

Cyclooxygenase-2 Inhibitors. Synthesis and Pharmacological Activities of 5-Methanesulfonamido-1-indanone Derivatives¹

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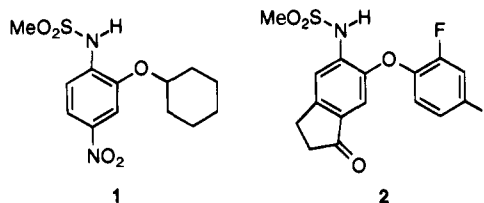
The recent discovery of an alternative form cyclooxygenase (cyclooxygenase-2, COX-2), which has been proposed to play a significant role in inflammatory conditions, may provide an opportunity to develop anti-inflammatory drugs with fewer side effects than existing non-steroidal anti-inflammatory drugs (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 substituents 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 ulcerogenic than **2** in rats.

Introduction

Prostaglandins, particularly prostaglandin E₂ (PGE₂), 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 described⁵⁻⁷ 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

We¹⁶ and others¹⁶⁻¹⁸ 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**

Chart 1

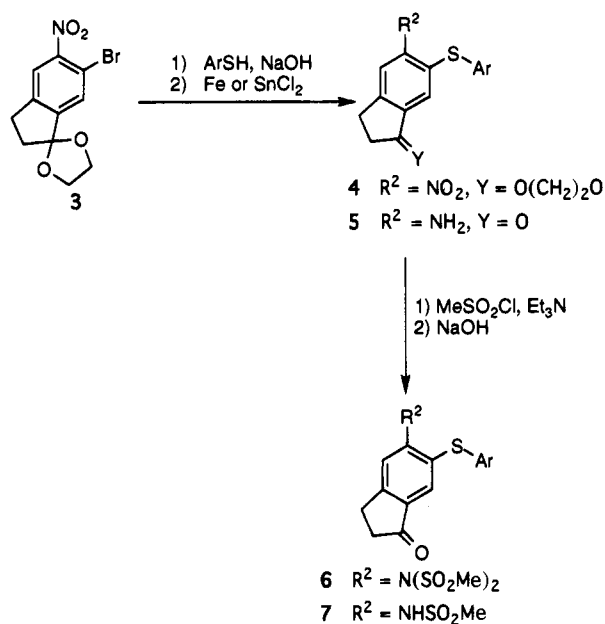


has good plasma levels after oral dosing in both rat and squirrel monkey while **1** has poor plasma levels in squirrel monkey. The low bioavailability of **1** in primate and the potential for toxicity due to the nitro group led us to focus on **2** as a lead structure.

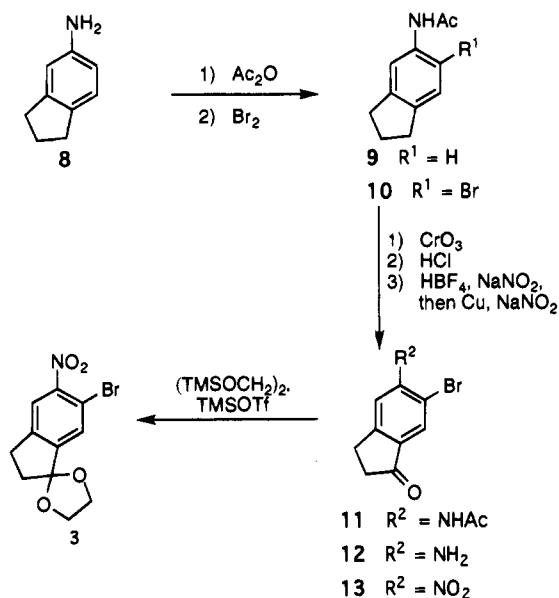
Ether-linked phenyl analogs of **2** have been previously synthesized, but the structure-activity relationship (SAR) of these compounds was not reported in the context of their COX-2 activity and selectivity.²⁰ The corresponding thioether-linked analogs of **2** have not been reported. Our discovery 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

