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Bioorganic & Medicinal Chemistry 13 (2005) 6810–6822
Bioorganic & Medicinal Chemistry

Indolyl esters and amides related to indomethacin are selective COX-2 inhibitors

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Received 5 August 2003; revised 15 June 2005; accepted 25 July 2005
Available online 19 September 2005

Abstract—Previous studies from our laboratory have revealed that esterification/amidation of the carboxylic acid moiety in the non-steroidal anti-inflammatory drug, indomethacin, generates potent and selective COX-2 inhibitors. In the present study, a series of reverse ester/amide derivatives were synthesized and evaluated as selective COX-2 inhibitors. Most of the reverse esters/amides displayed time-dependent COX-2 inhibition with IC_{50} values in the low nanomolar range. Replacement of the 4-chlorobenzoyl group on the indole nitrogen with a 4-bromobenzyl moiety resulted in compounds that retained selective COX-2 inhibitory potency. In addition to inhibiting COX-2 activity in vitro, the reverse esters/amides also inhibited COX-2 activity in the mouse macrophage-like cell line, RAW264.7. Overall, this strategy broadens the scope of our previous methodology of neutralizing the carboxylic acid group in NSAIDs as a means of generating COX-2-selective inhibitors and is potentially applicable to other NSAIDs.

1. Introduction

The utility of non-steroidal anti-inflammatory drugs (NSAIDs) in the treatment of inflammation and pain is often limited by gastrointestinal liabilities including ulceration and bleeding. Inhibition of cyclooxygenase (COX), the enzyme that catalyzes arachidonic acid oxygenation, was initially considered to be responsible for the shared therapeutic benefits and gastrointestinal side effects of NSAIDs.[1,2] However, the discovery of a second COX gene, COX-2, provided important insights into NSAID side effects that translated into more effective drugs.[3,4] COX-2 is inducible, short-lived, and the product of an immediate early gene.[5] Its expression is stimulated by a host of growth factors, cytokines, and mitogens.[6] Importantly, COX-2 is responsible for prostaglandin biosynthesis in inflammatory cells and the central nervous system.[7–9] In contrast, COX-1 is a constitutive enzyme and appears responsible for the biosynthesis of cytoprotective prostaglandins in the gastric mucosa and the kidney.[10] Classical NSAIDs inhibit both isoforms non-selectively.[11] The differential tissue distribution of the COX enzymes was the basis for the development of COX-2-selective inhibitors as anti-inflammatory and analgesic agents with reduced ulcerogenic potency.[8]

The first COX-2 inhibitors developed clinically were members of the diarylheterocycle class of compounds (Fig. 1).[12] Extensive structure–activity studies exist in this series.[13] In contrast, relatively few groups have utilized non-selective NSAIDs as leads in the search for selective COX-2 inhibitors. The Merck–Frosst group reported that replacement of the 4-chlorobenzoyl group in the NSAID, indomethacin, with a 4-bromobenzyl group generates a selective COX-2 inhibitor (Fig. 1).[14,15] Our laboratory reported that neutralization of the carboxylic acid moiety in indomethacin to esters or amides afforded selective COX-2 inhibitors (Fig. 1).[16] More recently, the Novartis group described conversion of diclofenac into lumiracoxib, which exhibits 500-fold selectivity for COX-2 over COX-1 (Fig. 1).[17]

Keywords: Indomethacin; COX-2; Indolylester; Indolylamide.
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doi:10.1016/j.bmc.2005.07.073

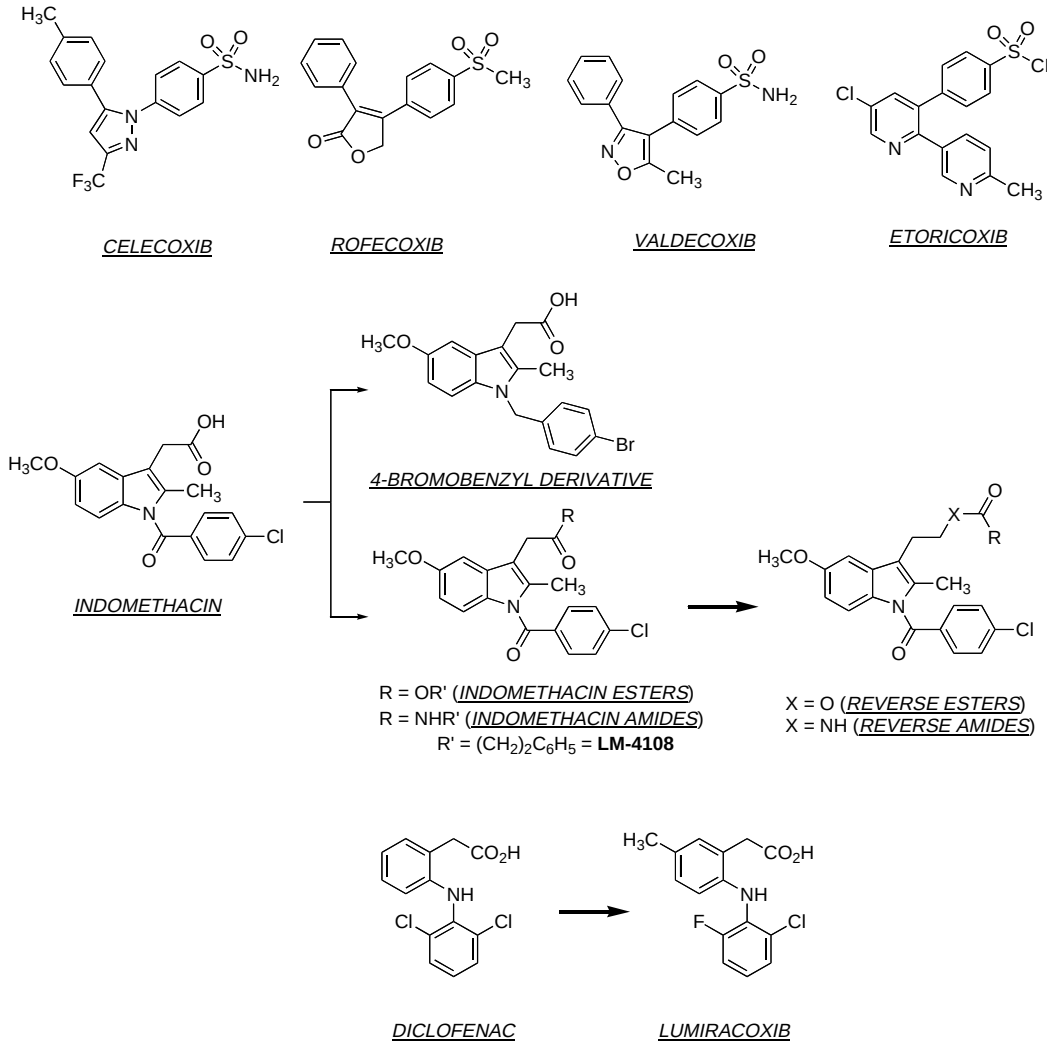


Figure 1. Structures of selective COX-2 inhibitors from the diarylheterocycle series and those derived from the modification of indomethacin and diclofenac.

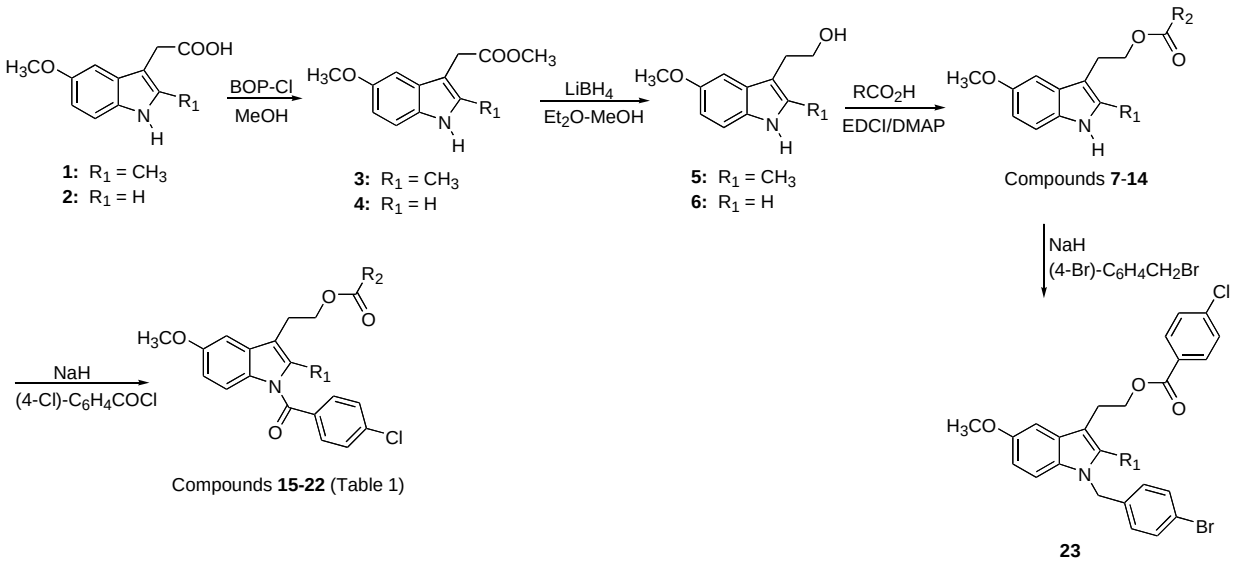
Conversion of non-selective NSAIDs to esters and amides is a facile strategy for generating COX-2 inhibitors from known drugs but it has the limitation that indomethacin esters and possibly some amides may be hydrolyzed to indomethacin in vivo.[18,19] Therefore, we investigated the possibility that indolyl esters and amides with essentially the ''reverse'' orientation would selectively inhibit COX-2. Hypothetically, such compounds eliminate or minimize the generation of indomethacin in vivo. We report herein that indolyl esters and amides are potent and selective inhibitors of COX-2 in vitro and in intact macrophages. Furthermore, reversing the orientation of the carboxyl group expands the universe of COX-2 inhibitors to 4-bromobenzyl analogs of indomethacin.

