3S_2$) C, H, N.

6-[(4-Fluorophenyl)thio]-5-methanesulfonamido-1-indanone (23): mp 175–176 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H), 7.80 (s, 1H), 7.76 (s, 1H), 7.16 (m, 2H), 6.98 (m, 2H), 3.16 (m, 2H), 2.88 (s, 3H), 2.72 (m, 2H). Anal. ($C_{16}H_{14}FNO_3S_2$) C, H, N.

6-[(2,6-Difluorophenyl)thio]-5-methanesulfonamido-1-indanone (24): mp 166–168 °C; ^{1}H NMR ($CDCl_3$) δ 8.22 (brs, 1H), 8.03 (s, 1H), 7.72 (s, 1H), 7.32 (m, 1H), 6.96 (m, 2H), 3.11 (m, 2H), 3.05 (s, 3H), 2.65 (m, 2H); exact mass (FAB) $C_{16}H_{13}F_2NO_3S_2 + H^+$ calcd 370.038 32, found 370.038 23.

6-[(4-Bromophenyl)thio]-5-methanesulfonamido-1-indanone (25): mp 189–191 °C; ^{1}H NMR (acetone-d_6) δ 9.15 (brs, 1H), 7.75 (s, 1H), 7.65 (s, 1H), 7.54 (d, 2H, J = 7.0 Hz), 7.22 (d, 2H, J = 7.0 Hz), 3.18 (m, 2H), 3.10 (s, 3H), 2.65 (m, 2H); exact mass (FAB) $C_{16}H_{14}BrNO_3S_2 + H^+$ calcd 411.967 67, found 411.967 58.

6-[(2,4-Dichlorophenyl)thio]-5-methanesulfonamido-1-indanone (26): mp 214–216 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H), 7.82 (s, 1H), 7.78 (s, 1H), 7.46 (d, 1H, J = 3.0 Hz), 7.10 (dd, 1H, J = 7.5, 3.0 Hz), 6.68 (d, 1H, J = 7.5 Hz), 3.20 (m, 2H), 3.02 (s, 3H), 2.78 (m, 2H).

6-(*o*-Tolylthio)-5-methanesulfonamido-1-indanone (27): mp 160–162 °C; ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1H), 7.82 (s, 1H), 7.68 (brs, 1H), 7.23 (m, 1H), 7.15 (m, 1H), 7.05 (m, 1H), 6.76 (m, 1H), 3.18 (m, 2H), 2.82 (s, 3H), 2.75 (m, 2H). Anal. ($C_{17}H_{17}NO_3S_2$) C, H, N.

6-(*m*-Tolylylthio)-5-methanesulfonamido-1-indanone (28): mp 166–167 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1H), 7.84 (s, 1H), 7.80 (s, 1H), 7.35–7.05 (m, 5H), 3.18 (m, 2H), 2.78 (s, 3H), 2.75 (m, 2H). Anal. ($C_{17}H_{17}NO_3S_2$) C, H, N.

6-(*p*-Tolylthio)-5-methanesulfonamido-1-indanone (29): mp 185–186 °C; ^{1}H NMR ($CDCl_3$) δ 8.04 (s, 1H), 7.82 (s, 1H), 7.78 (s, 1H), 7.10 (s, 4H), 3.16 (m, 2H), 2.78 (s, 3H), 2.72 (m, 2H), 2.30 (s, 3H). Anal. ($C_{17}H_{17}NO_3S_2$) C, H, N.

6-[(4-Ethylphenyl)thio]-5-methanesulfonamido-1-indanone (30): mp 125–126 °C; ^{1}H NMR ($CDCl_3$) δ 8.04 (s, 1H), 7.80 (brs, 1H), 7.77 (s, 1H), 7.12 (s, 4H), 3.18 (m, 2H), 2.77 (s, 3H), 2.73 (m, 2H), 2.61 (q, 2H, J = 7.5 Hz), 1.19 (t, 3H, J = 7.5 Hz). Anal. ($C_{18}H_{19}NO_3S_2$) C, H, N.

6-[(4-Isopropylphenyl)thio]-5-methanesulfonamido-1-indanone (31): mp 134–135 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1H), 7.85 (s, 1H), 7.80 (s, 1H), 7.14 (m, 4H), 3.16 (m, 2H), 2.85 (m, 1H), 2.71 (m, 2H), 2.70 (s, 3H), 1.20 (d, 6H, J = 7.5 Hz). Anal. ($C_{19}H_{21}NO_3S_2$) C, H, N.

6-[(4-*tert*-Butylphenyl)thio]-5-methanesulfonamido-1-indanone (32): mp 159–160 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (s, 1H), 7.85 (brs, 1H), 7.80 (s, 1H), 7.78 (d, 2H, J = 7.5 Hz), 7.12 (d, 2H, J = 7.5 Hz), 3.16 (m, 2H), 2.74 (m, 2H), 2.70 (s, 3H), 1.28 (s, 9H). Anal. ($C_{20}H_{23}NO_3S_2$) C, H, N.

6-[(2-Methoxyphenyl)thio]-5-methanesulfonamido-1-indanone (33): mp 166–168 °C; ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1H), 8.10 (s, 1H), 7.74 (s, 1H), 7.29–7.24 (m, 1H), 7.20–7.15 (m, 1H), 6.91–6.81 (m, 2H), 3.95 (s, 3H), 3.15–3.10 (m, 2H), 2.80 (s, 3H), 2.71–2.65 (m, 2H); exact mass (FAB) $C_{17}H_{17}NO_4S_2 + H^+$ calcd 364.067 73, found 364.067 84.

