Ester and Amide Derivatives of the Nonsteroidal Antiinflammatory Drug, Indomethacin, as Selective Cyclooxygenase-2 Inhibitors

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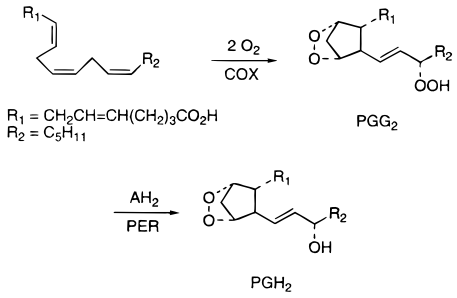
Recent studies from our laboratory have shown that derivatization of the carboxylate moiety in substrate analogue inhibitors, such as 5,8,11,14-eicosatetraynoic acid, and in nonsteroidal antiinflammatory drugs (NSAIDs), such as indomethacin and meclofenamic acid, results in the generation of potent and selective cyclooxygenase-2 (COX-2) inhibitors (Kalgutkar et al. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 925−930). This paper summarizes details of the structure−activity studies involved in the transformation of the arylacetic acid NSAID, indomethacin, into a COX-2-selective inhibitor. Many of the structurally diverse indomethacin esters and amides inhibited purified human COX-2 with IC_{50} values in the low-nanomolar range but did not inhibit ovine COX-1 activity at concentrations as high as 66 μM. Primary and secondary amide analogues of indomethacin were more potent as COX-2 inhibitors than the corresponding tertiary amides. Replacement of the 4-chlorobenzoyl group in indomethacin esters or amides with the 4-bromobenzyl functionality or hydrogen afforded inactive compounds. Likewise, exchanging the 2-methyl group on the indole ring in the ester and amide series with a hydrogen also generated inactive compounds. Inhibition kinetics revealed that indomethacin amides behave as slow, tight-binding inhibitors of COX-2 and that selectivity is a function of the time-dependent step. Conversion of indomethacin into ester and amide derivatives provides a facile strategy for generating highly selective COX-2 inhibitors and eliminating the gastrointestinal side effects of the parent compound.

Introduction

The committed step in prostaglandin and thromboxane biosynthesis involves the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), a reaction catalyzed by the sequential action of the cyclooxygenase (COX) and peroxidase activities of prostaglandin endoperoxide synthase or cyclooxygenase (PGHS or COX, EC 1.14.99.1) (Scheme 1).[1] COX activity originates from two distinct and independently regulated enzymes, termed COX-1 and COX-2.[2−4] COX-1 is the constitutive isoform and is mainly responsible for the synthesis of cytoprotective prostaglandins in the gastrointestinal tract (GI) and of the proaggregatory thromboxane in blood platelets.[5] COX-2 is inducible and short-lived; its expression is stimulated in response to endotoxin, cytokines, and mitogens.[6−8] COX-2 plays a major role in prostaglandin biosynthesis in inflammatory cells (monocytes/macrophages) and in the central nervous system.[9−12] Overall, these observations suggest that COX-1 and COX-2 serve different physiological and pathophysiological functions. Classical nonsteroidal antiinflammatory drugs (NSAIDs) inhibit both COX-1 and COX-2 to varying extents.[13] The differential tissue distribution of COX-1 and COX-2 provides a rationale for the development of selective COX-2 inhibitors as antiinflammatory and analgesic agents that lack the GI and hematologic liabilities

Scheme 1



exhibited by currently marketed NSAIDs. This hypothesis has been validated in animal models and has led to the marketing of two diarylheterocycles, celecoxib and rofecoxib (Figure 1), as COX-2 inhibitors.[14−20]

Although diarylheterocycles and other compounds have been extensively studied as selective COX-2 inhibitors, there are very few reports on the utilization of well-established NSAID templates in the design of selective COX-2 inhibitors.[21−24] Of all NSAIDs, indomethacin, zomepirac, aspirin, and flurbiprofen are the only examples of compounds that have been successfully elaborated into selective COX-2 inhibitors (see Figure 1). However, the methodology utilized in NSAID modification is not general and consists of extensive modification of individual compounds. For instance, replacement of the 4-chlorobenzoyl group of indomethacin with a 4-bromobenzyl moiety generates a COX-2-selective inhibitor.[21] In contrast, exchanging the carboxylate

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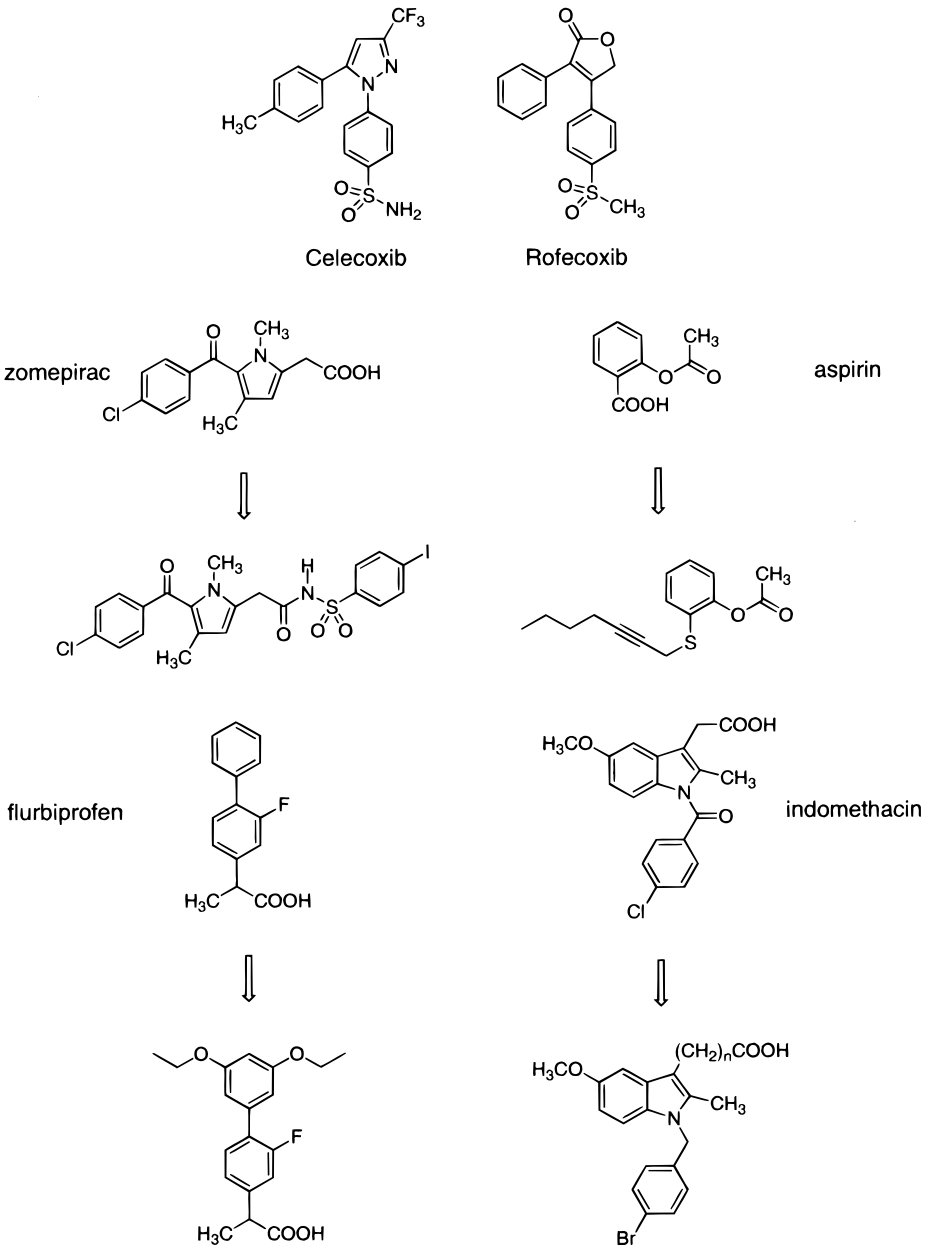


Figure 1. Structures of celecoxib and rofecoxib along with examples of conversion of nonselective COX inhibitors to COX-2-selective inhibitors.

moiety in aspirin with alkyl sulfide functionalities affords specific COX-2 inhibitors.[23]