2. Results

2.1. Chemistry

The synthesis of the target reverse ester derivatives was achieved in four steps starting from commercially available 5-methoxy-2-methylindole-3-acetic acid (**1**) (Scheme 1). Esterification of **1** in the presence of MeOH and BOP-Cl[20] afforded the corresponding methyl ester **3**[21] that upon reduction with lithium borohydride in THF–MeOH[22] yielded the corresponding alcohol **5**.[23] Alcohol **5** served as the synthon in the preparation of all reverse esters. Esterification of appropriate carboxylic acid derivatives with alcohol **5** employing EDCI as catalyst resulted in the formation of esters **7**–**14** in moderate to good yields (60–75%). N-acylation on the indole nitrogen in esters **7**–**14** with 4-chlorobenzoyl chloride in the presence of NaH afforded the final compounds **15**–**21** (Table 1). N-alkylation of **11** with 4-bromobenzyl bromide afforded the *N*-(4-bromobenzyl)indole derivative **23**. Similar synthetic manipulations on 5-methoxyindole-3-acetic acid (**2**) afforded the corresponding 2-*des*-methylindolyl alkyl ester **22** (see Scheme 1).

Two approaches were employed for the synthesis of reverse amides. The first utilized **1** as starting material and proceeded as outlined in Scheme 2. Reaction of **1** with ammonium chloride[24] in the presence of EDCI, HOBt, and DIPEA afforded primary amide **24** in 64%



Scheme 1.

yield. LAH mediated reduction of **24** afforded the primary amine **25**,[25] which was characterized as its stable oxalate salt. EDCI-promoted coupling of **25** with 4-chlorobenzoic acid, hydrocinnamic acid, or propionic acid afforded amides **26**, **27**, and **30**, respectively, that upon N-acylation with 4-chlorobenzoyl chloride or 4-bromobenzyl bromide furnished target compounds **31**–**33**, and **37**, respectively. N-Acylation/alkylation of melatonin (**28**) afforded the *N*-4-chlorobenzoyl- and *N*-4-bromobenzyl *des*-methylindole analogs **34** and **35**, respectively (see Scheme 2). The synthesis of **36** (see Scheme 2) was achieved via N-acetylation of **25** with acetyl chloride in the presence of Et_3N resulting in the formation of the previously described 2-methylmelatonin derivative **29**.[25] N-alkylation on the indole nitrogen in **29** with 4-bromobenzyl bromide afforded **36**.

Alkylation of the acylindolylamines, **26**–**30**, with 4-bromobenzyl bromide proceeded to some extent on the amide nitrogen as well as the indole nitrogen. Thus, a second route was developed for reverse amide synthesis in which the amine of **25** was BOC-protected then selectively alkylated on the indole nitrogen (Scheme 2). Removal of the protecting group and acylation of the amine produced reverse amides **41**–**43**.

The synthesis of *N*-(4-bromobenzyl)-3-butyl-5-methoxy-2-methylindole (**47**) is depicted in Scheme 3. Reaction of 4-methoxyphenylhydrazine (**44**) and 2-heptanone (**45**) following the Fisher-indole protocol afforded indole **46** that upon N-alkylation with 4-bromobenzyl bromide generated **47**.

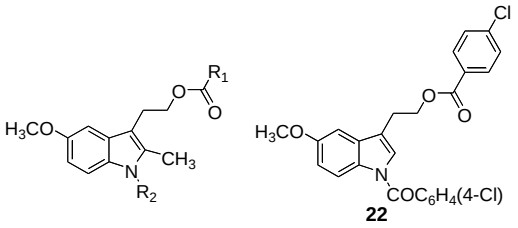
2.2. Enzymology

2.2.1. In vitro inhibition of COX activity. IC_{50} values for the inhibition of purified human COX-2 or ovine COX-1 by test compounds were determined by a thin layer chromatography (TLC) assay. Hematin-reconstituted COX-2 (66 nM) or COX-1 (44 nM) in 100 mM Tris–HCl, pH 8.0, containing 500 μM phenol was treated with several concentrations of inhibitors (0–66 μM) at 25 °C for 20 min. Since the recombinant COX-2 had specific activity lower than that of ovine COX-1, the protein concentrations were adjusted such that the percentages of total products obtained following arachidonic acid oxygenation by the two isozymes were comparable. The cyclooxygenase reaction was initiated by the addition of [1-^{14}C]arachidonic acid (50 μM) at 37 °C for 30 s. Control experiments in the absence of inhibitor indicated ~25–30% conversion of arachidonic acid to products, which was sufficient for assessing the inhibitory properties of all compounds described in this study.

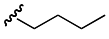
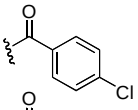
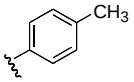
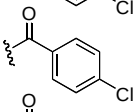
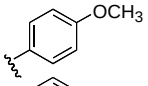
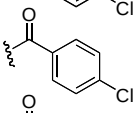
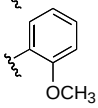
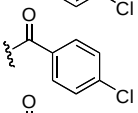
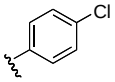
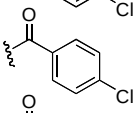
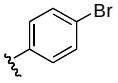
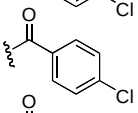
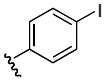
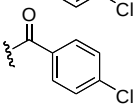
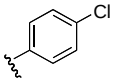
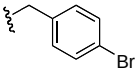
As displayed in Table 1, all of the reverse esters **15**–**21** displayed potent and selective inhibition of COX-2 with IC_{50} values in the low nanomolar range and very high COX-2 selectivity ratios. No COX-1 inhibition was discernible with these compounds even at very high concentrations (>66 μM). Under these assay conditions, indomethacin demonstrated modest COX-1-selective inhibition [(IC_{50} (COX-1) ~ 0.050 μM; IC_{50} (COX-2) ~ 0.75 μM)]. Removal of the 2-methyl group on the indole ring of the reverse ester **19** generated **22**, an inactive compound. Lack of COX inhibitory potency also was observed with the 2-*des*-methyl analogs of the simple indomethacin esters.[26] Replacement of the 4-chlorobenzoyl group on the indole nitrogen in **19** with a 4-bromobenzyl group afforded **23**, which also displayed selective COX-2 inhibitory properties. Compound **23**, however, was 40-fold less potent as a COX-2 inhibitor than **19**. In contrast, replacement of the 4-chlorobenzoyl group on the indole nitrogen with a 4-bromobenzyl group in the previously reported indomethacin ester series afforded inactive compounds.[16]

Examples from the *N*-(substituted)-5-methoxy-2-methylindole alkyl amide series included the 4-chlorobenzoyl and the phenethyl amide derivatives **31** and **33**, which

Table 1. IC_{50} values for selective COX-2 inhibition by reverse esters of indomethacin



22

Compound	R_1	R_2	IC_{50} (μM)[a]		Selectivity[b]	IC_{50}(μM)[c]
			COX-2	COX-1		Intact cells
15			0.065	>66	>1000	
16			0.050	>66	>1300	
17			0.040	>66	>1500	0.32
18			0.050	>66	>1300	
19			0.050	>66	>1500	
20			0.040	>66	>1300	>0.085
21			0.050	>66	>1330	
22	—	—	>66	66	—	
23			2.0	>66	>33	