6-[(4-Methoxyphenyl)thio]-5-methanesulfonamido-1-indanone (34): mp 136–137 °C; ^{1}H NMR ($CDCl_3$) δ 7.98 (s, 1H), 7.80 (brs, 1H), 7.72 (s, 1H), 7.20 (d, 2H, J = 7.0 Hz), 6.72 (d, 2H, J = 7.0 Hz), 3.76 (s, 3H), 3.13 (m, 2H), 2.79 (s, 3H), 2.69 (m, 2H); exact mass (FAB) $C_{17}H_{17}NO_4S_2 + H^+$ calcd 364.067 73, found 364.067 84.

6-[(4-Methylthio)phenoxy]-5-methanesulfonamido-1-indanone (35): mp 174–176 °C; ^{1}H NMR (acetone-d_6) δ 8.65 (brs, 1H), 7.79 (s, 1H), 7.38 (d, 2H, J = 10 Hz), 7.08 (d, 1H, J = 10 Hz), 7.01 (s, 1H), 3.20 (s, 3H), 3.14 (m, 2H), 2.62 (m, 2H), 2.50 (s, 3H). Anal. ($C_{17}H_{17}NO_3S_3$) C, H, N.

6-[(4-Methylsulfonyl)phenoxy]-5-methanesulfonamido-1-indanone (36): mp 220–222 °C; ^{1}H NMR ($CDCl_3$) δ 7.90 (d, 2H, J = 10 Hz), 7.72 (s, 1H), 7.22 (s, 1H), 7.12 (d, 2H, J = 10 Hz), 3.10 (m, 2H), 3.08 (s, 3H), 3.04 (s, 3H), 2.67 (m, 1H); MS (FAB) m/z 396 ($M^+ + H$).

6-[(4-Carboxyphenyl)thio]-5-methanesulfonamido-1-indanone (37): mp 258 °C dec; ^{1}H NMR (acetone-d_6) δ 7.90 (d, 2H, J = 7.0 Hz), 7.80 (s, 1H), 7.70 (s, 1H), 7.14 (d, 2H, J = 7.0 Hz), 3.22 (m, 2H), 3.00 (s, 3H), 2.72 (m, 2H). Anal. ($C_{17}H_{15}NO_5S_2$) C, H, N.

6-(2-Thiazolylthio)-5-methanesulfonamido-1-indanone (38): mp 194–195 °C; ^{1}H NMR ($CDCl_3$) δ 8.90 (brs, 1H), 8.10 (s, 1H), 7.88 (s, 1H), 7.70 (d, 1H, J = 3.4 Hz), 7.30 (d, 1H, J = 3.4 Hz), 3.16 (m, 2H), 3.06 (s, 3H), 2.70 (m, 2H). Anal. ($C_{13}H_{12}N_2O_3S_3$) C, H, N.

6-(4-Thiazolylthio)-5-methanesulfonamido-1-indanone (39): mp 161–163 °C; ^{1}H NMR ($CDCl_3$) δ 8.84 (s, 1H), 8.00 (s, 1H), 7.95 (s, 1H), 7.80 (brs, 1H), 7.74 (s, 1H), 3.14 (m, 2H), 3.00 (s, 3H), 2.70 (m, 2H). Anal. ($C_{13}H_{12}N_2O_3S_3$) C, H, N.

6-(5-Thiazolylthio)-5-methanesulfonamido-1-indanone (40): mp 169–171 °C; ^{1}H NMR ($CDCl_3$) δ 9.10 (s, 1H), 8.78 (s, 1H), 8.06 (s, 1H), 7.80 (s, 1H), 7.36 (s, 1H), 3.15 (m, 2H), 3.08 (s, 3H), 2.68 (m, 2H); exact mass (FAB) $C_{13}H_{12}N_2O_3S_3 + H^+$ calcd 341.008 83, found 341.008 73.

6-(2-Thienylthio)-5-methanesulfonamido-1-indanone (41): mp 159–160 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H), 7.80 (brs, 1H), 7.75 (s, 1H), 7.38 (m, 1H), 7.25 (m, 1H), 7.00 (m, 1H), 3.14 (m, 2H), 2.90 (s, 3H), 2.70 (m, 2H). Anal. ($C_{14}H_{13}NO_3S_3$) C, H, N.

6-(3-Thienylthio)-5-methanesulfonamido-1-indanone (42): mp 166–167 °C; ^{1}H NMR (acetone-d_6) δ 8.32 (s, 1H), 7.75 (s, 1H), 7.72 (s, 1H), 7.61–7.56 (m, 1H), 7.56–7.53 (m, 1H), 7.07–7.04 (m, 1H), 3.20–3.13 (m, 2H), 3.08 (s, 3H), 2.66–2.60 (m, 2H). Anal. ($C_{14}H_{13}NO_3S_3$) C, H, N.

6-(2-Pyridylthio)-5-methanesulfonamido-1-indanone (43): mp 174–175 °C; ^{1}H NMR ($CDCl_3$) δ 8.90 (brs, 1H), 8.38 (m, 1H), 8.08 (s, 1H), 7.88 (s, 1H), 7.60 (m, 1H), 7.25–7.05 (m, 2H), 3.20 (m, 2H), 3.06 (s, 3H), 2.75 (m, 2H). Anal. ($C_{15}H_{14}N_2O_3S_2$) C, H, N.