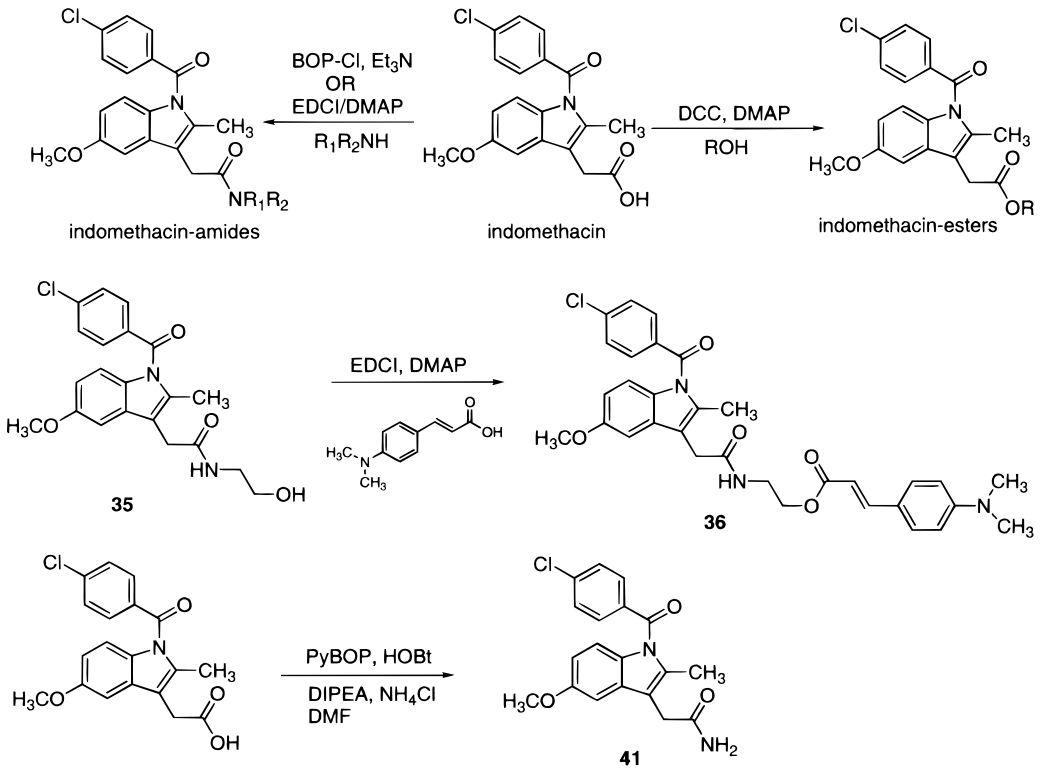
We recently described a biochemically based strategy for the facile conversion of carboxylic acid-containing NSAIDs. Derivatization of these compounds to esters or amides produces molecules capable of binding tightly to COX-2 but not COX-1.[25] The facile nature of this strategy is evident from the observation that a single chemical derivatization (amidation or esterification) of the carboxylate moiety of these compounds generates an impressive array of potent and highly selective COX-2 inhibitors. Several of the esters or amides tested exhibit antiinflammatory activity and antiangiogenic activity.[26]

In this paper, we disclose the details of the structure–activity relationship (SAR) analysis on indomethacin ester and amide derivatives as COX-2-selective inhibitors. These results are discussed with particular reference to previous site-directed mutagenesis experiments that reveal the molecular basis for selective COX-2 inhibition by the indomethacin derivatives.

Results

Chemistry. Well-established methodology was utilized in the synthesis of ester and amide derivatives of indomethacin as indicated in Scheme 2. Indomethacin esters were prepared by treatment with the appropriate alcohol or phenol in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). Indomethacin amides were prepared in a similar fashion, utilizing bis(2-oxo-3-oxazolidinyl)phosphonic chloride (BOP-Cl)[27] or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl (EDCI) as the carboxylate activator instead of DCC. The fluorescent indomethacin derivative **36** was obtained by esterification of the indomethacin ethanol amide derivative **35** with *N*,*N*-dimethylaminocinnamic acid in the presence of EDCI or DCC (see Scheme 2). No reaction was discernible when BOP-Cl

Scheme 2



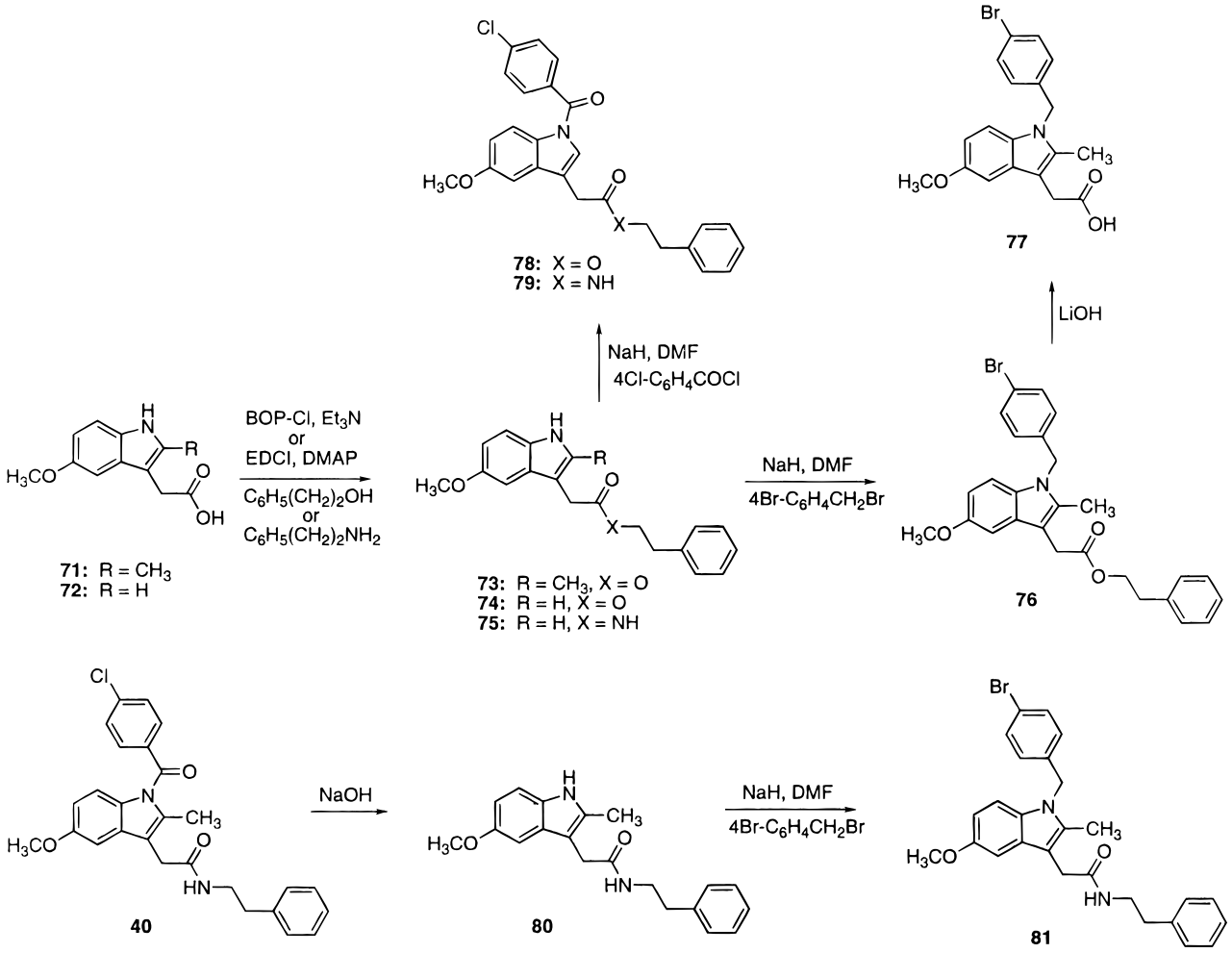
was used as the carboxylate activator instead of EDCI or DCC. The primary amide derivative **44** was synthesized in near quantitative yields by reacting indomethacin with NH_4Cl in the presence of (benzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (PyBOP), HOBt, and DIPEA.[28] The structures of all compounds were established by NMR and mass spectrometry. HPLC analyses in two different solvent systems of 20 representative target esters and amides indicated a minimum purity of 96.0%. Most of the compounds were in excess of 98.5% pure.