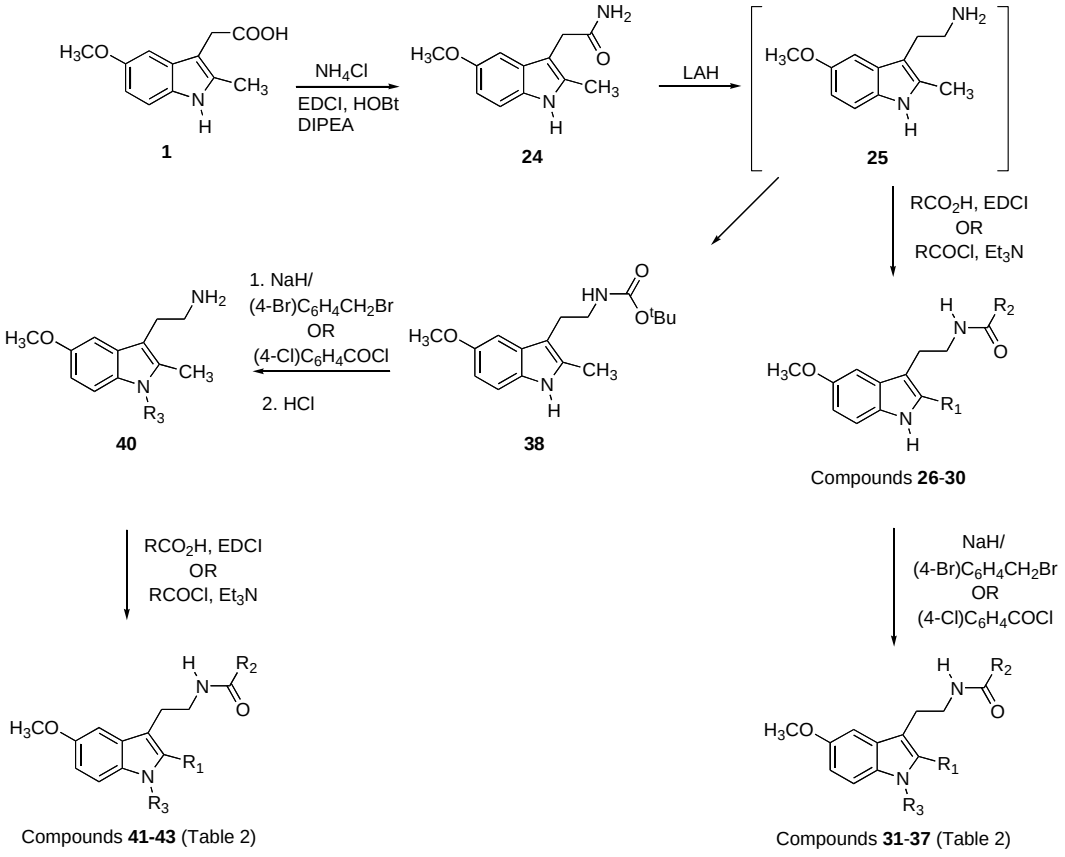
[a] Ovine COX-1 (44 nM) or human COX-2 (66 nM) was preincubated with inhibitors at 25 °C for 20 min followed by the addition of [1-^{14}C]arachidonic acid (50 μM) at 37 °C for 30 s. All assays were conducted in duplicate.

[b] Ratio of IC_{50}(COX-1)/IC_{50} (COX-2).

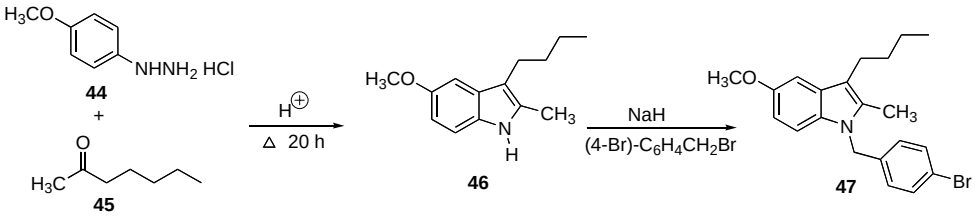
[c] RAW264.7 murine macrophages were activated with LPS and IFN-γ for 7 h. Vehicle or inhibitor was added for 30 min at 37 °C. Inhibition of prostaglandin D_2 synthesis was determined by adding [1-^{14}C]arachidonic acid (20 μM) for 15 min at 25 °C.

also displayed potent COX-2 inhibition (Table 2). But these compounds also displayed increased COX-1 inhibition such that the COX-1 over COX-2 selectivity ratio of **31** and **33** was 80 and 425, respectively, compared to the esters which displayed selectivity ratios of 1500 or greater. Interestingly, increasing the length of the incubation time between the COX-1/inhibitor reaction mixture and [1-^{14}C]arachidonic acid from 30 s to 10 min abolished all COX-1 inhibition by **31** and **33** [compound **31**: IC_{50} (COX-1) ~ 4.0 μM (30 s assay); IC_{50} (COX-1) > 66 μM (10 min assay); IC_{50} (COX-2) ~ 0.050 μM (30 s assay); IC_{50} (COX-2) ~ 0.060 μM (10 min assay)]. This result suggests that the off-rates for the dissociation of **31** and **33** from COX-1 are relatively high so they exchange with arachidonic acid substrate on prolonged incubation. The inhibitory potency of **31** as a competitive inhibitor of COX-1/-2 also was evaluated. Ovine COX-1 (2.8 nM) or human COX-2 (5 nM) was added to an incubation mixture containing [1-^{14}C]arachidonic acid (2 μM) and several concentrations of **31** (0.050 μM, 0.25 μM, 1.0 μM, or 4.0 μM). Although **31** was a potent competitive inhibitor of COX-1 (IC_{50} = 0.25 μM), no competitive inhibition of COX-2 by **31** was discernible under these experimental conditions (IC_{50} > 4 μM). Competitive inhibition of COX-1 activity has been reported with other time-dependent, selective COX-2 inhibitors.[27,28]

Inhibition of COX-1 by **31** and other reverse amides was abolished by replacement of the 4-chlorobenzoyl group in these compounds with a 4-bromobenzyl group as illustrated with **32**, **36**, and **37**, respectively (see Table 2). The 4-bromobenzyl derivative of 2-methylmelatonin (**36**) also displayed COX-2-selective inhibitory



Scheme 2.



Scheme 3.

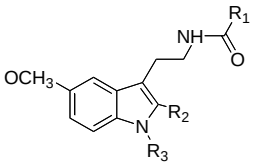
properties, albeit with lower COX-2 potency than **32**. Increasing the length of the alkyl substituent on the amide linkage from methyl (**36**) to ethyl (**37**) increased somewhat COX-2 potency and selectivity. In contrast to these observations, replacement of the 4-chlorobenzoyl moiety in simple indomethacin amides with a 4-bromobenzyl group affords inactive compounds.[16] As observed with simple indomethacin esters/amides and with reverse esters, replacement of the 2-methyl group on the indole ring in the reverse amides with hydrogen generated inactive compounds (analogs **34** and **35**).[26] Finally, replacement of the 3-alkylester or -amide linkage in these compounds with a long chain alkyl substituent resulted in the 3-butyl derivative, **47**, which was devoid of COX inhibitory activity (Table 2).

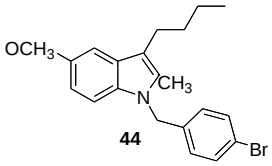
2.2.2. Inhibition of COX-2 activity in intact cells. The ability of reverse esters **17** and **19** or reverse amides **31**, **32**, **36**, and **37** to inhibit COX-2 in intact cells was assayed in RAW264.7 murine macrophage-like cell line.[29] The cells were treated with lipopolysaccharide (LPS) (1.0 μg/mL) and γ-interferon (10 U/mL) for 7.0 h to induce COX-2 and then treated with several concentrations of the test compounds. The IC_{50} values for inhibition of production of prostaglandin D_2 (PGD_2) by **17**, **19**, **31**, **32**, **36**, and **37** were 0.32, 0.085, 0.03, 0.05, 1.5, and 0.14 μM, respectively. Thus, in addition to inhibition of purified COX-2, these compounds are potent inhibitors of COX-2 activity in cultured inflammatory cells.