6-(3-Pyridylthio)-5-methanesulfonamido-1-indanone (44): mp 150–152 °C; ^{1}H NMR (acetone-d_6) δ 8.50–8.40 (m, 3H), 7.87–7.81 (m, 2H), 7.65–7.57 (m, 1H), 7.36–7.30 (m, 1H), 3.27–3.18 (m, 2H), 3.04 (s, 3H), 2.70–2.63 (m, 2H). Anal. ($C_{15}H_{14}N_2O_3S_2$) C, H, N.

6-(4-Pyridylthio)-5-methanesulfonamido-1-indanone (45): mp 215 °C dec; ^{1}H NMR ($CDCl_3$) δ 8.40 (m, 2H), 8.05 (s,

1H), 7.88 (s, 1H), 7.80 (brs, 1H), 6.95 (m, 2H), 3.22 (m, 2H), 3.04 (s, 3H), 2.75 (m, 2H); exact mass (FAB) $C_{15}H_{14}N_2O_3S_2$ + H^+ calcd 335.052 41, found 335.052 35.

Preparation of Cycloalkyl Ether Analogs. 6-(Cyclohexyloxy)-5-methanesulfonamido-1-indanone (46). Step 1: 5-(Cyclohexyloxy)indane. To a room temperature suspension of 5-indanol (6.1 g, 45.2 mmol) in 20 mL of pyridine was added powdered KOH (2.5 g, 44.9 mmol). The resulting viscous solution was treated with cyclohexyl bromide (7.0 mL, 56.8 mmol), heated to reflux for 3 h, cooled, and partitioned between 1 M NaOH and ether. The organic layer was washed with 1 M HCl (2×) and brine and then dried over anhydrous Na_2SO_4. Purification by flash chromatography (5% EtOAc: hexanes) provided 1.70 g (17%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 7.10 (d, 1H, J = 7.5 Hz), 6.80 (d, 1H, J = 2.0 Hz), 6.69 (dd, 1H, J = 7.5, 2.0 Hz), 4.18 (m, 1H), 2.86 (m, 4H), 2.08 (m, 2H), 2.00 (m, 2H), 1.80 (m, 2H), 1.50 (m, 3H), 1.35 (m, 3H).

Step 2: 5-(Cyclohexyloxy)-6-nitroindane. To a solution of 5-(cyclohexyloxy)indane (1.7 g, 7.9 mmol) at 10 °C was added dropwise a solution of $NaNO_3$ (737 mg, 8.7 mmol) in 3 mL of H_2O. After the mixture was stirred for 10 min, it was diluted with ether, and washed successively with 1 M NaOH, H_2O, and brine, dried over anhydrous $MgSO_4$, and concentrated. Chromatography on silica gel, eluting with 15% EtOAc: hexanes afforded 723 mg (35%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 7.63 (s, 1H), 6.93 (s, 1H), 2.90 (m, 4H), 2.12 (m, 2H), 1.94 (m, 2H), 1.80 (m, 2H), 1.65 (m, 2H), 1.35 (m, 4H).

Step 3: 5-Amino-6-(cyclohexyloxy)indane. To a solution of 5-(cyclohexyloxy)-6-nitroindane (720 mg, 2.8 mmol) in EtOAc (5 mL) and EtOH (40 mL) was added 10% Pd/C (730 mg). The mixture was shaken under 60 psi H_2 for 1.5 h and then filtered through Celite. The Celite was washed with EtOAc, and the filtrate was concentrated to give 582 mg (90%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 6.74 (s, 1H), 6.61 (s, 1H), 4.16 (m, 1H), 3.70 (brs, 2H), 2.82 (m, 4H), 2.05 (m, 4H), 1.78 (m, 2H), 1.60 (m, 3H), 1.35 (m, 3H).

Step 4: 5-(Cyclohexyloxy)-6-methanesulfonamidoindane. To a solution of 5-amino-6-(cyclohexyloxy)indane (580 mg, 2.5 mmol) in CH_2Cl_2 (30 mL) were added pyridine (3 mL) and MsCl (300 μL, 3.9 mmol). After the mixture was stirred for 40 min, it was partitioned between water and CH_2Cl_2. The organic layer was washed with 1 M HCl, 1 M $CuSO_4$, and brine, filtered through cotton, and evaporated. Chromatography on silica gel with EtOAc:hexanes (1:2) provided 700 mg (90%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 7.48 (s, 1H), 6.80 (s, 1H), 6.65 (s, 1H), 4.25 (m, 1H), 2.92 (s, 3H), 2.84 (m, 4H), 2.08 (m, 2H), 2.00 (m, 2H), 1.79 (m, 2H), 1.60–1.30 (m, 6H).

Step 5: 6-(Cyclohexyloxy)-5-methanesulfonamido-1-indanone (46). To a solution of 5-(cyclohexyloxy)-6-methanesulfonamidoindane (655 mg, 2.1 mmol) in benzene (15 mL) at reflux was added portionwise a ground mixture of PCC (2.2 g, 10.2 mmol) and Celite (4.1 g). The black slurry mixture was stirred for 1 h, cooled, and diluted with EtOAc. The mixture was filtered through Celite, washed with EtOAc, and evaporated. Chromatography on silica gel and elution with EtOAc:hexanes (1:1) afforded 233 mg (34%) of **46**: mp 171–172 °C; ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1H), 7.24 (s, 1H), 7.20 (s, 1H), 4.48 (m, 1H), 3.10 (s, 3H), 3.06 (m, 2H), 2.68 (m, 2H), 2.10–1.25 (m, 10H); exact mass (FAB) $C_{16}H_{21}NO_4S$ + H^+ calcd 324.1270, found 324.1269.

Following the procedure described for compound **46**, the following compounds were obtained.