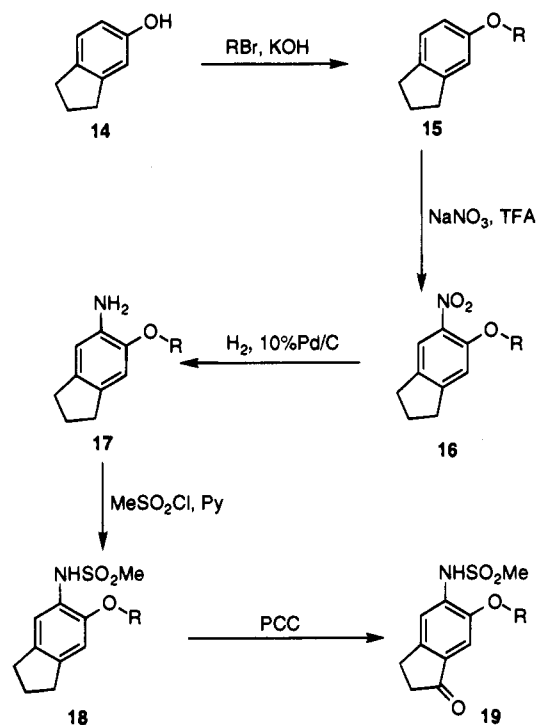


Chemistry

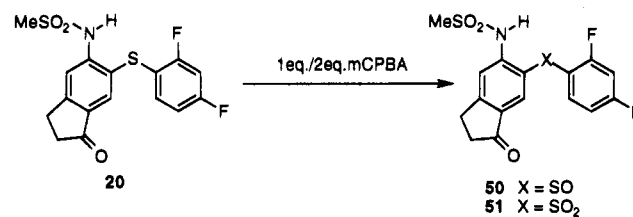
The thioether analogs of **2** were synthesized from a common intermediate, 5-nitro-6-bromo-1-indanone ethylene ketal (**3**), as shown in Scheme 1. Coupling with an appropriate nucleophile proceeded under basic conditions to give **4**. Reduction of the nitro group with iron powder or tin(II) chloride in aqueous ethanol with concomitant hydrolysis of the ketal group provided aminoindanone **5**. Sulfonation 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

Scheme 3



Scheme 4

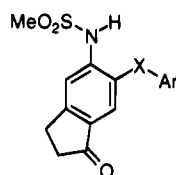


to diazotization followed by treatment of the diazonium salt with sodium nitrite in the presence of copper powder to provide nitro compound **13**. Attempts at displacing the bromine atom with nucleophiles instead resulted in *ipso* substitution of the nitro group. We reasoned that this was due to activation by the carbonyl group at the *para* position. Therefore, this carbonyl group was protected as a ketal using the conditions of Noyori²⁵ to provide **3**, which underwent substitution reactions with the desired regioselectivity.

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**. Sulfonation 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-

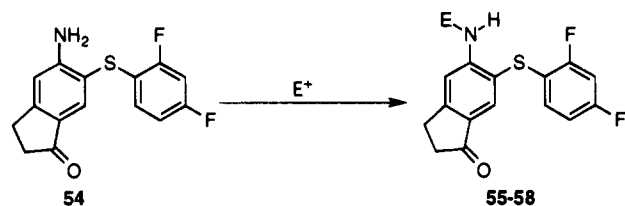
Table 1. Phenyl Analogs of Methanesulfonamido-1-indanone



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 ₂ Ph	0.021 ± 0.006 (n = 3)	>50	0.60
20	S	2,4-F ₂ Ph	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 ₂ Ph	0.1-1	>50*	
25	S	4-BrPh	0.01	>10	~3.0
26	S	2,4-Cl ₂ Ph	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>i</i> -PrPh	>10*		
32	S	4- <i>t</i> -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 ₂ HPh	>10		

^a Each IC₅₀ value is an average of at least two independent determinations. Those identified with an asterisk are the result of a single titration.

Scheme 5



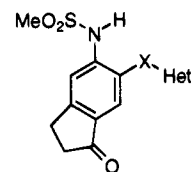
fied sulfonamide group were prepared from amine **54** using the appropriate electrophiles (Scheme 5).