3. Discussion

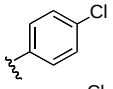
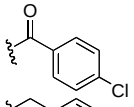
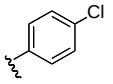
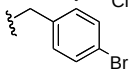
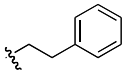
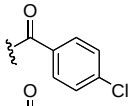
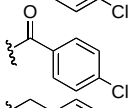
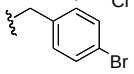
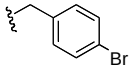
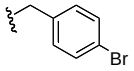
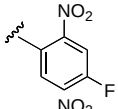
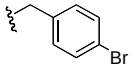
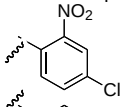
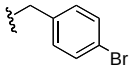
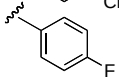
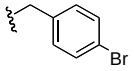
The present study broadens the scope and utility of our earlier strategy involving the esterification/amidation of NSAIDs as means of generating selective COX-2 inhibitors. As observed with the earlier indomethacin esters/

Table 2. IC_{50} values for selective COX-2 inhibition by reverse amides of indomethacin





44

Compound	R_1	R_2	R_3	IC_{50} (μM)[a]		Selectivity[b]	IC_{50}(μM)[c]
				COX-2	COX-1		Intact cells
31		CH_3		0.05	4.0	80	0.03
32		CH_3		0.04	>66	>1650	0.05
33		CH_3		0.04	17	425	
34	CH_3	H		>66	>66	—	
35	CH_3	H		>66	>66	—	
36	CH_3	CH_3		2.0	>66	>33	1.50
37	C_2CH_3	CH_3		0.40	>66	>165	0.14
41		CH_3		2.4	>3	>1.2	
42		CH_3		0.22	>3	>14	
43		CH_3		0.05	>1	>20	
47	—	—		>66	>66	—	

[a] Ovine COX-1 (44 nM) or human COX-2 (66 nM) was preincubated with inhibitors at 25 °C for 20 min followed by the addition of [1-^{14}C]arachidonic acid (50 μM) at 37 °C for 30 s. All assays were conducted in duplicate.

[b] Ratio of IC_{50} (COX-1)/IC_{50} (COX-2).

[c] RAW264.7 murine macrophages were activated with LPS and IFN-γ for 7 h. Vehicle or inhibitor was added for 30 min at 37 °C. Inhibition of prostaglandin D_2 synthesis was determined by adding [1-^{14}C]arachidonic acid (20 μM) for 15 min at 25 °C.

amides, structurally diverse groups can be part of the reverse ester/amide linkage in the present series leading to COX-2 inhibitors. An unexpected outcome from this study is the potent and selective COX-2 inhibition exhibited by the reverse ester and amide derivatives, in which the 4-chlorobenzoyl group on the indole nitrogen is replaced by the 4-bromobenzyl substituent. Similar manipulations in the simple indomethacin ester/amide series afforded inactive compounds.[16,26] Thus, in addition to the array of substituents as part of the reverse ester/amide linkage, we envision broad structural flexibility on groups linked to the indole nitrogen as well. The indomethacin template appears to be most flexible in delivering COX-2-selective inhibitors following functional group manipulations. For instance, in addition to Merck–Frosst's and our own efforts, Woods et al.[27] have reported that replacement of indomethacin's carboxylic acid group with 4-substituted thiazolyl groups results in potent and selective COX-2 inhibitors. Similarities in the SAR analysis between the reverse ester/amides and the simple indomethacin ester/amide derivatives include the lack of COX inhibition by the 2-*des*-methyl analogs in the two series. The 2-methyl group on the indole ring was recently demonstrated to insert into a hydrophobic depression in the side of the active site of both COX-2 and COX-1.[30] This appears to be a major determinant of the time-dependent inhibition of COX enzymes by indomethacin. It is likely that the methyl group plays a similar role in COX-2 inhibition by indomethacin esters and amides.

Preliminary biochemical analysis of the reverse ester/amide and the simple indomethacin analogs reveals that they conform to the two-step kinetic mechanism, typical of slow, tight-binding NSAIDs including selective COX-2 inhibitors.[28,31,32] However, the time course of COX-2 inhibition is slower for indomethacin derivatives than diarylheterocycles. These results reflect a slow on-rate for the initial association of these indomethacin derivatives with COX-2.[30] Although most of the simple indomethacin amides tested in our previous study were devoid of COX-1/-2 competitive inhibitory activity, some reverse amide derivatives (compound **31**) in the present study competitively inhibited COX-1. This feature seems unique to reverse amide analogs containing the 4-chlorobenzoyl group on the indole nitrogen, since its replacement with the 4-bromobenzyl group abolished all COX-1 competitive inhibition (e.g., **32**). These differences between the two series of indomethacin analogs suggest subtle differences in their interaction at the active sites of the two enzymes. Preliminary studies on the molecular basis for selective COX-2 inhibition by the reverse esters/amides reveal that these compounds bind at the COX-2 active site in a fashion similar to that observed with the simple indomethacin esters/amides.[16,26] For example, the Y355F mutant is resistant to the inhibitory effects of the simple indomethacin esters/amides as well as the reverse esters/amides but the R120Q mutant is not (data not shown). These results validate our design strategy and suggest that hydrogen bonding from the amide nitrogens in the simple indomethacin amides and the reverse amides to the hydroxyl group of Tyr-355 is an important determinant of binding.

4. Experimental

4.1. Chemistry

Melting points were determined with a Gallenkamp melting point apparatus and are uncorrected. Chemical yields are unoptimized specific examples of one preparation. All chemicals were purchased from Aldrich (Milwaukee, WI). Methylene chloride was purchased as ''anhydrous'' from Aldrich and was used as received. All other solvents were of HPLC grade. Analytical TLC (Analtech uniplates^{TM}) was used to follow the course of reactions. Silica gel (Fisher, 60-100 mesh) was used for column chromatography. ^{1}H NMR spectra in $CDCl_3$ or DMSO-d_6 were recorded on Bruker WP-360 or AM-400 spectrometers; 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. The purity of products was judged to be at least 99% on the basis of their chromatographic homogeneity.

4.1.1. Methyl ester of 5-methoxy-2-methylindole-3-acetic acid (3). A reaction mixture containing 5-methoxy-2-methylindole-3-acetic acid (**1**, 800 mg, 3.64 mmol) and BOP-Cl (926 mg, 3.64 mmol) in 10 mL of anhydrous CH_2Cl_2 was treated with Et_3N (735 mg, 7.28 mmol) and allowed to stir at rt for 5 min. The mixture was then treated with anhydrous methanol (0.5 mL) and stirred overnight at rt. Following dilution with CH_2Cl_2 (30 mL), the organic solution was washed with water (2×25 mL), dried ($MgSO_4$), filtered, and the solvent concentrated in vacuo. The crude ester was purified by chromatography on silica gel (EtOAc:hexanes; 25:75) to afford a pale yellow oil (648 mg, 76%). ^{1}H NMR ($CDCl_3$) δ 7.75 (br s, 1H, NH), 7.13–7.16 (d, 1H, $J = 8.7$ Hz, ArH), 6.98–6.99 (d, 1H, $J = 2.2$ Hz, ArH), 6.75–6.79 (dd, 1H, $J = 8.7$, 2.3 Hz, ArH), 3.85 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.66 (s, 2H, CH_2), 2.39 (s, 3H, CH_3).

4.1.2. Methyl ester of 5-methoxyindole-3-acetic acid (4). Compound **4** was obtained upon chromatography on silica gel (EtOAc:hexanes; 15:85) as a pale yellow oil[33] (450 mg, 78%). ^{1}H NMR ($CDCl_3$) δ 7.95 (br s, 1H, NH), 7.14–7.23 (m, 1H, ArH), 7.05 (s, 1H, ArH), 6.96 (s, 1H, ArH), 6.84–6.88 (dd, 1H, $J = 8.7$, 2.3 Hz, ArH), 3.86 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3).

4.1.3. 3-(2-Hydroxyethyl)-5-methoxy-2-methylindole (5). A reaction mixture containing the methyl ester **2** (648 mg, 2.78 mmol) in anhydrous ether (20 mL) and dry MeOH (150 μL) was treated with $LiBH_4$ (122 mg, 5.6 mmol) at 0 °C. The reaction mixture was allowed to attain rt and stirred at that temperature for 5 h. The mixture was diluted with water and extracted with ether (2×30 mL). The combined organic solution was washed with water (2×25 mL), dried ($MgSO_4$), filtered, and the solvent was concentrated in vacuo. The crude alcohol was purified by recrystallization in CH_2Cl_2/hexanes to afford white needles (384 mg, 67%). mp = 102–103 °C (lit. mp:[23] 98–101 °C); ^{1}H NMR ($CDCl_3$) δ 7.72 (br s, 1H, NH), 7.15–7.18 (d, 1H, $J = 8.7$ Hz, ArH), 6.97–6.98 (d, 1H, $J = 2.3$ Hz, ArH), 6.76–6.80 (dd, 1H, $J = 8.7$, 2.3 Hz, ArH), 3.83–3.85 (m, 5H, CH_2 and OCH_3), 2.92–2.97 (t, 2H, $J = 6.4$ Hz, CH_2), 2.39 (s, 3H, CH_3).

4.1.4. 3-(2-Hydroxyethyl)-5-methoxyindole (6). Compound **6** was obtained in a similar fashion from **4** as a colorless oil[34] (306 mg, 74%). ^{1}H NMR ($CDCl_3$) δ 7.92 (br s, 1H, NH), 7.25–7.28 (m, 1H, ArH), 7.05–7.07 (dd, 2H, $J = 2.4$ Hz, ArH), 6.85–6.89 (dd, 1H, $J = 8.8$, 2.4 Hz, ArH), 3.88–3.92 (t, 2H, $J = 6.3$ Hz, CH_2), 3.86 (s, 3H, CH_3), 2.99–3.03 (t, 2H, $J = 6.3$ Hz, CH_2).