6-(Cyclopentyloxy)-5-methanesulfonamido-1-indanone (47): mp 167–168 °C; ^{1}H NMR (acetone-d_6) δ 8.02 (brs, 1H), 7.60 (s, 1H), 7.16 (s, 1H), 5.0 (m, 1H), 3.10 (s, 3H), 3.06 (m, 2H), 2.60 (m, 2H), 2.10–1.55 (m, 8H); exact mass (FAB) $C_{15}H_{19}NO_4S$ + H^+ calcd 310.111 31, found 310.111 29.

6-(4-Tetrahydropyranyloxy)-5-methanesulfonamido-1-indanone (48): mp 217–218 °C; ^{1}H NMR (DMSO-d_6) δ 7.50 (s, 1H), 7.24 (s, 1H), 4.79–4.69 (m, 1H), 3.93–3.82 (m, 2H), 3.53–2.42 (m, 2H), 3.13 (s, 3H), 3.04–2.95 (m, 2H), 2.64–2.57 (m, 2H), 1.99–1.89 (m, 2H), 1.77–1.62 (m, 2H). Anal. ($C_{15}H_{19}O_5SN$) C, H, N.

The cycloalkyl thioether analog **49** was obtained from the procedure described for the preparation of **20** by using cyclohexylthiol in the coupling reaction with **3**.

6-(Cyclohexylthio)-5-methanesulfonamido-1-indanone (49): ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H), 7.96 (s, 1H), 7.70 (s, 1H), 3.16 (m, 2H), 3.12 (s, 3H), 2.90 (m, 1H), 2.70 (m, 2H), 1.95–1.10 (m, 10H); exact mass (FAB) $C_{16}H_{21}NO_3S_2$ calcd 340.104 11, found 340.104 02.

Modifications of the Linkage Unit. 6-(2,4-Difluorophenylsulfinyl)-5-methanesulfonamido-1-indanone (50). To a solution of **20** (120 mg, 0.32 mmol) in CH_2Cl_2 at 0 °C was added mCPBA (70 mg, ~0.32 mmol, Aldrich, 57–86% pure) in one portion. The mixture was stirred at room temperature for 1 h. Solvent was evaporated *in vacuo*. The residue was swished with EtOH and then chromatographed on silica gel, eluting with hexanes:EtOAc (1:3) containing 5% of MeOH, to give **50** (80 mg, 65%) as a white solid: mp 208–210 °C; ^{1}H NMR ($CDCl_3$) δ 10.16 (s, 1H), 8.00 (m, 2H), 7.66 (s, 1H), 7.20 (m, 1H), 6.85 (m, 1H), 3.15 (m, 2H), 3.00 (s, 3H), 2.72 (m, 2H); exact mass (FAB) $C_{16}H_{13}F_2NO_4S_2$ + H^+ calcd 386.033 23, found 386.033 33.

6-(2,4-Difluorophenylsulfonyl)-5-methanesulfonamido-1-indanone (51). The title compound was prepared by the oxidation of **20** with 2.5 equiv of mCPBA: ^{1}H NMR ($CDCl_3$) δ 9.38 (brs, 1H), 8.36 (s, 1H), 8.16 (m, 1H), 7.80 (s, 1H), 7.14 (m, 1H), 6.90 (m, 1H), 3.20 (m, 2H), 3.15 (s, 3H), 2.75 (m, 2H); exact mass (FAB) $C_{16}H_{13}F_2NO_5S_2$ + H^+ calcd 402.028 18, found 402.027 97.

The nitrogen linkage analog **52** was obtained from the procedure described for the preparation of **20** by using 2,4-difluoroaniline in the coupling reaction with **3**.

6-[(2,4-Difluorophenyl)amino]-5-methanesulfonamido-1-indanone (52): ^{1}H NMR ($CDCl_3$) δ 7.70 (s, 1H), 7.52 (s, 1H), 7.45 (brs, 1H), 6.90 (m, 1H), 6.75 (m, 1H), 6.56 (m, 1H), 5.34 (s, 1H), 3.16 (m, 2H), 3.12 (s, 3H), 2.70 (m, 2H).

The methylene-linked compound **53** was periliminarily prepared from **12** in low overall yield and has not been optimized.

6-(2,4-Difluorobenzyl)-5-methanesulfonamido-1-indanone (53). The amino group of **12** was protected as a *tert*-butyl carbamate; the carbonyl group was reduced and protected as a *tert*-butyl silyl ether. The resulting intermediate 6-bromo-5-[(*tert*-butoxycarbonyl)amino]-1-[(*tert*-butyldimethylsilyl)oxy]indane was treated with 3 equiv of *tert*-butyllithium at −78 °C, converted to the cuprate by reaction with copper(I) cyanide, and reacted with 2,4-difluorobenzyl bromide. The silyl ether of the coupling product was cleaved, and the resulting hydroxyl group was oxidized with PDC in DMF at room temperature to provide 6-(2,4-difluorobenzyl)-5-[(*tert*-butoxycarbonyl)amino]-1-indanone. Removal of the *tert*-butyloxycarbonyl group followed by bis-mesylation and hydrolysis yielded **53**: mp 171–173 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H), 7.58 (s, 1H), 7.02 (m, 1H), 6.85 (m, 2H), 6.65 (brs, 1H), 3.94 (s, 2H), 3.10 (m, 2H), 2.92 (s, 3H), 2.68 (m, 2H); exact mass (FAB) $C_{17}H_{15}F_2NO_3S$ + H^+ calcd 352.081 90, found 352.081 79.