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 IC₅₀ < 0.1 μM were also tested in a whole cell cyclooxygenase-1 assay. These assays measured prostaglandin E₂ 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 E₂ 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 IC₅₀ 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.

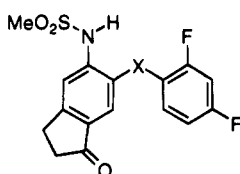
For the heterocyclic analogs (Table 2), only the 2-thiazolyl analog **38** showed reasonable potency; the 2-thienyl analogs **41** and the 2-pyridyl analog **43** were moderately active. Other heterocyclic analogs investigated were found to be significantly less active. Further investigation of other 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

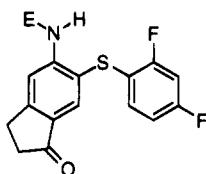
compd no.	X	R	COX-2 ^a IC ₅₀ (μM)	COX-1 IC ₅₀ (μM)	rat paw edema (ED ₃₀ , mpk)
46	O	cyclohexyl	0.05	≥1	~10
47	O	cyclopentyl	≥1	>10	
48	O	4-tetrahydropyranyl	>10		
49	S	cyclohexyl	0.05	>50*	>3

^a Each IC₅₀ 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 IC₅₀ 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 ₂ Et	>10	≥10
56	SO ₂ CF ₃	0.04	
57	Ac	>10	
58	COCF ₃	>10	

^a Each IC₅₀ 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 trifluoromethylsulfonamide 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** (ED₅₀ = 2.0 mg/kg) and indomethacin (ED₅₀ = 2.0 mg/kg) were equipotent, but **20** was approximately 4 times more potent than indomethacin against carrageenan-induced rat paw hyperalgesia (ID₅₀ values of 0.37 and 1.47 mg/kg, respectively)³⁰ and 2 times more potent than indomethacin in the endotoxin-induced rat pyresis (ID₅₀ values of 3.8 and 7 mg/kg, respectively).²⁹ Compound **20** significantly inhibited the production of PGE₂ (80% at 3 mg/kg) in the inflammatory exudate in carrageenan-induced pleurisy in rats.²⁹ The tissue content of PGE₂ 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 nociceptive³⁰ 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 ⁵¹Cr 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 ⁵¹Cr excretion assay³¹⁻³⁴ used to determine gastrointestinal integrity, compound **20** had no effect on fecal ⁵¹Cr 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 ⁵¹Cr 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. Comparison 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 ⁵¹Cr 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 commercial sources or prepared from the corresponding anilines according to the procedures of Klages and Bott³⁶ 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-Acetamidoin-dane (**9**). To a solution of 5-aminoindane (**8**) (10.0 g, 7.5 mmol) in CH₂Cl₂ (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 CH₂Cl₂ layer was separated, washed successively with 1 M aqueous HCl and brine, dried over anhydrous MgSO₄, 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 (CDCl₃) 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 Br₂ (19.0 mL, 0.37 mol) in HOAc. The mixture was further stirred at 10 °C for 15 min and then diluted with H₂O until no more precipitate formed. The precipitate was collected, washed with H₂O, and dried under vacuum to give 61 g (80%) of **10**: ¹H NMR (CDCl₃) 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 CrO₃ (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 H₂O (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 MgSO₄, 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 (CDCl₃) 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 H₂O, 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 HBF₄ (120 mL) at 0 °C was added dropwise 4 M aqueous NaNO₂ (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 NaNO₂ (120 g, 1.74 mol) in H₂O (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 Et₂O. 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 MgSO₄, 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 (CDCl₃) 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/CH₄) *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)-oxyethane (22.0 mL, 90 mmol) in CH₂Cl₂ (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 NaHCO₃ (100 mL). The CH₂Cl₂ layer was separated, washed with brine, dried over anhydrous MgSO₄, 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 (CDCl₃) 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 H₂O, 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 MgSO₄, 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 (CDCl₃) 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 NH₄Cl (50 mg, 0.93 mmol) in 30 mL of EtOH:H₂O (2:1) was refluxed for 1 h. The hot mixture was filtered through Celite and concentrated *in vacuo*. The residue was diluted with H₂O and extracted with EtOAc. The EtOAc extract was dried over anhydrous MgSO₄ and concentrated to give **5a** (410 mg, 81%) as a light brown solid: ¹H NMR (CDCl₃)