The preparation of the 4-bromobenzyl derivatives **76** and **81** is outlined in Scheme 3. Commercially available 5-methoxy-2-methylindole-3-acetic acid (**71**) was converted to the corresponding phenethyl ester **73** by reaction with phenethyl alcohol and BOP-Cl. *N*-Alkylation of **73** with 4-bromobenzyl bromide in the presence of NaH afforded **76**. Base-catalyzed hydrolysis of the ester linkage in **76** afforded the indomethacin analogue **77**.[21] The corresponding amide **81** was synthesized in a slightly different fashion. Hydrolysis of the 4-chlorobenzoyl group in indomethacin phenethyl amide **40** afforded indole **80** that upon alkylation with 4-bromobenzyl bromide generated **81**. The desmethyl phenethyl ester and amide analogues **78** and **79** were synthesized in analogous fashion starting from 5-methoxyindole-3-acetic acid (**72**). Esterification or amidation of **72** with phenethyl alcohol or phenethylamine afforded **74** and **75**, respectively, which upon acylation with 4-chlorobenzoyl chloride gave **78** and **79**.

Enzymology. A. Selective COX-2 Inhibition by Ester Derivatives of Indomethacin. IC_{50} values for the inhibition of purified human COX-2 or ovine COX-1 by test compounds were determined by thin-layer chromatography (TLC). HoloCOX-2 (66 nM) or holoCOX-1 (44 nM) in 100 mM Tris-HCl, pH 8.0, containing 500 μM phenol was treated with several concentrations of test compounds at 25 °C for 20 min. Since the recombinant COX-2 had a lower specific activity than ovine COX-1, the protein concentrations were adjusted so that the percentages of total products obtained following oxygenation of arachidonic acid by the two isoforms were comparable. The cyclooxygenase reaction was initiated by the addition of [1-^{14}C]arachidonic acid (50 μM) at 37 °C and continued for 30 s. Control experiments in the absence of inhibitor indicated ~25−30% conversion of fatty acid substrate to products which was sufficient for assessing the inhibitory properties of all compounds described in this study. Under these conditions, indomethacin displayed selective time- and concentration-dependent inhibition of COX-1 [IC_{50}(COX-1) ~ 0.050 μM, IC_{50}(COX-2) ~ 0.75 μM], whereas NS-398[29] and SC-299[30] displayed selective COX-2 inhibition [NS-398: IC_{50}(COX-2) ~ 0.12 μM, IC_{50}(COX-1) > 66 μM; SC-299: IC_{50}(COX-2) ~ 0.060 μM, IC_{50}(COX-1) > 66 μM].

1. Aliphatic Esters. Conversion of the free carboxylate group in indomethacin to the methyl ester afforded **1**[31] which displayed selective COX-2 inhibition [**1:** IC_{50}(COX-2) ~ 0.25 μM, IC_{50}(COX-1) ~ 33 μM] (Table 1). Thus *esterification of the carboxylate moiety in indomethacin increased the inhibitory potency against COX-2 but had a detrimental effect on the potency for COX-1 inhibition*. Chain length extension studies of the methyl group in **1** to higher alkyl homologues revealed significant increases in potency and selectivity against COX-2. For example, the heptyl ester **10** was more potent and selective as a COX-2 inhibitor than **1** [compound **10**: IC_{50}(COX-2) ~ 0.040 μM, IC_{50}(COX-1) > 66 μM]. Incorporation of functionalities in the alkyl chain led to compounds such as the butoxyethyl (**11**) or *trans*-heptenyl (**12**) ester derivatives, which also dis-

Scheme 3

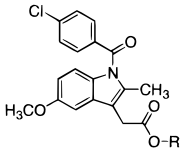


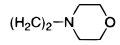
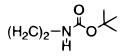
played potent and selective COX-2 inhibition. Compared to the olefin **12**, the heptynyl ester **13** was less potent as a COX-2 inhibitor. Likewise, cycloalkyl esters illustrated by the cyclohexyl, cyclohexylethyl, or morpholinoethyl analogues **8**, **9**, and **16**, respectively, were less potent as COX-2 inhibitors. However, the bulky BOC-protected 2-aminoethyl ester **17** exhibited potent and selective COX-2 inhibition, indicating the structural diversity of functionalities which can be tolerated in the COX-2 active site.

2. Aromatic Esters. Transformation of the carboxylate moiety in indomethacin to a phenyl ester **18** also led to selective COX-2 inhibition (Table 2). Potency and selectivity were increased by the introduction of methylene spacers between the phenyl ring and the ester oxygen as illustrated with the 2-phenylethyl ester **22**. However, no significant COX inhibition was discernible with the β- and α-naphthyl esters **19** and **20**, respectively, or with the 3,5-dimethylphenyl ester derivative **21**, even at very high concentrations. Interestingly, selective COX-2 inhibition by the aromatic esters was extremely sensitive to the type and position of substituents on the phenyl ring. For instance, the presence of a methylmercapto group in the 4-position of the phenyl group afforded **23** which was only approximately 10-fold selective as a COX-2 inhibitor. The corresponding 2-methylmercaptophenyl isomer **24** was greater than 1100-fold selective as a COX-2 inhibitor. Furthermore, replacement of the 4-methylmercapto group with a 4-methoxy group yielded **25**, which was one of the most potent ester derivatives in the series with a COX-1 over COX-2 selectivity ratio greater than 1650. Like the 4-methylmercaptophenyl ester **23**, the 4-fluorophenyl (**27**) and 3-pyridyl (**28**) ester analogues also demonstrated dramatic losses in selectivity.

B. Selective COX-2 Inhibition by Amide Derivatives of Indomethacin. 1. Aliphatic Amides. Like the methyl ester **1**, *the N-methyl amide* ***29***[31] *also displayed selective COX-2 inhibition* [IC_{50}(COX-2) $\sim$ 0.70 μM, IC_{50}(COX-1) > 66 μM] (Table 3). Increments in COX-2 potency and selectivity were observed with higher alkyl homologues such as the octyl derivative **32**, but a further increase in chain length to the nonyl derivative **33** resulted in a loss of COX-2 selectivity [octyl amide **32**: IC_{50}(COX-2) $\sim$ 0.040 μM, IC_{50}(COX-1) $\sim$ 66 μM; nonyl amide **33**: IC_{50}(COX-2) $\sim$ 0.040 μM, IC_{50}(COX-1) $\sim$ 17 μM]. Incorporation of terminal functionalities on the alkyl chain afforded compounds that retained COX-2 potency and selectivity as illustrated by the 3-chloropropyl and the 2-ethanol amide derivatives **34** and **35**. Esterification of the hydroxy group in **35** with *N*,*N*-dimethylaminocinnamic acid generated the corresponding fluorescent analogue **36**, which also exhibited excellent COX-2-selective inhibition. Although the methyl ester of the indomethacin-glycine amide conjugate, **37**, was a poor inhibitor of COX-2, significant improvements in COX-2 potency were discernible with the corresponding methyl esters of indomethacin-ala-

Table 1. In Vitro COX-2 Potencies of Aliphatic Esters of Indomethacin



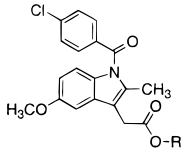
Compound	R	IC_{50} (μM)[a]		Selectivity[b]
		COX-2	COX-1	
NS-398		0.12	> 66	> 600
SC-299		0.060	> 66	> 1000
Indomethacin	H	0.75	0.05	0.067
1	CH_3	0.25	33	130
2	C_2H_5	0.10	> 66	700
3	C_3H_7	0.10	> 66	700
4	*i*-C_3H_7	0.25	37	150
5	C_4H_9	0.050	> 66[c]	> 1300
6	C_5H_{11}	0.050	> 66[c]	> 1300
7	C_6H_{13}	0.06	> 66[c]	> 1100
8	*cyc*-C_6H_{11}	0.12	> 66[c]	> 500
9	$(CH_2)_2$-cyc-C_6H_{11}	1.0	> 66	> 66
10	C_7H_{15}	0.040	> 66[c]	> 1700
11	$(CH_2)_2O(CH_2)_3CH_3$	0.060	> 66[c]	> 1000
12	*trans*-$CH_2CHCH(CH_2)_3CH_3$	0.050	> 66[c]	> 1300
13	$H_2CC{\equiv}C(CH_2)_3CH_3$	0.25	> 66	> 270
14	$CH(CH_3)CH_2CCCH_2CH_3$	0.12	> 66	> 550
15	C_8H_{17}	0.090	> 66	> 730
16		0.68	> 66[c]	> 100
17		0.045	> 66[c]	> 1500

[a] IC_{50} values were determined by incubating several inhibitor concentrations in DMSO with human COX-2 (66 nM) or ovine COX-1 (44 nM) for 20 min at rt followed by initiation of the cyclooxygenase reaction with the addition of ^{14}C-AA (50 μM) at 37 °C for 30 s. Isolation and quantification of prostanoid products were conducted as described before. Assays were run in duplicate. [b] Ratio of IC_{50}(COX-1):IC_{50}(COX-2). [c] >90% activity remains at these inhibitor concentrations.

nine amide derivatives **38** and **39**, respectively. The L-enantiomer **39** was 2 times more potent at inhibiting COX-2 than the corresponding D-enantiomer **38**.