Sulfonamide Modifications. Following the procedure described for the preparation of **20** from **5a**, the following compounds were obtained by using the appropriate electrophiles.

6-[(2,4-Difluorophenyl)thio]-5-ethanesulfonamido-1-indanone (55): mp 133–134 °C; ^{1}H NMR ($CDCl_3$) δ 7.95 (two overlapping s, 2H), 7.75 (s, 1H), 7.25 (m, 1H), 6.85 (m, 2H), 3.25 (q, 2H, J = 7.5 Hz), 3.15 (m, 2H), 2.70 (m, 2H), 1.35 (t, 3H, J = 7.5 Hz). Anal. ($C_{17}H_{15}F_2NO_3S_2$) C, H, N.

6-[(2,4-Difluorophenyl)thio]-5-trifluoromethanesulfonamido-1-indanone (56): ^{1}H NMR (acetone-d_6) δ 7.58 (m, 1H), 7.54 (s, 1H), 7.14 (m, 2H), 6.72 (s, 1H), 2.94 (m, 2H), 2.44 (m, 2H); exact mass (FAB) $C_{16}H_{10}F_5NO_3S_2$ + H^+ calcd 424.010 07, found 424.010 02.

6-[(2,4-Difluorophenyl)thio]-5-acetamido-1-indanone (57): mp 154–155 °C; ^{1}H NMR ($CDCl_3$) δ 8.78 (brs, 1H), 8.60 (s, 1H), 8.00 (s, 1H), 7.25 (m, 1H), 6.85 (m, 2H), 3.15 (m, 2H), 2.68 (m, 2H), 2.25 (s, 3H). Anal. ($C_{17}H_{13}F_2NO_2S$) C, H, N.

6-[(2,4-Difluorophenyl)thio]-5-(trifluoroacetamido)-1-indanone (58): mp 151–153 °C; ^{1}H NMR ($CDCl_3$) δ 9.70 (brs,

1H), 8.58 (s, 1H), 8.06 (s, 1H), 7.35 (m, 1H), 6.85 (m, 2H), 3.18 (m, 2H), 2.70 (m, 2H). Anal. ($C_{17}H_{10}F_5NO_2S$) C, H, N.

Whole Cell COX-1 and COX-2 Assays. The human osteosarcoma cell line 143.98.2 has been shown to selectively express COX-2 by the reverse transcriptase polymerase chain reaction, Northern blot, and immunoblot techniques,[26] while the undifferentiated human histiocytic lymphoma U-937 cells selectively express COX-1.[27] The production of PGE_2 by these cell lines following stimulation by arachidonic acid was used as a cell-based assay for COX-2 and COX-1, respectively. Osteosarcoma cells were cultured in 1 mL of media in 24-well multidishes (Nunclon) until confluent ($(1-2) \times 10^5$ cells/well). U-937 cells were grown in spinner flasks and resuspended to a final density of 1.5×10^6 cells/mL in 24-well multidishes. Following washing and resuspension of osteosarcoma and U-937 cells in 1 mL of Hank's balanced salts solution (HBSS), 1 μL of a DMSO solution of test compound or DMSO vehicle was added, and samples were gently mixed. All assays were performed in triplicate. Samples were then incubated for 15 min at 37 °C, prior to the addition of arachidonic acid. Arachidonic acid was prepared as a 10 mM stock solution in ethanol and further diluted 10-fold in HBSS. An aliquot of 10 μL of this diluted solution was added to the cells to give a final arachidonic acid concentration of 10 μM. Samples were again gently mixed and incubated for a further 10 min at 37 °C. For osteosarcoma cells, reactions were then stopped by the addition of 100 μL of 1 N HCl, with mixing, and by the rapid removal of the solution from cell monolayers. For U-937 cells, reactions were stopped by the addition of 100 μL of 1 N HCl and with mixing. Samples were then neutralized by the addition of 100 μL of 1 N NaOH and PGE_2 levels measured by radioimmunoassay. In these assays, 100% activity is defined as the difference between PGE_2 synthesis in the absence and presence of arachidonic acid.

Pharmacological Methods. Detailed experiments have been described by Chan.[29] The carrageenan-induced rat paw edema assay was carried out using procedures described by Otterness and Moore.[40] Carrageenan-induced rat paw hyperalgesia was measured using the procedures originally described by Randall and Selitto.[41] Carrageenan-induced pleurisy in rats was carried out according to the method described by Di Rosa.[42]

Endotoxin-Induced Pyresis in Rats.[29] Male Sprague–Dawley rats (150–200 g) were used. They were fasted for 16–18 h before use. At approximately 9:30 a.m., the animals were placed in plexiglass restrainers temporarily, and the resting rectal temperature was recorded using a flexible temperature probe (YSI series 400) connected to a digital thermometer (Model 08502, Cole Parmer). The same probe and thermometer were used for all animals to reduce experimental error. The animals were returned to their cages after the temperature measurements. At time zero, the rats were injected interperitoneally with either saline or LPS (1.8 mg/kg) and the rectal temperature was measured 5, 6, and 7 h following LPS injection. After the measurement at 5 h, when the increase in rectal temperature had reached a plateau, the LPS-injected rats were given orally either the vehicle or a test compound to determine whether the rise in temperature could be reversed. Percent reversal (antipyretic activity) was calculated using the rectal temperature obtained at 7 h, taking this value in the vehicle control group as zero reversal.