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), Et₃N (1.0 mL, 7.2 mmol), and MsCl (300 μ L, 3.9 mmol) in CH₂Cl₂ (10 mL) was stirred at room temperature for 1 h. After the mixture was diluted with CH₂Cl₂ (20 mL), it was washed successively with saturated aqueous NaHCO₃, 1 M aqueous HCl, and brine. The CH₂Cl₂ layer was separated, dried over anhydrous MgSO₄, 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 μ L, 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 H₂O and extracted with EtOAc. The EtOAc layer was separated, washed with brine, dried over anhydrous MgSO₄, 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; ¹H NMR (CDCl₃) 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. (C₁₆H₁₃F₂NO₃S₂) 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; ¹H NMR (CDCl₃) 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, 3H), 2.70 (m, 2H). Anal. (C₁₆H₁₄FNO₃S₂) C, H, N.

6-[(3-Fluorophenyl)thio]-5-methanesulfonamido-1-indanone (22): mp 153–154 °C; ¹H NMR (CDCl₃) 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. (C₁₆H₁₄FNO₃S₂) C, H, N.

6-[(4-Fluorophenyl)thio]-5-methanesulfonamido-1-indanone (23): mp 175–176 °C; ¹H NMR (CDCl₃) 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. (C₁₆H₁₄FNO₃S₂) C, H, N.

6-[(2,6-Difluorophenyl)thio]-5-methanesulfonamido-1-indanone (24): mp 166–168 °C; ¹H NMR (CDCl₃) 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) C₁₆H₁₃F₂NO₃S₂ + H⁺ calcd 370.038 32, found 370.038 23.

6-[(4-Bromophenyl)thio]-5-methanesulfonamido-1-indanone (25): mp 189–191 °C; ¹H 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) C₁₆H₁₄BrNO₃S₂ + H⁺ calcd 411.967 67, found 411.967 58.

6-[(2,4-Dichlorophenyl)thio]-5-methanesulfonamido-1-indanone (26): mp 214–216 °C; ¹H NMR (CDCl₃) 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; ¹H NMR (CDCl₃) 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. (C₁₇H₁₇NO₃S₂) C, H, N.

6-(*m*-Tolylthio)-5-methanesulfonamido-1-indanone (28): mp 166–167 °C; ¹H NMR (CDCl₃) 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. (C₁₇H₁₇NO₃S₂) C, H, N.

6-(*p*-Tolylthio)-5-methanesulfonamido-1-indanone (29): mp 185–186 °C; ¹H NMR (CDCl₃) 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. (C₁₇H₁₇NO₃S₂) C, H, N.

6-[(4-Ethylphenyl)thio]-5-methanesulfonamido-1-indanone (30): mp 125–126 °C; ¹H NMR (CDCl₃) 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. (C₁₈H₁₉NO₃S₂) C, H, N.

6-[(4-Isopropylphenyl)thio]-5-methanesulfonamido-1-indanone (31): mp 134–135 °C; ¹H NMR (CDCl₃) 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. (C₁₉H₂₁NO₃S₂) C, H, N.

6-[(4-*tert*-Butylphenyl)thio]-5-methanesulfonamido-1-indanone (32): mp 159–160 °C; ¹H NMR (CDCl₃) 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. (C₂₀H₂₃NO₃S₂) C, H, N.

6-[(2-Methoxyphenyl)thio]-5-methanesulfonamido-1-indanone (33): mp 166–168 °C; ¹H NMR (CDCl₃) 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) C₁₇H₁₇NO₄S₂ + H⁺ calcd 364.067 73, found 364.067 84.

6-[(4-Methoxyphenyl)thio]-5-methanesulfonamido-1-indanone (34): mp 136–137 °C; ¹H NMR (CDCl₃) 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) C₁₇H₁₇NO₄S₂ + H⁺ calcd 364.067 73, found 364.067 84.