2. Arylalkyl and Aromatic Amides. Incorporation of a terminal phenyl ring in the alkyl amides also generated potent and selective COX-2 inhibitors as illustrated with the 2-phenethyl amide analogue **40** (see Table 3). As observed with the aromatic esters, COX-2 selectivity was dependent on the type and position of substituents on the phenyl ring. For instance, the 2-methylbenzyl amide **45** was greater than 440-fold selective as a COX-2 inhibitor, whereas the 4-methylbenzyl isomer **46** was only 130-fold selective as a COX-2 inhibitor (see Table 3). Replacement of the 4-methyl group in **46** with an acetyl group (analogue **49**) led to significant improvements in COX-2 potency and selectivity. Furthermore, some enantioselective COX-2 inhibition also was observed with the *R* and *S* enantiomers of α-methyl-4-methylbenzyl amide derivatives of indomethacin. The *R*-α-methyl enantiomer **47** was a better inhibitor of COX-2 than the corresponding *S*-α-

Table 2. In Vitro COX-2 Potencies of Aromatic Esters of Indomethacin



Compound	R	IC_{50} (μM)[a]		Selectivity[b]
		COX-2	COX-1	
18	C_6H_5	0.40	> 66	170
19	β-$C_{10}H_7$	> 8.0	> 66	- - -
20	α-$C_{10}H_7$	5.0	> 66	> 10
21	C_6H_3(3,5-CH_3)	> 8.0	> 66	- - -
22	$(CH_2)_2C_6H_5$	0.040	> 66[c]	> 1700
23	C_6H_4(4-SCH_3)	0.30	3.0	10
24	C_6H_4(2-SCH_3)	0.060	> 66[c]	> 1100
25	C_6H_4(4-OCH_3)	0.040	> 66	> 1700
26	C_6H_4(4-$NHCOCH_3$)	0.050	66	1300
27	C_6H_4(4-F)	0.075	3.0	40
28	3-Pyridyl	0.050	2.5	50

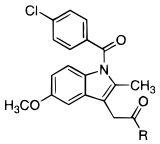
[a] IC_{50} values were determined by incubating several inhibitor concentrations in DMSO with human COX-2 (66 nM) or ovine COX-1 (44 nM) for 20 min at rt followed by initiation of the cyclooxygenase reaction with the addition of ^{14}C-AA (50 μM) at 37 °C for 30 s. Isolation and quantification of prostanoid products were conducted as described before. Assays were run in duplicate. [b] Ratio of IC_{50}(COX-1):IC_{50}(COX-2). [c] >90% activity remains at these inhibitor concentrations.

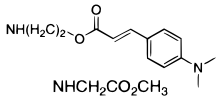
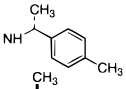
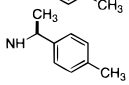
methyl enantiomer **48**. It is noteworthy that all of the aromatic amides containing the 4-fluoro (compound **50**), 4-methylmercapto (compound **52**), or 3-pyridyl substituent (compound **62**) displayed potent and selective COX-2 inhibition (Table 4). In contrast, the corresponding aromatic esters with identical substituents suffered severe losses in COX-2 selectivity.

SAR studies with the indomethacin amides also revealed that only the secondary amides displayed potent and selective COX-2 inhibition, since no significant inhibition of either COX isozyme was discernible with the corresponding tertiary amides at the concentration range studied. This is illustrated by the *N*,*N*-dimethyl (**30**), *N*,*N*-diethyl (**31**), *N*,*N*-methyl-2-phenethyl (**41**), piperidinyl (**42**), and morpholide (**43**) amide derivatives (see Table 3). The primary amide analogue **44** displayed potency and selectivity similar to many of the secondary amides.

Although many of the (monosubstituted)phenyl amide derivatives displayed potent and selective COX-2 inhibition, the 3,4,5-trimethoxyphenyl amide **56** did not display COX-2 inhibition over the concentration range studied. In contrast, COX-2-selective inhibition was discernible with the bulky 4-benzamido-2-methoxy-5-methylphenyl and biphenyl amide analogues **60** and **61**, respectively (see Table 4). Several examples of heterocyclic amide derivatives of indomethacin were analyzed for COX-2-selective inhibition. Although the 3-pyridyl amide derivative **62** was a potent and selective COX-2 inhibitor, the corresponding pyrazinyl amide **65** was a poor inhibitor. Likewise, the thiazolyl amide derivative **67** demonstrated modest COX-2-selective inhibitory properties. Replacement of the methylene group adjacent to the amide linkage in the phenethyl amide analogue **40** generated the previously described hydrox-

Table 3. In Vitro COX-2 Potencies of Aliphatic and Arylalkyl Amide Derivatives of Indomethacin



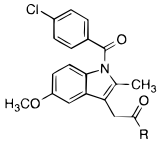
Compound	R	IC_{50} (μM)[a]		Selectivity[b]
		COX-2	COX-1	
Indomethacin	OH	0.75	0.050	0.067
29	$NHCH_3$	0.70	> 66[c]	> 90
30	$N(CH_3)_2$	18	> 66	> 4.0
31	$N(C_2H_5)_2$	25	> 66	> 3.0
32	NHC_8H_{17}	0.040	66	1800
33	NHC_9H_{19}	0.040	17	410
34	$NH(CH_2)_3Cl$	0.050	45	900
35	$NH(CH_2)_2OH$	0.25	> 66	290
36		0.19	> 66[c]	> 350
37	$NHCH_2CO_2CH_3$	4.0	> 66	> 17
38	(D)- $NHCH(CH_3)CO_2CH_3$	0.40	> 66	> 17
39	(L)- $NHCH(CH_3)CO_2CH_3$	0.19	> 66	> 350
40	$NH(CH_2)_2C_6H_5$	0.060	> 66	> 1000
41	$N(CH_3)(CH_2)_2C_6H_5$	> 17	> 66	- - -
42	NC_5H_{10}	> 17	> 66	- - -
43		> 33	> 66	- - -
44	NH_2	0.70	> 20	> 29
45	$NHCH_2C_6H_4(2\text{-}CH_3)$	0.15	> 66[c]	> 440
46	$NHCH_2C_6H_4(4\text{-}CH_3)$	0.060	8.0	130
47		0.060	4.0	64
48		0.20	4.0	20
49	$NHCH_2C_6H_4(4\text{-}COCH_3)$	0.080	> 66	> 800

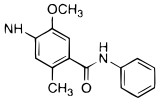
[a] IC_{50} values were determined by incubating several concentrations of inhibitor in DMSO with human COX-2 (66 nM) or ovine COX-1 (44 nM) for 20 min followed by treatment with 1-^{14}C-AA (50 μM) at 37 °C for 30 s. Assays were run in duplicate. [b] Ratio of IC_{50}(COX-1):IC_{50}(COX-2). [c] >80% remaining COX-1 activity at this concentration.

amate derivative **68**[32] that was a nonselective COX inhibitor. Incorporation of a 4-nitro group in the phenyl ring of **68** or replacement of the hydroxamate linkage with a hydrazinyl moiety somewhat improved the COX-2-selective inhibitory properties of the resultant compounds **69** and **70**.