4.1.5. General procedure for the esterification of 5 and 6. To a solution of the appropriate carboxylic acid (2.18 mmol) in 5 mL of anhydrous CH_2Cl_2 were added EDCI (2.44 mmol), DMAP (0.244 mmol), and **5** or **6** (2.44 mmol) after which time the reaction mixture was stirred overnight at rt. Upon dilution with water, the aqueous solution was extracted with CH_2Cl_2 (2×20 mL). The combined organic was washed with

water (2 × 25 mL), dried ($MgSO_4$), filtered, and the solvent concentrated in vacuo. The residue was chromatographed on silica gel to afford the esters **7–14**.

4.1.6. 4-Methylbenzoic acid-2-[5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (8). Compound **8** was obtained as a pale yellow solid upon chromatography on silica gel (EtOAc:hexanes; 10:90) in 59% yield. ^{1}H NMR (DMSO-d_6) δ 10.59 (br s, 1H, NH), 7.73–7.76 (dd, 1H, J = 6.7, 1.8 Hz, ArH), 7.30–7.44 (m, 1H, ArH), 7.23–7.30 (m, 2H, ArH), 7.08–7.11 (d, 1H, J = 8.7 Hz, ArH), 6.96–6.97 (d, 1H, J = 2.0 Hz, ArH), 6.58–6.62 (dd, 1H, J = 8.7, 2.3 Hz, ArH), 4.33–4.37 (t, 2H, J = 6.8 Hz, CH_2), 3.67 (s, 3H, OCH_3), 3.01–3.05 (t, 2H, J = 6.8 Hz, CH_2), 2.44 (s, 3H, CH_3), 2.29 (s, 3H, CH_3).

4.1.7. 4-Methoxybenzoic acid-2-[5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (9). Compound **9** was obtained as a bright yellow oil upon chromatography on silica gel (EtOAc:hexanes; 20:80) in 84% yield. ^{1}H NMR ($CDCl_3$) δ 7.60–7.99 (m, 2H, ArH), 7.39 (s, 1H, NH), 7.15–7.17 (d, 2H, J = 8.7 Hz, ArH), 7.02–7.03 (d, 1H, J = 2.1 Hz, ArH), 6.88–6.91 (d, 1H, J = 9.0 Hz, ArH), 6.75–6.79 (dd, 1H, J = 8.7, 2.1 Hz, ArH), 4.42–4.47 (t, 2H, J = 7.2 Hz, CH_2), 3.83 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.09–3.14 (t, 2H, J = 7.2 Hz, CH_2), 2.40 (s, 3H, CH_3).

4.1.8. 4-Chlorobenzoic acid-2-[5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (11). Compound **11** was obtained as a white solid upon chromatography on silica gel (EtOAc:hexanes; 10:90) in 73% yield. mp = 119–120 °C; ^{1}H NMR ($CDCl_3$) δ 7.92–7.97 (dd, 2H, J = 6.7, 1.8 Hz, ArH), 7.70 (br s, 1H, NH), 7.37–7.41 (dd, 2H, J = 6.8, 1.9 Hz, ArH), 7.14–7.19 (d, 1H, J = 8.7 Hz, ArH), 7.01–7.02 (d, 1H, J = 2.0 Hz, ArH), 6.75–6.80 (dd, 1H, J = 8.7, 2.3 Hz, ArH), 4.43–4.51 (t, 2H, J = 7.2 Hz, CH_2), 3.83 (s, 3H, OCH_3), 3.09–3.16 (t, 2H, J = 7.2 Hz, CH_2), 2.39 (s, 3H, CH_3).

4.1.9. 4-Bromobenzoic acid-2-[5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (12). Compound **12** was obtained as a pale yellow solid upon chromatography on silica gel (EtOAc:hexanes; 5:95) in 72% yield. mp = 122–123 °C; ^{1}H NMR ($CDCl_3$) δ 7.92–7.97 (dd, 2H, J = 6.7, 1.8 Hz, ArH), 7.70 (br s, 1H, NH), 7.37–7.41 (dd, 2H, J = 6.8, 1.9 Hz, ArH), 7.14–7.19 (d, 1H, J = 8.7 Hz, ArH), 7.01–7.02 (d, 1H, J = 2.0 Hz, ArH), 6.75–6.80 (dd, 1H, J = 8.7, 2.3 Hz, ArH), 4.43–4.51 (t, 2H, J = 7.2 Hz, CH_2), 3.83 (s, 3H, OCH_3), 3.09–3.16 (t, 2H, J = 7.2 Hz, CH_2), 2.40 (s, 3H, CH_3).

4.1.10. 4-Iodobenzoic acid-2-[5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (13). Compound **13** was obtained as a pale yellow solid upon chromatography on silica gel (EtOAc:hexanes; 5:95) in 71% yield. mp = 131–132 °C; ^{1}H NMR ($CDCl_3$) δ 7.73–7.76 (m, 4 H, ArH), 7.14–7.19 (d, 1H, J = 8.7 Hz, ArH), 7.01–7.02 (d, 1H, J = 2.0 Hz, ArH), 6.75–6.80 (dd, 1H, J = 8.7, 2.3 Hz, ArH), 4.43–4.51 (t, 2H, J = 7.2 Hz, CH_2), 3.83 (s, 3H, OCH_3), 3.09–3.16 (t, 2H, J = 7.2 Hz, CH_2), 2.39 (s, 3H, CH_3).

4.1.11. 4-Chlorobenzoic acid-2-[5-methoxy-1*H*-indol-3-yl]ethyl ester (14). Compound **14** was obtained as a crystalline white solid upon chromatography on silica gel (EtOAc:hexanes; 10:90–20:80). mp = 98–100 °C; ^{1}H NMR ($CDCl_3$) δ 7.95–7.99 (d and br s, 3H, J = 8.5 Hz, 2 ArH and NH), 7.37–7.42 (d, 2H, J = 8.5 Hz, ArH), 7.25–7.29 (m, 1H, ArH), 7.08–7.09 (m, 2H, ArH), 6.84-6.90 (dd, 1H, J = 8.9, 2.4 Hz, ArH), 4.55–4.62 (t, 2H, J = 7.0 Hz, CH_2), 3.85 (s, 3H, CH_3), 3.16–3.24 (t, 2H, J = 7.0 Hz, CH_2).

4.1.12. General procedure for the *N*-acylation and *N*-alkylation of esters 7-14. To a solution of the appropriate ester (1.57 mmol) in 5 mL of anhydrous DMF was added sodium hydride (60% dispersion in mineral oil) (1.88 mmol) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 20 min and then treated with 4-chlorobenzoyl chloride or 4-bromobenzyl bromide (1.88 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 solution was washed with water (2 × 25 mL), dried ($MgSO_4$), filtered, and the solvent concentrated in vacuo. The residue was chromatographed on silica gel (EtOAc:hexanes; 5:95–10:90) to afford the target compounds.

4.1.13. Valeric acid-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (15). Compound **15** was obtained as a colorless oil in 34% yield. ^{1}H NMR ($CDCl_3$) δ 7.47–7.50 (d, 2H, J = 8.4 Hz, ArH), 7.29–7.32 (d, 2H, J = 8.4 Hz, ArH), 6.80–6.81 (d, 1H, J = 2.4 Hz, ArH), 6.68–6.71 (d, 1H, J = 9.0 Hz, ArH), 6.48–6.51 (d, 1H, J = 9.0 Hz, ArH), 4.07–4.11 (t, 2H, J = 7.2 Hz, CH_2), 3.66–3.71 (s, 3H, CH_3), 2.80–2.85 (t, 2H, J = 7.2 Hz, CH_2), 2.21 (s, 3H, CH_3), 2.10–2.15 (t, 2H, J = 7.2 Hz, CH_2), 1.39–1.46 (m, 4 H, CH_2), 0.70–0.76 (t, 3H, CH_3).

4.1.14. 4-Methylbenzoic acid-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (16). Compound **16** was obtained as a fluffy white solid upon recrystallization with CH_2Cl_2/hexanes in 31% yield. mp = 127–128 °C; ^{1}H NMR ($CDCl_3$) δ 7.83–7.86 (d, 1H, J = 8.7 Hz, ArH), 7.62–7.65 (d, 2H, J = 8.4 Hz, ArH), 7.37–7.45 (m, 3H, ArH), 7.19–7.26 (m, 2H, ArH), 7.00–7.01 (d, 1H, J = 2.2 Hz, ArH), 6.90–6.93 (d, 1H, J = Hz, ArH), 6.65–6.69 (dd, 1H, J = 8.9, 2.3 Hz, ArH), 4.47–4.52 (t, 2H,J = 6.9 Hz, CH_2), 3.80 (s, 3H, OCH_3), 3.11–3.15 (t, 2H, J = 6.9 Hz, CH_2), 2.56 (s, 3H, CH_3), 2.36 (s, 3H, CH_3); ESI-CID 462 (MH^+), m/z 326, 139.