6-[(4-Methylthio)phenoxy]-5-methanesulfonamido-1-indanone (35): mp 174–176 °C; ¹H 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. (C₁₇H₁₇NO₃S₃) C, H, N.

6-[(4-Methylsulfonyl)phenoxy]-5-methanesulfonamido-1-indanone (36): mp 220–222 °C; ¹H NMR (CDCl₃) 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; ¹H 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. (C₁₇H₁₅NO₅S₂) C, H, N.

6-(2-Thiazolylthio)-5-methanesulfonamido-1-indanone (38): mp 194–195 °C; ¹H NMR (CDCl₃) 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. (C₁₃H₁₂N₂O₃S₃) C, H, N.

6-(4-Thiazolylthio)-5-methanesulfonamido-1-indanone (39): mp 161–163 °C; ¹H NMR (CDCl₃) 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. (C₁₃H₁₂N₂O₃S₃) C, H, N.

6-(5-Thiazolylthio)-5-methanesulfonamido-1-indanone (40): mp 169–171 °C; ¹H NMR (CDCl₃) 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) C₁₃H₁₂N₂O₃S₃ + H⁺ calcd 341.008 83, found 341.008 73.

6-(2-Thienylthio)-5-methanesulfonamido-1-indanone (41): mp 159–160 °C; ¹H NMR (CDCl₃) 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. (C₁₄H₁₃NO₃S₃) C, H, N.

6-(3-Thienylthio)-5-methanesulfonamido-1-indanone (42): mp 166–167 °C; ¹H 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. (C₁₄H₁₃NO₃S₃) C, H, N.

6-(2-Pyridylthio)-5-methanesulfonamido-1-indanone (43): mp 174–175 °C; ¹H NMR (CDCl₃) 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. (C₁₅H₁₄N₂O₃S₂) C, H, N.

6-(3-Pyridylthio)-5-methanesulfonamido-1-indanone (44): mp 150–152 °C; ¹H 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. (C₁₅H₁₄N₂O₃S₂) C, H, N.

6-(4-Pyridylthio)-5-methanesulfonamido-1-indanone (45): mp 215 °C dec; ¹H NMR (CDCl₃) 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 \times) 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: 1H 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 $^\circ C$ 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: 1H 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: 1H 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: 1H 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 $^\circ C$; 1H 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-(Cyclopentylloxy)-5-methanesulfonamido-1-indanone (47): mp 167–168 $^\circ C$; 1H 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-Tetrahydropyranloxy)-5-methanesulfonamido-1-indanone (48): mp 217–218 $^\circ C$; 1H 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): 1H 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-Difluorophenylsulfanyl)-5-methanesulfonamido-1-indanone (50). To a solution of **20** (120 mg, 0.32 mmol) in CH_2Cl_2 at 0 $^\circ C$ 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 $^\circ C$; 1H 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: 1H 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): 1H 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 preliminarily 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*-butyldimethylsilyloxy)oxy]indane was treated with 3 equiv of *tert*-butyllithium at -78 $^\circ C$, 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*-butoxycarbonyl group followed by bis-mesylation and hydrolysis yielded **53**: mp 171–173 $^\circ C$; 1H 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 $^\circ C$; 1H 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): 1H 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 $^\circ C$; 1H 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 $^\circ C$; 1H 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. (C₁₇H₁₀F₃NO₂S) 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 PGE₂ 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 (Nunc) until confluent ((1–2) × 10⁵ cells/well). U-937 cells were grown in spinner flasks and resuspended to a final density of 1.5 × 10⁶ 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 PGE₂ levels measured by radioimmunoassay. In these assays, 100% activity is defined as the difference between PGE₂ 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) **⁵¹Cr Fecal Excretion in Rats.**²⁹ Test compounds or vehicles were dosed orally as above, followed immediately by

injection into the tail vein of 0.5 mL of ⁵¹Cr-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 ⁵¹Cr fecal excretion was calculated as a percent of total injected dose. ⁵¹Cr-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 Na₂⁵¹CrO₄ 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 Na₂⁵¹CrO₄. 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) **⁵¹Cr 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, ⁵¹CrCl₂ 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. ⁵¹Cr 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).

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