***In Vivo* Assays for Ulcerogenicity.**[29] **(1) Visible Gastric Lesions in Rats.** Fasted male Sprague–Dawley rats (150–200 g) were dosed orally with a test compound or vehicle (1% methocel, 1 mL/100 g of body weight). Four hours later, the animals were euthanized and the stomachs excised along their greater curvature. After the mucosa was rinsed with normal saline, it was examined for the presence of petechiae or frank lesions. Petechiae were assigned a score of 1 and lesions were scored according to their length (a score of 5 for lesions with length between 1 and 3 mm, a score of 10 for lesions greater than 3 mm). The sum of the total scores was used for comparison. All treatment groups were coded to prevent measurement bias.

(2) ^{51}Cr Fecal Excretion in Rats.[29] Test compounds or vehicls were dosed orally as above, followed immediately by injection into the tail vein of 0.5 mL of ^{51}Cr-labeled red blood cells from a donor rat (see procedures below). The animals were placed individually in metabolism cages with food and water *ad lib*. Feces were collected for a 48 h period, and ^{51}Cr fecal excretion was calculated as a percent of total injected dose. ^{51}Cr-labeled red blood cells were prepared using the following procedures. Ten mL of blood was obtained via the vena cava from a donor rat in heparinized tubes. Plasma was removed by centrifugation and replenished with an equal volume of HBSS. The red blood cells were incubated with 400 μCi of $Na_2{}^{51}CrO_4$ for 30 min at 37 °C. At the end of the incubation, the red blood cells were washed twice with 20 mL of HBSS to remove free $Na_2{}^{51}CrO_4$. The red blood cells were reconstituted finally in 10 mL of HBSS, and 0.5 mL of the solution (about 20 μCi) was injected per rat.

(3) ^{51}Cr Fecal Excretion in Squirrel Monkeys.[29] Squirrel monkeys (*Saimiri sciureus*, 0.8–1.4 kg) were dosed orally with a test compound or vehicle (1% methocel, 1 mL/kg) twice daily at 8 h apart for 1–5 days as indicated. Food and water were allowed *ad lib*. One hour after administration of the last dose, $^{51}CrCl_2$ in sterile saline (1 mL/kg, equivalent to 4–5 μCi per animal) was injected via a saphenous vein and plasma obtained for measurement of drug concentration. The monkeys were then housed individually in metabolism cages, and feces were collected for a 24 h period. ^{51}Cr fecal excretion was calculated as a percent of total injected dose.

Measurement of Plasma Levels of Compound 20. In the po studies, the compound was dosed at 10 mg/kg as a solution in 1% methocel (dose volume = 1 mL/100 g of body weight). Blood was taken 0, 0.25, 0.5, 1, 2, 4, and 6 h after dosing. In the iv studies, compounds were dissolved in 5% dextrose and injected intravenously at a dose of 5 mg/kg (dose volume = 0.1 mL/100 g of body weight). Blood was taken 0, 0.08, 0.25, 0.5, 1, 2, 4, and 6 h after dosing. Blood was centrifuged and plasma collected. To 150 μL of each plasma sample was added an equal volume of acetonitrile to precipitate the protein. An aliquot (30 μL) of the supernatant after centrifugation was subjected to reversed-phase HPLC on a 4 μm Nova Pak C18 column (3.9 × 150 mm, Waters, Milford, MA). The solvent system was absolute methanol:10 mM phosphate buffer (pH 7.1) (35:65) at a flow rate of 1 mL/min; samples were monitored at 340 nm. The parent compound was quantitated from the area of the corresponding peak, relative to the standard (plasma sample at time 0 spiked with the parent compound corresponding to a plasma concentration of 6.7 μg/mL).

References

(1) Preliminary report: Li, C.-S.; Boily, C.; Black, C.; Chan, C. C.; Ford-Hutchinson, A. W.; Guay, D.; Kargman, S.; Lau, C. K.; Mancini, J.; Ouimet, N.; Prasit, P.; Roy, B.; Roy, P.; Scheigetz, J.; Targari, P.; Vickers, P.; Wong, E.; Young, R. N.; Zamboni, R. Synthesis and Pharmacological Activities of A New Cyclooxygenase-2 Inhibitor. Presented at the 208th National Meeting of the American Chemical Society, Washington DC, August 1994; MEDI 0116.

(2) DeWitt, D. L.; Smith, W. L. Primary Structure of Prostaglandin G/H Synthase from Sheep Vesicular Gland Determined from the Complementary DNA Sequence. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1412–1416.

(3) DeWitt, D. L.; El-Harith, E. A.; Kraemer, S. A.; Andrews, M. J.; Yao, E. F.; Armstrong, R. L.; Smith, W. L. The Aspirin and Heme-Binding Sites of Ovine and Murine Prostaglandin Endoperoxide Synthase. *J. Biol. Chem.* **1990**, *265*, 5192–5198.

(4) Yokoyama, C.; Tanabe, T. Cloning of Human Gene Encoding Prostaglandin Endoperoxide Synthase and Primary Structure of the Enzyme. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 888–894.

(5) Xie, W.; Robertson, D. L.; Simmons, D. L. Mitogen-inducible Prostaglandin G/H Synthase: A New Target for Nonsteroidal Antiinflammatory Drugs. *Drug Devel. Res.* **1992**, *25*, 249–265.

(6) O'Neill, G. P.; Kennedy, B. P.; Mancini, J. A.; Kargman, S.; Ouellet, M.; Yergey, J.; Falgueyret, J.-P.; Cromlish, W. A.; Payette, P.; Chan, C.-C.; Culp, S. A.; Vincent, C.; Boily, C.; Abramovitz, M.; Evans, J. F.; Ford-Hutchinson, A. W.; Vickers, P. J.; Percival, M. D. Selective Inhibitors of Cox-2 in Novel Molecular Approaches to Anti-Inflammatory Therapy. *Proceedings of the XIIth International Congress of Pharmacology*, Inflammation Satellite, Toronto, Canada, July 22–23, 1994.