4.1.15. 4-Methoxybenzoic acid-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (17). Compound **17** was obtained as a pale yellow solid upon recrystallization with CH_2Cl_2/hexanes in 28% yield. mp = 95–97 °C; ^{1}H NMR ($CDCl_3$) δ 7.95–7.97 (d, 2H, J = 8.7 Hz, ArH), 7.62–7.64 (d, 2H, J = 8.4 Hz, ArH), 7.42–7.44 (d, 2H, J = 8.3 Hz, ArH), 7.01–7.02 (d, 1H, J = 2.2 Hz, ArH), 6.89–6.93 (m, 3H, ArH), 6.66–6.69 (dd, 1H, J = 8.9, 2.3 Hz, ArH), 4.47–4.51 (t, 2H, J = 6.9 Hz, CH_2), 3.86 (s, 3H, OCH_3), 3.82 (s, 3H,

OCH_3), 3.10–3.14 (t, 2H, J = 6.9 Hz, CH_2), 2.36 (s, 3H, CH_3); ESI-CID 478 (MH^+), *m/z* 326, 308, 188, 139.

4.1.16. 2-Methoxybenzoic acid-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (18). Compound **18** was obtained as a white solid upon recrystallization with CH_2Cl_2/hexanes in 28% yield. mp = 88–90 °C; ^1H NMR ($CDCl_3$) δ 7.95–7.97 (d, 2H, J = 8.7 Hz, ArH), 7.62–7.66 (m, 2H, ArH), 7.41–7.45 (m, 3H, ArH), 6.89–7.01 (m, 5H, ArH), 6.66–6.69 (dd, 1H, J = 8.9, 2.3 Hz, ArH), 4.47–4.51 (t, 2H, J = 6.9 Hz, CH_2), 3.87 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.10–3.14 (t, 2H, J = 6.9 Hz, CH_2), 2.36 (s, 3H, CH_3); ESI-CID 478 (MH^+), *m/z* 326, 139.

4.1.17. 4-Chlorobenzoic acid-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (19). Compound **19** was obtained as a pale yellow solid upon recrystallization with CH_2Cl_2/hexanes in 35% yield. mp = 102–104 °C; ^1H NMR ($CDCl_3$) δ 7.91–7.96 (d, 2H, J = 8.5 Hz, ArH), 7.62–7.66 (d, 2H, J = 8.5 Hz, ArH), 7.38-7.46 (m, 4H, ArH), 7.00–7.01 (d, 1H, J = 2.3 Hz, ArH), 6.86–6.91 (d, 1H, J = 9.0 Hz, ArH), 6.66–6.70 (dd, 1H, J = 9.0, 2.4 Hz, ArH), 4.47–4.54 (t, 2H, J = 7.0 Hz, CH_2), 3.82 (s, 3H, OCH_3), 3.10–3.17 (t, 2H, J = 7.0 Hz, CH_2), 2.38 (s, 3H, CH_3); ESI-CID 482 (MH^+), 326, 188, 139.

4.1.18. 4-Bromobenzoic acid-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (20). Compound **20** was obtained as a pale yellow solid upon recrystallization with CH_2Cl_2/hexanes in 41% yield. mp = 97–99 °C; ^1H NMR ($CDCl_3$) δ 7.84–7.88 (m, 3H, ArH), 7.54–7.66 (m, 3H, ArH), 7.42–7.46 (m, 2H, ArH), 6.99–7.00 (d, 1H, J = 2.4 Hz, ArH), 6.86–6.91 (d, 1H, J = 9.0 Hz, ArH), 6.69–6.70 (dd, 1H, J = 9.0, 2.4 Hz, ArH), 4.47–4.54 (t, 2H, J = 7.0 Hz, CH_2), 3.82 (s, 3H, OCH_3), 3.09–3.16 (t, 2H, J = 7.0 Hz, CH_2), 2.37 (s, 3H, CH_3); ESI-CID 527 (MH^+), 326, 188, 139.

4.1.19. 4-Iodobenzoic acid-2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (21). Compound **21** was obtained as a pale yellow solid upon recrystallization with CH_2Cl_2/hexanes in 40% yield. mp = 128–129 °C; ^1H NMR ($CDCl_3$) δ 7.84–7.88 (m, 3H, ArH), 7.54–7.66 (m, 3H, ArH), 7.42–7.46 (m, 2H, ArH), 6.99–7.00 (d, 1H, J = 2.4 Hz, ArH), 6.86–6.91 (d, 1H, J = 9.0 Hz, ArH), 6.69–6.70 (dd, 1H, J = 9.0, 2.4 Hz, ArH), 4.47–4.54 (t, 2H, J = 7.0 Hz, CH_2), 3.82 (s, 3H, OCH_3), 3.09–3.16 (t, 2H, J = 7.0 Hz, CH_2), 2.37 (s, 3H, CH_3); ESI-CID 574 (MH^+), 326, 188, 139.

4.1.20. 4-Chlorobenzoic acid-2-[1-(4-chlorobenzoyl)-5-methoxy-1*H*-indol-3-yl]ethyl ester (22). Compound **22** was obtained as a white solid upon recrystallization with CH_2Cl_2/hexanes in 67% yield. ^1H NMR ($CDCl_3$) δ 7.91–7.96 (d, 2H, J = 8.5 Hz, ArH), 7.62–7.66 (d, 2H, J = 8.5 Hz, ArH), 7.38–7.46 (m, 4 H, ArH), 7.00–7.01 (d, 1H, J = 2.3 Hz, ArH), 6.86–6.91 (d, 1H, J = 9.0 Hz, ArH), 6.66–6.70 (dd, 1H, J = 9.0, 2.4 Hz, ArH), 4.55–4.59 (t, 2H, J = 6.9 Hz, CH_2), 3.87 (s, 3H, OCH_3), 3.09–3.13 (t, 2H, J = 6.6 Hz, CH_2).

4.1.21. 4-Chlorobenzoic acid-2-[1-(4-bromobenzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl ester (23). Compound **23** was obtained as a white solid upon recrystallization with CH_2Cl_2/hexanes in 14% yield. mp = 96–98 °C; ^1H NMR ($CDCl_3$) δ 7.89–7.93 (d, 2H, J = 9.0 Hz, ArH), 7.62–7.66 (d, 2H, J = 9.0 Hz, ArH), 7.33–7.38 (m, 4H, ArH), 7.04-7.07 (d, 1H, J = 9.0 Hz, ArH), 6.86–6.91 (d, 1H, J = 2.1 Hz, ArH), 6.76–6.79 (dd, 1H, J = 9.0, 2.1 Hz, ArH), 5.20 (s, 2H, CH_2), 4.46–4.51 (t, 2H, J = 7.0 Hz, CH_2), 3.82 (s, 3H, OCH_3), 3.14–3.19 (t, 2H, J = 7.0 Hz, CH_2), 2.24 (s, 3H, CH_3); ESI-CID 512 (MH^+), *m/z* 358, 187, 171.

4.1.22. 5-Methoxy-2-methylindole-3-acetamide (24). A reaction mixture containing **1** (880 mg, 4.02 mmol), EDCI (1.16 g, 6.04 mmol), HOBt (816 mg, 6.04 mmol), DIPEA (2.8 mL, 16.08 mmol), and NH_4Cl (430 mg, 8.04 mmol) in anhydrous (DMF) 16 mL (4 mL DMF/1 mmol **1**) was stirred at rt for 5 h. The reaction was diluted with water and extracted with EtOAc (3 × 10 mL). The combined EtOAc extracts were washed with satd $NaHCO_3$ (2 × 10 mL), water, dried ($MgSO_4$), filtered, and the solvent concentrated in vacuo until a minimum volume of EtOAc remained. Upon cooling, the desired amide crystallized out as a white crystalline solid. mp = 146–148 °C (lit. mp[35] =1 47–150 °C). ^1H NMR (DMSO-d_6) δ 10.56 (s, 1H, NH), 7.34 (br s, 1H, CONH), 6.96–7.22 (m, 1H, ArH), 6.81 (s, 1H, ArH), 6.74 (br s, 1H, CONH), 6.58–6.62 (m, 1H, ArH), 3.78 (s, 3H, CH_3), 3.34 (s, 2H, CH_2), 2.29 (s, 3H, CH_3); ESI-CID 219 (MH^+), *m/z* 187, 174, 148.