Another interesting aspect in the SAR studies with the indomethacin amides was the observation that the inhibitory potency of indomethacin esters and amides was dependent on the *N*-acyl substituent on the indole ring (Table 5). Replacement of the 4-chlorobenzoyl moiety in the potent and COX-2-selective phenethyl ester **22** and the phenethyl amide **40** with a 4-bromobenzyl group produced inactive compounds (analogues **76** and **81**, respectively). The unacylated indole **80** (see Scheme 3) also was devoid of COX-2-selective inhibitory properties. COX-2 potency also was dependent on the 2-methyl substituent in the indole ring of indomethacin amides and esters. For instance, its replacement with hydrogen in the ester and amide derivatives **22** and **40**, respectively, afforded inactive

Table 4. In Vitro COX-2 Potencies of Aromatic and Heterocyclic Amide Derivatives of Indomethacin



Compound	R	IC_{50} (μM)[a]		Selectivity[b]
		COX-2	COX-1	
50	$NHC_6H_4(4\text{-}F)$	0.060	> 66[c]	> 1000
51	$NHC_6H_4(4\text{-}Cl)$	0.055	> 66[c]	> 1200
52	$NHC_6H_4(4\text{-}SCH_3)$	0.12	> 66	> 550
53	$NHC_6H_4(3\text{-}SCH_3)$	0.22	> 66[c]	> 300
54	$NHC_6H_4(4\text{-}OCH_3)$	0.056	> 66[c]	> 1200
55	$NHC_6H_4(3\text{-}OC_2H_5)$	0.65	53	80
56	$NHC_6H_2(3,4,5\text{-}OCH_3)$	> 1.0	> 66	- - -
57	$NHC_6H_4(4\text{-}NHCOCH_3)$	0.12	> 66[c]	> 550
58	$NHC_6H_4(4\text{-}CH_2CO_2CH_3)$	0.058	> 66[c]	> 1100
59	$NHC_6H_4(4\text{-}CONH_2)$	0.14	> 66[c]	> 471
60		0.60	17	28
61	$NHC_6H_4(4\text{-}C_6H_5)$	0.50	> 66	> 130
62	NH(3-pyridyl)	0.052	> 66	> 1300
63	NH(5-Chloro-3-pyridyl)	0.047	> 66[c]	> 1400
64	NH(2-Chloro-3-pyridyl)	0.050	45	900
65		4.0	> 66	> 17
66		0.70	> 66[c]	> 90
67		4.0	> 66	> 17
68	$NHOCH_2C_6H_5$	0.050	0.06	1.2
69	$NHOCH_2C_6H_4(4\text{-}NO_2)$	0.060	4.0	66
70	$NHNHCH_2C_6H_5$	2.5	> 66	> 26

[a] IC_{50} values were determined by incubating several concentrations of inhibitor in DMSO with human COX-2 (66 nM) or ovine COX-1 (44 nM) for 20 min followed by treatment with 1-^{14}C-AA (50 μM) at 37 °C for 30 s. Assays were run in duplicate. [b] Ratio of IC_{50}(COX-1):IC_{50}(COX-2). [c] >80% remaining COX-1 activity at this concentration.

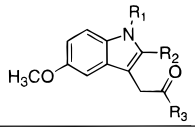
compounds (ester **78** and ester **79**). Likewise, replacement of the 2-methyl group in **77** with hydrogen resulted in an inactive compound.

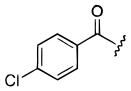
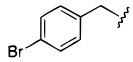
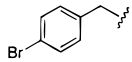
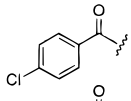
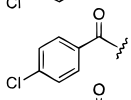
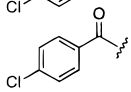
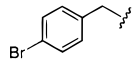
C. Inhibition of COX-2 Activity in RAW264.7 Murine Macrophages. The ability of indomethacin amide derivatives to inhibit COX-2 in intact cells was assayed in RAW264.7 macrophages in which COX-2 activity was induced by pathologic stimuli. The macrophages were exposed to lipopolysaccharide and γ-interferon to induce COX-2 and then treated with several concentrations of 4-methoxyphenyl ester **25** or amide **54**. The IC_{50} values for inhibition of prostaglandin D_2 (PGD_2) by **25** and **54** were 0.20 and 0.062 μM, respectively. Under comparable conditions, indomethacin inhibited PGD_2 synthesis with an IC_{50} of 0.010 μM.

Discussion

The present report describes our initial SAR studies of the conversion of indomethacin into COX-2-selective inhibitors using a biochemically based strategy that we recently reported.[25] Neutral derivatives of NSAIDs such as indomethacin and meclofenamic acid retain the

Table 5. Replacement of the *N*-Acyl and 2-Methyl Group in Indomethacin Esters and Amides



Compd	R_1	R_2	R_3	IC_{50} (μM)[a]		Selectivity[b]
				COX-2	COX-1	
22		CH_3	$C_6H_5(CH_2)_2O$	0.050	> 66[c]	> 1300
76		CH_3	$C_6H_5(CH_2)_2O$	> 66	> 66	- - -
77		CH_3	OH	2.5	> 66	> 30
78		H	$C_6H_5(CH_2)_2O$	> 66	> 66	- - -
40		CH_3	$C_6H_5(CH_2)_2NH$	0.060	> 66	> 1100
79		H	$C_6H_5(CH_2)_2NH$	> 66	> 66	- - -
81		CH_3	$C_6H_5(CH_2)_2NH$	> 66	> 66	- - -

[a] IC_{50} values were determined by incubating several inhibitor concentrations in DMSO with human COX-2 (66 nM) or ovine COX-1 (44 nM) for 20 min at rt followed by initiation of the cyclooxygenase reaction with the addition of ^{14}C-AA (50 μM) at 37 °C for 30 s. Isolation and quantification of prostanoid products were conducted as described before. Assays were run in duplicate. [b] Ratio of IC_{50}(COX-1): IC_{50}(COX-2). [c] >90% activity remains at these inhibitor concentrations.

ability of the parent compounds to bind tightly to COX-2, but they do not bind to COX-1. Therefore, the conversion of these carboxylic acid-containing NSAIDs into ester or amide derivatives generates derivatives that are potent and highly selective COX-2 inhibitors. The potency of some of the derivatives reported in Tables 1−5 is underscored by the fact that the lowest IC_{50} values achieved against COX-2 are comparable to the enzyme concentration used in the assay (routinely 66 nM). The selectivity for COX-2 exhibited by the some of the most potent inhibitors is actually underestimated because many of the compounds assayed exhibited no inhibition of COX-1 at the highest inhibitor concentrations tested (66 μM). Thus, our relatively straightforward strategy produces excellent COX-2 inhibitors.

A striking observation from the present study is the breadth of substitutions tolerated in the ester or amide functionality that yield COX-2 inhibitors. Relatively large alkyl, aryl, aralkyl, and heterocyclic esters or amides of indomethacin exhibit high potency and selectivity. This is consistent with the model we proposed for the binding of these esters or amides to COX-2.[25] The indomethacin moiety is located in the cyclooxygenase active site, but the ester and amide groups project through the constriction at the base of the active site and into a wide "lobby" in the membrane-binding domain (Figure 2). This model is consistent with the crystal structure of a complex of COX-2 with a carboxyl chain-extended analogue of zomepirac.[22a] A comparable lobby is present in COX-1, but there are a number of conserved sequence changes in this region between the proteins that may contribute to the COX-2 selectivity exhibited by ester and amide inhibitors.[22b]

Despite the fact that many of the esters and amides exhibited high COX-2 selectivity, there were notable exceptions, particularly in the ester series. For example, the 4-thiomethoxyphenyl ester of indomethacin was only 10-fold selective for COX-2, whereas the 2-thiomethoxyphenyl ester was greater than 1100-fold selective. Interestingly, the 4-methoxyphenyl ester was greater than 1700-fold selective, and the 4-thiomethoxyphenyl amide of indomethacin was greater than 550-fold selective. Likwise, the 4-fluorophenyl and 3-pyridyl esters exhibited only 40- and 50-fold selectivity, respectively, whereas the analogous amides were greater than 1000- and 1300-fold selective.