(7) Smith, W. L.; Marnett, L. J. Prostaglandin Endoperoxide Synthases. *Met. Ions Biol. Syst.* **1994**, *30*, 163–199.

(8) Kujubu, D. A.; Fletcher, B. S.; Varnum, B. C.; Lim, R. W.; Herschman, H. R. TIS10, A Phorbol Ester Tumor Promoter Inducible mRNA from Swiss 3T3 Cells, Encodes A Novel Prostaglandin Synthase/Cyclooxygenase Homologue. *J. Biol. Chem.* **1991**, *266*, 12866–12872.
(9) Lee, S. H.; Soyoola, E.; Chanmugam, P.; Hart, S.; Sun, W.; Zhong, H.; Liou, S.; Simmons, D.; Hwang, D. Selective Expression of Mitogen-Inducible Cyclooxygenase in Macrophages Stimulated with Lipopolysaccharide. *J. Biol. Chem.* **1992**, *267*, 25934–25938.
(10) O'Sullivan, M. G.; Huggins, E. M., Jr.; Mccall, C. E. Lipopolysaccharide-Induced Expression of Prostaglandin H Synthase-2 in Alveolar Macrophages is Inhibited by Dexamethasone but not by Aspirin. *Biochem. Biophys. Res. Commun.* **1993**, *191*, 1294–1300.
(11) Kennedy, B. P.; Chan, C.-C.; Culp, S. A.; Cromlish, W. A. Cloning and Expression of Rat Prostaglandin Endoperoxide Synthase (Cyclooxygenase)-2 cDNA. *Biochem. Biophys. Res. Commun.* **1993**, *197*, 494–500.
(12) Masferrer, J. L.; Zweifel, B. S.; Manning, P. T.; Hauser, S. D.; Leahy, K. M.; Smith, W. G.; Isakson, P. C.; Seibert, K. Selective Inhibition of Inducible Cyclooxygenase 2 *in vivo* is Antiinflammatory and Nonulcerogenic. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3228–3232.
(13) Vane, J. R.; Mitchell, J. A.; Appleton, I.; Tomlinson, A.; Bishop-Bailey, D.; Croxtall, J.; Willoughby, D. A. Inducible Isoforms of Cyclooxygenase and Nitric-Oxide Synthase in Inflammation. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2046–2050.
(14) Harada, Y.; Hatanaka, K.; Saito, M.; Majima, M.; Ogino, M.; Kawamura, M.; Ohno, T.; Yang, Q.; Katori, M.; Yamamoto, S. Detection of Inducible Prostaglandin H Synthase-2 in Cells in the Exudate of Rat Carrageenin-Induced Pleurisy. *Biomed. Res.* **1994**, *15*, 127–130.
(15) Mitchell, J. A.; Akarasereenont, P.; Thiemermann, C.; Flower, R. J.; Vane, J. R. Selectivity of Nonsteroidal Antiinflammatory Drugs as Inhibitors of Constitutive and Inducible Cyclooxygenase. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11693–11697.
(16) In our *in vitro* COX-1 and COX-2 assays (see the Experimental Section), both NS-398 (see ref 6) and flosulide were shown to be potent and selective COX-2 inhibitors.
(17) Futaki, N.; Takahashi, S.; Yokoyama, M.; Arai, I.; Higuchi, S.; Otomo, S. NS-398, A New Antiinflammatory Agent, Selectively Inhibits Prostaglandin G/H Synthase/Cyclooxygenase (COX-2) Activity *in vitro*. *Prostaglandins* **1994**, *47*, 55–59.
(18) Klein, T.; Nüsing, R. M.; Pfeilschifter, J.; Ullrich, V. Selective Inhibition of Cyclooxygenase 2. *Biochem. Pharmacol.* **1994**, *48*, 1605–1610.
(19) Futaki, N.; Yoshikawa, K.; Hamasaka, Y.; Arai, I.; Higuchi, S.; Iizuka, H.; Otomo, S. NS-398, A Novel Non-steroidal Antiinflammatory Drug with Potent Analgesic and Antipyretic Effects, which Cause Minimal Stomach Lesions. *Gen. Pharmacol.* **1993**, *24*, 105–110.
(20) Rufer, C.; Bahnlmann, F.; Schröder, E.; Böttcher, I. Nichtsteroidale Entzündungshemmer.8. Entzündungshemmende Methansulfonanilide III (Non-steroidal antiinflammatory agents. 8. Methanesulfonanilides. III). *Eur. J. Med. Chem.* **1982**, *17*, 173–189.
(21) Wiesenberg-Boettcher, I.; Schweizer, A.; Müller, K. The Pharmacological Profile of CGP 28238, A Highly Potent Anti-inflammatory Compound. *Agents Actions* **1989**, *26*, 240–242.
(22) Wiesenberg-Boettcher, I.; Schweizer, A.; Green, J. A.; Müller, K.; Maerki, F.; Pfeilschifter, J. The Pharmacological Profile of CGP 28238, A Highly Potent Anti-inflammatory Compound. *Drug Exp. Clin. Res.* **1989**, *15*, 501–509.
(23) Unpublished results: Methylation of the sulfonamide nitrogen or reduction of the carbonyl group on the indanone ring of flosulide to a hydroxyl group or methylene group resulted in complete loss of *in vitro* COX-2 activity.