4.1.23. 3-(2-Aminoethyl)-5-methoxy-2-methylindole (25). To a suspension of LAH (370 mg, 9.74 mmol) in anhydrous tetrahydrofuran (THF) (80 mL) was added **24** (760 mg, 3.38 mmol) under argon at 0 °C. The reaction mixture was allowed to stir at rt under argon for 60 h. The reaction was carefully quenched by the addition of ice-cold water (~100 mL) and then extracted with Et_2O (3 × 25 mL). The combined ether extracts were washed with 1 N HCl (2 × 25 mL). The combined acidic extract was washed once with Et_2O (50 mL) and then neutralized with 1 N NaOH. Following neutralization, the aqueous solution was extracted with Et_2O (3 × 25 mL). The combined organic solution was washed with water, dried ($MgSO_4$), filtered, and the solvent concentrated in vacuo to afford a yellow oil (530 mg, 73%). A portion of the oil was treated with a solution containing one equivalent of oxalic acid in Et_2O to furnish the oxalate salt of **25**, which was recrystallized from MeOH/Et_2O to afford a light brown crystalline solid. mp = 176–178 °C; ^1H NMR (CD_3OD) δ 7.12–7.16 (d, 1H, J = 8.7 Hz, ArH), 6.94–6.95 (d, 1H, J = 2.3 Hz, ArH), 6.67–6.71 (dd, 1H, J = 8.7, 2.3 Hz, ArH), 3.80–3.84 (s, 3H, OCH_3), 3.01–3.11 (dd, 4 H, J = 8.3 Hz, CH_2), 2.36 (s, 3H, CH_3); ESI-CID 205 (MH^+), *m/z* 188, 173, 158, 145, 130.

4.1.24. 4-Chloro-*N*-{2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl}-benzamide (31). A reaction mixture containing **25** (520 mg, 2.55 mmol), EDCI (489 mg, 2.55 mmol), DMAP (31 mg, 0.255 mmol), and 4-chlorobenzoic acid (354 mg, 2.26 mmol) in

anhydrous CH_2Cl_2 (15 mL) was stirred at rt for 3 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (2 × 15 mL). The combined CH_2Cl_2 extracts were washed with water, dried ($MgSO_4$), filtered, and the solvent concentrated in vacuo. The crude amide was chromatographed on silica gel (EtOAc:hexanes; 25:75 then 60:40) to afford **26** as a yellow oil (390 mg, 45%), which was used in the next step without further characterization owing to its unstable nature. To a reaction mixture comprising **26** (390 mg, 1.14 mmol) in anhydrous DMF (3 mL) was added sodium hydride (60% dispersion in mineral oil) (56 mg, 1.4 mmol) at 0 °C under argon. After stirring for 20 min, the reaction was treated with 4-chlorobenzoyl chloride (180 μL, 1.4 mmol) and the reaction was allowed to stir overnight at rt. The reaction mixture was quenched with water and extracted with Et_2O (3 × 10 mL). The combined Et_2O extracts were washed with satd $NaHCO_3$ (3 × 10 mL), water, dried ($MgSO_4$), filtered, and the solvent concentrated in vacuo to afford a yellow residue which was chromatographed (EtOAc:hexanes; 20:80 then 40:60) to generate **31** as a pale yellow solid (recrystallized from EtOAc/hexanes) (371 mg, 67%). mp = 165–166 °C; ^{1}H NMR ($CDCl_3$) δ 7.58–7.65 (m, 4 H, ArH), 7.43–7.47 (d, 2H, J = 8.6 Hz, ArH), 7.36–7.39 (d, 2H, J = 8.6 Hz, ArH), 6.96–6.97 (d, 1H, J = 2.4 Hz, ArH), 6.88–6.90 (d, 1H, J = 9.0 Hz, ArH), 6.65–6.69 (dd, 1H, J = 9.0, 2.5 Hz, ArH), 6.17 (br t, 1H, CONH), 3.76 (s, 3H, OCH_3), 3.67–3.71 (q, 2H, J = 6.7 Hz, CH_2), 2.99–3.04 (t, 2H, J = 6.7 Hz, CH_2), 2.33 (s, 3H, CH_3); ESI-CID 482 (MH^+), 312, 173, 139.

4.1.25. 4-Chloro-*N*-{2-[1-(4-bromobenzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl}-benzamide (32). It was obtained from **26** in a similar manner as **31**, using 4-bromobenzyl bromide as the alkylating agent. Upon workup, silica gel chromatography (EtOAc:hexanes; 20:80 then 25:75) afforded **32** as a pale yellow solid (recrystallized from CH_2Cl_2/hexanes) (184 mg, 57%). mp = 172–173 °C; ^{1}H NMR ($CDCl_3$) δ 7.50–7.53 (d, 2H, J = 8.3 Hz, ArH), 7.28–7.43 (m, 4 H, ArH), 7.13-7.10 (d, 1H, J = 8.9 Hz, ArH), 7.01–7.02 (d, 1H, J = 2.3 Hz, ArH), 6.77–6.81 (m, 3H, ArH), 6.09 (br t, 1H, CONH), 3.78 (s, 3H, OCH_3), 3.66–3.73 (q, 2H, J = 6.3 Hz, CH_2), 3.02–3.07 (t, 2H, J = 6.4 Hz, CH_2), 2.24 (s, 3H, CH_3); ESI-CID 512 (MH^+), 341, 173, 170.

4.1.26. *N*-{2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl}-2-phenethylamide (33). A reaction mixture containing **25** (277 mg, 1.36 mmol), EDCI (261 mg, 1.36 mmol), DMAP (17 mg, 0.136 mmol), and hydrocinnamic acid (179 mg, 1.19 mmol) in anhydrous CH_2Cl_2 (15 mL) was stirred at rt for 3 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (2 × 15 mL). The combined CH_2Cl_2 extracts were washed with water, dried ($MgSO_4$), filtered, and the solvent concentrated in vacuo. The crude amide was chromatographed on silica gel (EtOAc:hexanes; 20:80 then 50:50) to afford **27** as a reddish brown oil (256 mg, 54%), which was used in the next step without further characterization. Acylation of **27** with 4-chlorobenzoyl chloride was conducted in a manner similar to that described in the preparation of **31**. Silica gel chromatography (EtOAc:hexanes; 20:80 then 40:60) yielded a yellow oil which upon crystallization from CH_2Cl_2/hexanes generated **33** as an off-white solid (recrystallized from EtOAc/hexanes) (60 mg, 17%). mp = 109–111 °C; ^{1}H NMR ($CDCl_3$) δ 7.62–7.66 (d, 2H, J = 8.3 Hz, ArH), 7.44–7.48 (d, 2H, J = 8.5 Hz, ArH), 7.15-7.23 (m, 5H, ArH), 6.94–6.95 (d, 1H, J = 2.4 Hz, ArH), 6.84-6.88 (d, 1H, J = 9.0 Hz, ArH), 6.65–6.69 (dd, 1H, J = 9.0, 2.5 Hz, ArH), 5.54 (br t, 1H, CONH), 3.84 (s, 3H, OCH_3), 3.45–3.48 (q, 2H, J = 6.4 Hz, CH_2), 2.90–2.98 (t, 2H, J = 7.4 Hz, CH_2), 2.80–2.87 (t, 2H, J = 7 Hz, CH_2), 2.38–2.46 (t, 2H, J = 8.1 Hz, CH_2), 2.30 (s, 3H, CH_3); ESI-CID 475 (MH^+), 341, 173, 170.

4.1.27. *N*-{2-[1-(4-Chlorobenzoyl)-5-methoxy-1*H*-indol-3-yl]ethyl}-acetamide (34). Compound **34** was obtained by the acylation of melatonin (**28**) with 4-chlorobenzoyl chloride as described in the preparation of **31**. Following work up, recrystallization from CH_2Cl_2/hexanes afforded **34** as a white solid in 54% yield. mp = 172–173 °C; ^{1}H NMR ($CDCl_3$) δ 8.25–8.28 (d, 1H, J = 8.4 Hz, ArH), 7.64–7.67 (dd, 2H, J = 6.6, 1.8 Hz, ArH), 7.50–7.52 (dd, 2H, J = 8.4, 1.8 Hz, ArH), 6.98–7.05 (m, 3H, ArH), 5.55 (br t, 1H, NH), 3.89 (s, 3H, OCH_3), 3.51–3.58 (q, 2H, J = 6.7 Hz, CH_2), 2.85–2.89 (t, 2H, J = 7.0 Hz, CH_2), 1.94 (s, 3H, CH_3).

4.1.28. *N*-{2-[1-(4-Bromobenzyl)-5-methoxy-1*H*-indol-3-yl]ethyl}-acetamide (35). Compound **35** was obtained as a white solid upon recrystallization from EtOAc/hexanes in 66% yield. mp = 152–153 °C. ^{1}H NMR ($CDCl_3$) δ 7.39–7.42 (dd, 2H, J = 6.7, 1.76 Hz, ArH), 7.09–7.11 (d, 1H, J = 8.8 Hz, ArH), 7.04–7.05 (d, 1H, J = 2.3 Hz, ArH), 6.92–6.97 (m, 3H, ArH), 6.82–6.86 (dd, 1H, J = 8.9, 2.4 Hz, ArH), 5.55 (br t, 1H, NH), 5.19 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.54–3.59 (q, 2H, J = 6.7 Hz, CH_2), 2.91–2.96 (t, 2H, J = 6.7 Hz, CH_2), 1.92 (s, 3H, CH_3).