Primary and secondary amide derivatives of indomethacin were potent inhibitors of COX-2, whereas tertiary amide derivatives were not. Several dialkyl amides, cycloalkyl amides, or pyridyl derivatives were synthesized and did not inhibit COX-2 under our standard assay conditions. This suggests that hydrogen bonding from the amide nitrogen to a protein acceptor is an important determinant of binding. Possible hydrogen bond acceptors near the carboxylate-binding region of COX-2 include Tyr355 and Glu524 (Figure 2). Mutation of either of these residues to Phe or Ala, respectively, renders the mutant proteins resistant to the inhibitory effects of indomethacin amides.[25]

Substitution of the *p*-chlorobenzoyl group of indomethacin with a *p*-bromobenzyl group generates a molecule, **77**, that is a reasonably effective COX-2 inhibitor[21a] (IC_{50} = 2.5 μM, selectivity > 30 in our hands). We anticipated that conversion of this compound to esters or amides would generate a family of highly selective COX-2 inhibitors, but this was not the case. Neither the

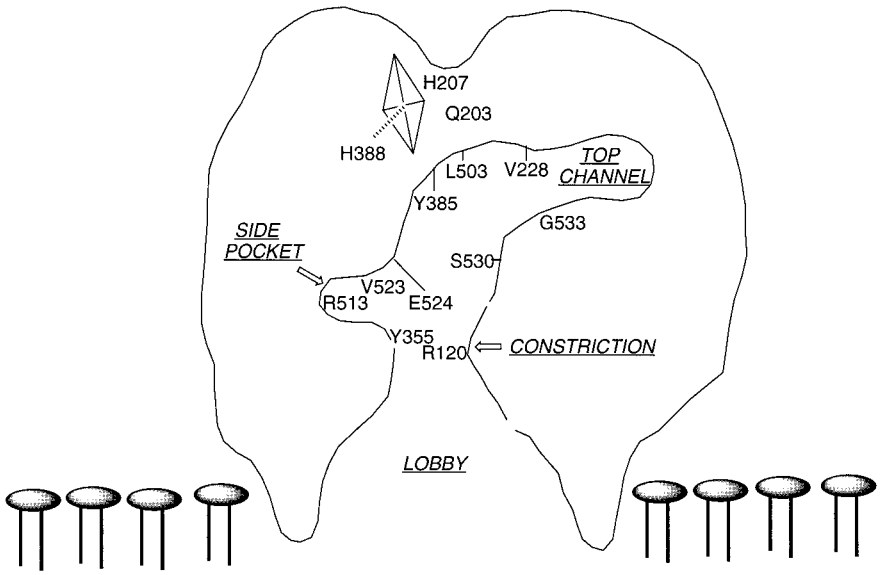


Figure 2. COX subunit with the key structural elements and important amino acid residues. The model for binding of indomethacin esters or amides posits that the substituted indole is bound above the constriction comprised of Arg120, Tyr355, and Glu524; the amide is hydrogen-bonded to residues in the vicinity of the constriction; and the substituted ester or amide group projects down into the lobby. Other structural elements highlighted are the side pocket into which the sulfonamide or sulfone of celecoxib or rofecoxib inserts; the heme group ligated by His388; and the top channel into which the ω-end of arachidonic acid inserts.

phenethyl ester **76** nor the phenethyl amide **81** exhibited any COX-2 inhibitory activity. The analogous derivatives of indomethacin, compounds **22** and **40**, were highly selective inhibitors so it was quite surprising that the derivatives of **77** were not. This suggests that not all carboxylate-containing NSAIDs or related compounds will be converted into COX-2 inhibitors by esterification or amidation.

Perhaps the most surprising result from our SAR analysis was the complete absence of inhibitory activity of indomethacin esters or amides that lacked a methyl group at the 2-position of the indole ring (Table 5). This finding uncovers what may be a previously unrecognized determinant of COX inhibitory activity. Clearly, neither the 2-desmethylphenethyl ester nor the 2-desmethylphenethyl amide were active against COX-2. Since esters or amides of indomethacin are not time-dependent inhibitors of COX-1,[38] it is not possible to speculate whether the 2-methyl group of nonselective inhibitors is also important for inhibition of COX-1. It will be interesting to prepare 2-desmethylindomethacin to explore this possibility.

Some of the molecules described herein (e.g., **40** and **57**) have been found to exhibit antiinflammatory activity in the carageenan-induced foot pad edema assay following oral administration.[25] The time dependence of antiiflammatory activity and in vitro analysis of metabolites generated by liver microsomes suggests that the antiinflammatory activity is due to the parent compound and not due to hydrolysis to indomethacin.[25] This observation, coupled with the structural flexibility revealed in the present study, suggests that conversion of NSAIDs to amide derivatives may be a very useful approach for the generation of novel and efficacious COX-2 inhibitors. The ease of amide synthesis by combinatorial approaches should make it easy to construct highly diverse libraries to maximize not only COX-2 inhibitory activity but also physical properties, distribution, metabolic profile, etc. Thus, our biochemically based method for generating COX-2-selective inhibitors is a promising new strategy for the generation of antiinflammatory, analgesic, and antiangiogenic agents.[26]

Experimental Section

Chemistry. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Chemical yields are unoptimized specific examples of one preparation. Indomethacin was purchased from Sigma (St. Louis, MO). All other chemicals were purchased from Aldrich (Milwaukee, WI). Methylene chloride was purchased as "anhydrous" from Aldrich and was used as received. All other solvents were HPLC grade. Analytical TLC (Analtech uniplates) was used to follow the course of reactions. Silica gel (Fisher, 60−100 mesh) was used for column chromatography. ^{1}H and ^{13}C NMR spectra in $CDCl_3$ were recorded on a Bruker WP-360 or AM-400 spectrometer; chemical shifts are expressed in parts per million (ppm, δ) relative to tetramethylsilane as internal standard. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) are given in hertz (Hz). Positive ion electrospray ionization (ESI) and collision-induced dissociation (CID) mass spectra were obtained on a Finnigan TSQ 7000 mass spectrometer. CID fragmentations were consistent with assigned structures.

General Procedure for the Esterification and Amidation of NSAIDs. Method A: 4-(Methylmercapto)phenyl 1-*p*-Chlorobenzoyl-5-methoxy-2-methylindole-3-acetate (23). A reaction mixture containing indomethacin (300 mg, 0.84 mmol) in 6 mL of anhydrous CH_2Cl_2 was treated with DCC (192 mg, 0.92 mmol), DMAP (10 mg, 84 μmol), and 4-methylmercaptophenol (129 mg, 0.92 mmol). After stirring at room temperature for 5 h, the reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue was diluted with water (~30 mL) and extracted with EtOAc (2 × 30 mL). The combined organic solution was washed with 5% AcOH (2 × 30 mL), 1 N NaOH (2 × 30 mL), and water (~100 mL), dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo. The crude product was purified by chromatography on silica gel (EtOAc:hexanes, 20:80) and obtained as a yellow oil that solidified upon freezing (307 mg, 76%): mp = 132−133 °C; ^{1}H NMR ($CDCl_3$) δ 7.66−7.69 (d, 2 H, J = 8.4 Hz, ArH), 7.46−7.49 (d, 2 H, J = 8.5 Hz, ArH), 7.22−7.23 (d, 1 H, J =

2.4 Hz, ArH), 7.04−7.05 (d, 1 H, J = 2.4 Hz, ArH), 6.97−7.00 (d, 2 H, J = 8.6 Hz, ArH), 6.87−6.90 (d, 1 H, J = 9.0 Hz, ArH), 6.67−6.71 (dd, 1 H, J = 9.0 and 2.5 Hz, ArH), 3.89 (s, 2 H, CH_2), 3.83 (s, 3 H, CH_3), 2.46 (s, 3 H, CH_3), 2.45 (s, 3 H, CH_3); ESI-CID 480 (MH^+), *m/z* 312, 139.