(24) Preliminary communication: Prasit, P.; Black, W. C.; Chan, C.-C.; Ford-hutchinson, A. W.; Gauthier, J. Y.; Gordon, R.; Guay, D.; Kargman, S.; Lau, C. K.; Li, C.-S.; Mancini, J.; Ouimet, N.; Roy, P.; Targari, P.; Vickers, P.; Wong, E.; Young, R. N.; Zamboni, R. L-745,337: A Selective Cyclooxygenase-2 Inhibitor. *Med. Chem. Res.* **1995**, *5*, 364–374.
(25) Tsunoda, T.; Suzuki, M.; Noyori, R. A Facile Procedure for Acetalization under Aprotic Conditions. *Tetrahedron Lett.* **1980**, *21*, 1357–1358.
(26) Cromlish, W. A.; Kennedy, B. P.; O'Neil, G.; Vickers, P. J.; Wong, E.; Mancini, J. A. *PCT* International Patent Application Number WO 9414977, 1994.
(27) Hoff, T.; DeWitt, T.; Kaever, V.; Resch, K.; Goppelt-Struebe, M. Differentiation-Associated Expression of Prostaglandin G/H Synthase in Monocytic Cells. *FEBS Lett.* **1993**, *320*, 38–42.
(28) Otterness, I. G.; Gans, D. J. Nonsteroidal Anti-inflammatory Drugs: An Analysis of the Relationship between Laboratory Animal and Clinical Doses, Including Species Scaling. *J. Pharm. Sci.* **1988**, *77*, 790–795.
(29) Chan, C.-C.; Black, C.; Boyce, S.; Brideau, C.; Ford-Hutchinson, A. W.; Gordon, R.; Guay, D.; Hill, R.; Li, C.-S.; Mancini, J.; Penneton, M.; Prasit, P.; Rasori, R.; Riendeau, D.; Roy, P.; Tagari, P.; Vickers, P.; Wong, E.; Rodger, I. W. Pharmacolgy of A selective Cyclooxygenase-2 Inhibitor L-745,337: A Novel Nonsteroidal Anti-inflammatory Agent with an Ulcerogenic Sparing Effect in Rat and Primate Stomach. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 1531–1537.
(30) Boyce, S.; Chan, C.-C.; Gordon, R.; Li, C.-S.; Rodger, I. W.; Webb, J. K.; Rupniak, N. M. J.; Hill, R. G. L-745,337: A Selective Inhibitor of Cyclooxygenase-2 Elicits Antinociception but not Gastric Ulceration in Rats. *Neuropharmacology* **1994**, *33*, 1609–1611.
(31) Rodger, R. F.; Bronson, R. T.; McIntyre, K. W.; Nicolosi, R. J. Protein-losing Enteropathy in Six Macaques. *J. Am. Vet. Med. Assoc.* **1980**, *177*, 863–866.
(32) Abakken, L.; Dybdahl, J. H.; Eidsaunet, W.; Haaland, A.: Larsen, S.; Osnes, M. Optimal Assessment of Gastrointestinal Side Effects Induced by Non-steroidal Anti-inflammatory Drugs. *Scand. J. Gastroenterol.* **1989**, *24*, 1007–1013.
(33) Warrington, S. J.; Debbas, N. M. G.; Farrthing, M.; Horton, M.; Johnston, A.; Thillainayagam, A.; Turner, P.; Ferber, H. Lornoxicam, Indomethacin and Placebo: Comparison of Effects on Fecal Blood Loss and Upper Gastrointestinal Endoscopic Appearances in Healthy Men. *Postgrad. Med. J.* **1990**, *66*, 622–626.
(34) Young, G. P.; St. John, D. J. B.; Lynch, N. M.; McHutchison, J. G.; Barraclough, D. Measurement of Occult Upper Gastrointestinal Tracts Blood Loss: A Direct Comparison of Radiochromium and Haem-porphyrin Assay Techniques. *J. Gastroenterol. Hepatol.* **1993**, *8*, 328–333.
(35) Chan, C.-C. Unpublished results.
(36) Klages, F.; Bott, K. Synthesen und Infrarotspektren Weitgehend Säurestabiler Aliphatischer Diazoverbindungen. *Chem. Ber.* **1964**, *97*, 735–740.
(37) Newman, M. S.; Karnes, H. A. The Conversion of Phenols to Thiophenols via Dialkylthiocarbamates. *J. Org. Chem.* **1966**, *31*, 3980–3984.
(38) Kwart, H.; Evans, E. R. The Thio-Claisen Rearrangement. The Mechanism of Thermal Rearrangement of Allyl Aryl Sulfides. *J. Org. Chem.* **1966**, *31*, 413–419.
(39) A synthesis of compound **20** in multigram quantity has been developed: Scheigetz, J.; Zamboni, R.; Roy, B. Synthesis of 5-Methanesulfonamide-6-(2,4-Difluorophenylthio)-1-Indanone. *Synth. Commun.* **1995**, *25*, 2791–2806.
(40) Otterness, I. G.; Moore, P. F. Carrageenan Foot Edema Test. *Methods Enzymol.* **1988**, *162*, 320–327.
(41) Randall, L. O.; Selitto, J. J. A Method for Measurement of Analgesic Activity on Inflamed Tissue. *Arch. Int. Pharmacodyn.* **1957**, *111*, 409–419.
(42) Di Rosa, M.; Giroud, J. P.; Willoughby, D. A. Studies on the Mediators of the Acute Inflammatory Reponses Induced in Rats in Different Sites by Carrageenan and Turpentine. *J. Pathol.* **1971**, *104*, 15–29.

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