Method B. *N*-(4-Acetamidophenyl)-1-*p*-chlorobenzoyl-5-methoxy-2-methylindole-3-acetamide (57). A reaction mixture containing indomethacin (300 mg, 0.84 mmol) and bis-(2-oxazolidinyl)phosphinic chloride (218 mg, 0.84 mmol) in 5 mL of anhydrous CH_2Cl_2 was treated with Et_3N (167 mg, 0.84 mmol) and allowed to stir at room temperature for 10 min. The mixture was then treated with 4′-aminoacetanilide (141 mg, 0.94 mmol) and stirred overnight at room temperature. The precipitate that formed was filtered and washed with cold CH_2Cl_2 and then recrystallized from hot MeOH to afford a pale yellow solid (221 mg, 54%): mp = 256−257 °C; ^{1}H NMR (DMSO-d_6) δ 10.14 (s, 1H, NH), 9.86 (s, 1H, NH), 7.62−7.70 (m, 4H, ArH), 7.48 (s, 4H, ArH), 7.18 (d, 1H, J = 2.3 Hz, ArH), 6.90−6.93 (d, 1H, J = 9.0 Hz, ArH), 6.68−6.72 (dd, 1H, J = 9.1 and 2.5 Hz, ArH), 3.73 (s, 3H, CH_3), 3.71 (s, 2H, CH_2), 2.27 (s, 3H, CH_3), 1.99 (s, 3H, CH_3); ESI-CID 492 (MH^+), *m/z* 340, 312, 174.

***N*-(2-Hydroxyethyl)-1-*p*-chlorobenzoyl-5-methoxy-2-methylindole-3-acetamide (35). 35** was obtained in a similar manner as described above. Chromatography of the crude product on silica gel (EtOAc) afforded **33** as a pale yellow solid (143 mg, 39%): mp = 162−164 °C; ^{1}H NMR ($CDCl_3$) δ 7.66−7.68 (dd, 2 H, J = 6.7 and 1.7 Hz, ArH), 7.47−7.50 (dd, 2 H, J = 6.9 and 1.9 Hz, ArH), 6.85−6.89 (d and s, 2 H, J = 9.2 Hz, ArH), 6.68−6.72 (dd, 1 H, J = 9.0 and 2.5 Hz, ArH), 6.03 (bs, 1 H, NH), 3.82 (s, 3 H, CH_3), 3.67 (bs, 4 H, $2CH_2$), 3.35−3.40 (q, 2 H, J = 4.8 Hz, CH_2), 2.44 (bs, 1 H, OH), 2.39 (s, 3 H, CH_3); ESI-CID 401 (MH^+), *m/z* 375.

***N*-[2-(*N*,*N*-Dimethylamino)cinnamyloxyethyl]-1-*p*-chlorobenzoyl-5-methoxy-2-methylindole-3-acetamide (36).** To a solution of *N*,*N*-dimethylaminocinnamic acid (85 mg, 0.44 mmol) in dry CH_2Cl_2 (10 mL) were added DCC (99 mg, 0.48 mmol) and DMAP (6 mg, 44 μmol) followed by **33** (200 mg, 0.5 mmol). The reaction was stirred at room temperature overnight. The mixture was diluted with water (∼30 mL) and extracted with EtOAc (2 × 30 mL). The combined organic solution was washed with water (2 × 50 mL), dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo. The crude product was purified by chromatography on silica gel (EtOAc: hexanes, 20:80 then 50:50) and obtained as a bright yellow solid (162 mg, 58%): mp = 138−140 °C; ^{1}H NMR (DMSO-d_6) δ 8.19 (bt, 1 H, NH), 7.64−7.66 (d, 2 H, J = 8.5 Hz ArH), 7.59−7.61 (d, 2 H, J = 8.6 Hz, ArH), 7.42−7.50 (m, 2 H, ArH), 7.10−7.11 (d, 1 H, J = 2.3 Hz, ArH), 6.90−6.92 (d, 1 H, J = 9.0 Hz, ArH), 6.68−6.72 (dd, 1 H, J = 9.0 Hz & 2.4 Hz, ArH), 6.66−6.69 (m, 3 H, ArH and olefinic H), 6.16−6.20 (d, 1 H, J = 16 Hz, olefinic H), 4.08−4.11 (t, 2 H, J = 5.5 Hz, CH_2), 3.72 (s, 3 H, CH_3), 3.51 (s, 2 H, CH_2), 3.32−3.35 (m, 2 H, CH_2), 2.96 (s, 6 H, $N(CH_3)_2$), 2.20 (s, 3 H, CH_3); UV (AcCN) λ_{max} 364 nm; ESI-CID 574 (MH^+), *m/z* 174.

1-*p*-Chlorobenzoyl-5-methoxy-2-methylindole-3-acetamide (44).[39] A reaction mixture containing indomethacin (750 mg, 2.01 mmol), PyBOP (1.57 g, 3.02 mmol), HOBt (409 mg, 3.02 mmol), DIPEA (1.4 mL, 8.04 mmol), and NH_4Cl (215 mg, 4.02 mmol) in dry DMF (8 mL) was stirred at room temperature overnight. The precipitated solid was filtered, washed with cold CH_2Cl_2 and then recrystallized from CH_2Cl_2/hexanes to afford a pale yellow solid (600 mg, 84%): mp = 225−227 °C; ^{1}H NMR (DMSO-d_6) δ 7.62−7.70 (q, 4 H, J = 8.6 Hz, ArH), 7.43 (bs, 1 H, NH), 7.09−7.10 (d, 1 H, J = 2.5 Hz, ArH), 6.97 (bs, 1 H, NH), 6.90−6.93 (d, 1 H, J = 8.9 Hz, ArH), 6.68−6.72 (dd, 1 H, J = 8.9 and 2.5 Hz, ArH), 3.75 (s, 3 H, CH_3), 3.45 (s, 2 H, CH_2), 2.21 (s, 3 H, CH_3); ESI-MS calcd for $C_{19}H_{17}ClN_2O_4$ (MH^+) 357.09, found 356.8; CID *m/z* 340, 312, 139.

5-Methoxy-3-methylindole 3-Phenethylacetate (73). This compound was prepared by the reaction of 5-methoxy-2-methylindole (**71**) with phenethyl alcohol in the presence of BOP-Cl in a similar manner as described for indomethacin amides. Chromatography on silica gel (EtOAc:hexanes, 50:50) gave the title compound as a yellow oil (394 mg, 67%): ^{1}H NMR ($CDCl_3$) δ 7.72 (bs, 1 H, NH), 7.10−7.25 (m, 6 H, ArH), 6.96−6.97 (d, 1 H, J = 2.2 Hz, ArH), 6.75−6.79 (dd, 1 H, J = 8.7 Hz & 2.3 Hz, ArH), 4.26−4.30 (t, 2 H, J = 6.9 Hz, CH_2), 3.83 (s, 3 H, OCH_3), 3.63 (s, 2 H, CH_2), 2.87−2.91 (t, 2 H, J = 6.9 Hz, CH_2), 2.34 (s, 3 H, CH_3).

Phenethyl 1-*p*-Bromobenzyl-5-methoxy-3-methylindole-3-acetate (76). To a solution of **73** (394 mg, 1.2 mmol) in 5 mL of anhydrous DMF was added NaH (53 mg, 1.3 mmol) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 20 min and then treated with 4-bromobenzyl bromide (330 mg, 1.3 mmol). The reaction mixture was stirred overnight and then diluted with water. The aqueous solution was extracted with ether (2 × 20 mL). The combined organic was washed with water (2 × 25 mL), dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo. The residue was chromatographed on silica gel (EtOAc:hexanes, 5:95−10:90) to afford **73** as a light brown solid (384 mg, 73%): mp = 104−106 °C; ^{1}H NMR ($CDCl_3$) δ 7.35−7.37 (d, 2 H, J = 8.3 Hz, ArH), 7.01−7.35 (m, 7 H, ArH), 6.96−6.97 (d, 1 H, J = 2.2 Hz, ArH), 6.75−6.81 (m, 3 H, ArH), 5.20 (s, 2 H, CH_2), 4.27−4.31 (t, 2 H, J = 6.9 Hz, CH_2), 3.83 (s, 3 H, OCH_3), 3.68 (s, 2 H, CH_2), 2.87−2.92 (t, 2 H, J = 6.9 Hz, CH_2), 2.24 (s, 3 H, CH_3); ESI-CID 494 (MH^+), *m/z* 344, 231, 170, 105.

1-*p*-Bromobenzyl-5-methoxy-3-methylindole-3-acetic Acid (77). A reaction mixture containing **76** (150 mg) and 1 N LiOH (∼1.5 mL) in MeOH (3 mL) was stirred at room temperature for 4 h. The reaction mixture was diluted with water and extracted with EtOAc (2 × 10 mL) (the organic washing was discarded) and then acidified with 1 N HCl and extracted with EtOAc (2 × 20 mL). The combined organic solution was washed with water (2 × 20 mL), dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo. The crude residue was recrystallized from CH_2Cl_2/hexanes to generate **77** as fluffy white crystals in 90% yield: mp = 198−199 °C; ^{1}H NMR ($CDCl_3$) δ 7.36−7.38 (d, 2 H, J = 8.4 Hz & 1.8 Hz, ArH), 7.02−7.07 (m, 2 H, ArH), 6.75−6.82 (m, 3 H, ArH), 5.21 (s, 2 H, CH_2), 3.48 (s, 3 H, OCH_3), 3.73 (s, 2 H, CH_2), 2.29 (s, 3 H, CH_3); NI-ESI 388 $(M - H)^-$.

5-Methoxy-2-methylindole-3-phenethylacetamide (80). A reaction mixture containing **40** (500 mg, 1.08 mmol) in MeOH (5 mL) was treated with 1 N NaOH (5 mL) was stirred at room temperature overnight. The reaction mixture was diluted with water, extracted with EtOAc (3 × 20 mL). The organic extracts were washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo to essentially yield the pure compound as a white solid (324 mg, 93%): ^{1}H NMR ($CDCl_3$) δ 7.82 (bs, 1 H, NH), 7.18−7.21 (d, 1 H, J = 8.2 Hz, ArH), 7.11−7.13 (m, 3 H, ArH), 6.90−6.93 (m, 2 H, ArH), 6.80−6.84 (m, 2 H, ArH), 5.65 (bt, 1 H, NH), 3.82 (s, 3 H, OCH_3), 3.59 (s, 2 H, CH_2), 3.38−3.44 (t, 2 H, J = 6.6 Hz, CH_2), 2.64−2.68 (t, 2 H, J = 6.8 Hz, CH_2), 2.25 (s, 3 H, CH_3).

1-*p*-Bromobenzyl-5-methoxy-2-methylindole-3-phenethylacetamide (81). This compound was prepared in a similar manner as described for **76**. Compound was obtained as an off-white solid (recrystallization from CH_2Cl_2/hexanes) (481 mg, 80%): mp = 141−143 °C; ^{1}H NMR ($CDCl_3$) δ 7.34−7.37 (d, 2 H, J = 8.4 Hz, ArH), 7.07−7.10 (m, 4 H, ArH), 6.88−6.91 (m, 3 H, ArH), 6.74−7.83 (m, 3 H, ArH), 5.64 (bt, 1 H, NH), 5.18 (s, 2 H, CH_2), 3.83 (s, 3 H, OCH_3), 3.64 (s, 2 H, CH_2), 3.40−3.46 (t, 2 H, J = 6.6 Hz, CH_2), 2.64−2.69 (t, 2 H, J = 6.8 Hz, CH_2), 2.14 (s, 3 H, CH_3); ESI-CID 491 (MH^+), *m/z* 344, 171, 122.

1-*p*-Chlorobenzoyl-5-methoxyindole-3-phenethylacetamide (79). To a solution of 5-methoxyindole-3-acetic acid (**72**; 450 mg, 2.19 mmol) in dry CH_2Cl_2 (10 mL) were added EDCI (497 mg, 2.6 mmol) and DMAP (27 mg, 0.22 mmol), followed by phenethylamine (315 mg, 2.6 mmol). The reaction was stirred at room temperature overnight. The mixture was diluted with water (∼30 mL) and extracted with EtOAc (2 × 30 mL). The combined organic solution was washed with water (2 × 50 mL), dried ($MgSO_4$), and filtered, and the solvent was removed in vacuo. The crude amide **75** upon purification by

chromatography on silica gel (EtOAc:hexanes, 30:70) was obtained as a yellow oil (600 mg, 88%) and was used in the next step without any further purification, owing to its instablity. To this amide (650 mg, 2.11 mmol) in dry DMF (5 mL) was added NaH (60% dispersion in mineral oil, 60 mg, 2.5 mmol) at 0 °C under argon. After stirring at 0 °C for 20 min, 4-chlorobenzoyl chloride (420 mg, 2.4 mmol) was added to the solution which was then allowed to attain room temperature and stirred for 15 h. The reaction was quenched with water and extracted with Et_2O (3 × 10 mL). The combined Et_2O extract was washed with water, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was chromatographed on silica gel (EtOAc:hexanes, 50:50) to afford **79** as a pale yellow solid (700 mg, 74%): mp = 125−126 °C; ^{1}H NMR ($CDCl_3$) δ 8.29−8.32 (d, 1 H, J = 8.8 Hz, ArH), 7.60−7.62 (d, 2 H, J = 6.9 Hz, ArH), 7.49−7.52 (d, 2 H, J = 7.0 Hz, ArH), 7.07−7.13 (m, 5 H, ArH), 6.87−6.95 (m, 3 H, ArH), 5.6 (bs, 1 H, NH), 3.86 (s, 3 H, CH_3), 3.57 (s, 2 H, CH_2), 3.44−3.46 (m, 2 H, CH_2), 2.65−2.69 (t, 2 H, J = 6.5 Hz, CH_2); ESI-CID 447 (MH^+), m/z 298, 139, 122, 105.

Enzymology. Arachidonic acid was purchased from Nu Chek Prep (Elysian, MN). [1-^{14}C]Arachidonic acid (∼55−57 mCi/mmol) was purchased from NEN Dupont or American Radiolabeled Chemicals (ARC, St. Louis, MO). Hematin was purchased from Sigma Chemical Co. (St. Louis, MO). COX-1 was purified from ram seminal vesicles (Oxford Biomedical Research, Inc., Oxford, MI) as described earlier.[36] The specific activity of the protein was 20 (μMO_2/min)/mg, and the percentage of holoprotein was 13.5%. ApoCOX-1 was prepared as described earlier.[37] Apoenzyme was reconstituted by the addition of hematin to the assay mixtures. Human COX-2 was expressed in SF-9 insect cells by means of the pVL 1393 expression vector (Pharmingen) and purified by ion-exchange and gel filtration chromatography. All of the purified proteins were shown by densitometric scanning of a 7.5% SDS PAGE gel to be >80% pure. SC-299 was a gift of Dr. John J. Talley, Monsanto/Searle (St. Louis, MO).

Time- and Concentration-Dependent Inhibition of Ovine COX-1 and Human COX-2 Using Thin Layer Chromatography (TLC) Assay. Cyclooxygenase activity of ovine COX-1 (44 nM) or human COX-2 (66 nM) was assayed by TLC. Reaction mixtures of 200 μL consisted of hematin-reconstituted protein in 100 mM Tris-HCl, pH 8.0, 500 μM phenol, and [1-^{14}C]arachidonic acid (50 μM, ∼55−57 mCi/mmol). For the time-dependent inhibition assay, hematin-reconstituted COX-1 (44 nM) or COX-2 (66 nM) was preincubated at room temperature for 20 min with varying inhibitor concentrations in DMSO followed by the addition of [1-^{14}C]-arachidonic acid (50 μM) for 30 s at 37 °C. Reactions were terminated by solvent extraction in Et_2O/CH_3OH/1 M citrate, pH 4.0 (30:4:1). The phases were separated by centrifugation at 2000g for 2 min and the organic phase was spotted on a TLC plate (J. T. Baker, Phillipsburg, NJ). The plate was developed in EtOAc/CH_2Cl_2/glacial AcOH (75:25:1) at 4 °C. Radiolabeled prostanoid products were quantitated with a radioactivity scanner (Bioscan, Inc., Washington, D.C.). The percentage of total products observed at different inhibitor concentrations was divided by the percentage of products observed for protein samples preincubated for the same time with DMSO.

Inhibition of COX-2 Activity in Activated RAW264.7. Protocols for COX-2 inhibition in RAW264.7 cells have been previously described.[23] Briefly, cells (6.2 × 10^6 cells/T25 flask) were activated with lipopolysaccharide (1 μg/mL) and γ-interferon (10 U/mL) in serum-free DMEM for 7 h and then treated with inhibitor (0−2 μM) for 30 min at 37 °C. Exogenous arachidonate metabolism was determined by adding [1-^{14}C]-arachidonic acid (20 μM) for 15 min at 37 °C. IC_{50} values are the average of two independent determinations.

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Supporting Information Available: ^{1}H NMR data for all compounds included in the study. This information is available free of charge via the Internet at http://pubs.acs.org.

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