4.1.29. *N*-{2-[1-(4-Bromobenzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl}-acetamide (36). A reaction mixture containing **25** (520 mg, 2.55 mmol) in 5 mL of anhydrous CH_2Cl_2 was treated with freshly distilled Et_3N (420 μL, 3 mmol) and acetyl chloride (213 μL, 3 mmol) at 0 °C and the reaction was allowed to proceed at rt for 5 h. The reaction mixture was quenched with water and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with satd $NaHCO_3$ (3 × 10 mL), water, dried ($MgSO_4$), filtered, and the solvent concentrated in vacuo. The crude residue was chromatographed on silica gel (EtOAc:hexane; 40:60) to afford **29** as a brown oil (250 mg, 40%), which was alkylated without further purification. N-alkylation of **29** with 4-bromobenzyl bromide afforded **36** as a white solid recrystallized from CH_2Cl_2/hexanes in 30% yield. mp = 168–170 °C; ^{1}H NMR ($CDCl_3$) δ 7.35–7.43 (dd, 2H, J = 6.7, 1.76 Hz, ArH), 7.09–7.11 (d, 1H, J = 8.8 Hz, ArH), 7.01–7.04 (d, 1H, J = 2.3 Hz, ArH), 6.76–6.81 (m, 3H, ArH), 5.47 (br t, 1H, NH), 5.21 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.45–3.52 (q, 2H, J = 6.7 Hz, CH_2), 2.90–2.95 (t, 2H, J = 6.7 Hz, CH_2), 2.27 (s, 3H, CH_3), 1.90 (s, 3H, CH_3); ESI-CID 416 (MH^+), 173, 170.

4.1.30. *N*-{2-[1-(4-Bromobenzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]ethyl}-propionamide (37). It was obtained in a similar fashion as **32**. Upon workup, the residue was recrystallized from CH_2Cl_2/hexanes to afford **37** as an off-white solid (160 mg, 33%). mp = 168–169 °C; ^{1}H NMR ($CDCl_3$) δ 7.32–7.39 (dd, 2H, J = 8.7, 2.3 Hz, ArH), 7.00–7.07 (m, 2H, ArH), 6.76-6.81 (m, 3H, ArH), 5.45 (br t, 1H, NH), 5.20 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.47–3.53 (q, 2H, J = 6.9 Hz, CH_2), 2.90–2.95 (t, 2H, J = 6.9 Hz, CH_2), 2.26 (s, 3H, CH_3), 2.08–2.15 (q, 2H, J = 7.6 Hz, CH_2), 1.04–1.08 (t, 3H, J = 7.6 Hz, CH_3); ESI-CID 430 (MH^+), 343, 173, 170.

4.1.31. *N*-{2-[5-Methoxy-2-methyl-1*H*-indol-3-yl]ethyl}-carbamic acid *tert*-butyl ester (38). Compound **25** (50 mg, 0.25 mmol) was stirred in dry methanol (2 mL) while di-*tert*-butyldicarbonate (64 mg, 0.29 mmol) in dry methanol (50 μL) was added dropwise to the stirred solution at 23 °C. The reaction mixture was stirred at rt for 18 h. The reaction was concentrated and the residue was dissolved in ethyl acetate (5 mL), washed with saturated sodium bicarbonate (2 mL), dried over sodium sulfate, and concentrated. Purification by flash chromatography yielded 75 mg (99%) product. ^{1}H NMR ($CDCl_3$) δ 9.95 (s, 1H, NH), 7.25 (d, J = 8.3 Hz, 1H, ArH), 6.88 (s, 1H, ArH), 6.70 (d, J = 9.0 Hz, 1H, ArH), 4.45 (s, 1H, NH), 3.78 (s, 3H, OCH_3), 3.60 (d, J = 2.2 Hz, 2H, CH_2), 2.80 (d, J = 2.4 Hz, 2H, CH_2), 2.28 (s, 3H), 1.36 (s, 9H, *t*-Bu); ESI-CID 305 ($M-H^+$).

4.1.32. *N*-{2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethyl}-carbamic acid *tert*-butyl ester (39). NaH (7 mg, 0.29 mmol) was stirred in DMF (2 mL) at 0 °C. Compound **38** (74 mg, 0.25 mmol) in DMF (1 mL) was added dropwise to the stirred solution. The reaction mixture was stirred for 20 min at 0 °C at which time *p*-bromobenzyl bromide (72 mg, 0.29 mmol) was added. The reaction mixture stirred overnight at room temperature. The reaction mixture was diluted slowly with water, extracted with ethyl acetate (2 × 10 mL) and washed with water (2 × 5 mL), dried over sodium sulfate, concentrated, and purified on silica 10:90 ethyl acetate/hexane to give 52 mg of a yellow solid, 40%. ^{1}H NMR ($CDCl_3$) δ 7.39 (d, J = 8 Hz, 2H, ArH), 7.10 (s, 1H, ArH), 7.06 (d, J = 8.6 Hz, 1H, ArH), 6.8 (d, J = 8.2 Hz, 2H, ArH), 6.77 (d, J = 8.7 Hz, 1H, ArH), 5.2 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.4 (d, J = 2.2 Hz, 2H, CH_2), 2.85 (d, J = 2.6 Hz, 2H, CH_2), 2.28 (s, 3H, CH_3), 1.40 (s, 9H, *t*-Bu); ESI-CID 474 ($M-H^+$).

4.1.33. 2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethylamine·HCl (40). $HCl_{(g)}$ was slowly bubbled through a 1 mL solution of compound **39** in dichloromethane. After 1 h, the product precipitated. The solvent was removed in vacuo to give 13 mg (84%) of brown solid, which was used as is without further purification. ^{1}H NMR (300 MHz, DMSO) 7.3 (s, 1H, ArH), 7.20 (d, J = 8.2 Hz, 1H, ArH), 7.05 (d, J = 2.4 Hz, 2H, ArH), 6.85 (d, J = 8.9 Hz, 2H, ArH), 6.65 (d, J = 8.9 Hz, 1H, ArH), 5.2 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 3.2 (d, J = 2.4 Hz, 2H, CH_2), 2.9 (d, J = 2.3 Hz, 2H, CH_2), 2.20 (s, 3H, CH_3); ESI-CID 373 ($M-H^+$).

4.1.34. *N*-{2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethyl}-4- fluoro-2-nitrobenzamide (41). 2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethylamine (50 mg, 0.21 mmol), EDCI (53 mg, 0.31 mmol), DIPEA (54 ul, 0.31 mmol), 4-fluoro-2-nitrobenzoic acid (58 mg, 0.31 mmol), and HOBt (42 mg, 0.31 mmol) were dissolved in DMF (dry, 5 ml) and allowed to stir for 18 h at room temperature. The reaction was quenched with saturated sodium bicarbonate (10 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic solution was washed with water (20 mL), dried with sodium sulfate, concentrated, and purified on silica (ethyl acetate 50% in hexane) to give a yellow solid (52 mg, 72%) ^{1}H NMR ($CDCl_3$) 8.08 (s, 1H, ArH), 7.63–7.58 (dd, J_1 = J_2 = 6.24, ArH), 7.37 (d, J = 8.4 Hz, ArH), 7.30–7.21 (m, 4H, ArH), 7.08 (s, 1H, ArH), 6.80-6.77 (dd, J_1 = J_2 = 6.3 Hz, 2H, ArH), 5.20 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.69–3.67 (m, 2H, CH_2), 3.08–3.02 (m, 2H, CH_2), 2.30 (s, 3H, CH_3); ESI-CID 541 ($M-H^+$).

4.1.35. *N*-{2-[1-(4-bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethyl}-4-chloro-2-nitrobenzamide (42). 2-[1-(4-bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethylamine (50 mg, 0.21 mmol), EDCI (53 mg, 0.31 mmol), DIPEA (54 μL, 0.31 mmol), 4-chloro-2-nitrobenzoic acid (63 mg, 0.31 mmol), and HOBt (42 mg, 0.31 mmol) were dissolved in DMF (dry, 5 mL) and allowed to stir for 18 h at room temperature. The reaction was quenched with saturated sodium bicarbonate (10 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic solution was washed with water (20 mL), dried with sodium sulfate, concentrated, and purified on silica (ethyl acetate 50% in hexane) to give a yellow solid (56 mg, 74%) ^{1}H NMR ($CDCl_3$) 8.0 (s, 1H, ArH), 7.62–7.57 (dd, J_1 = J_2 = 6.24, ArH), 7.38 (d, J = 8.4 Hz, ArH), 7.29–7.22 (m, 4H, ArH), 7.06 (s, 1H, ArH), 6.80–6.77 (dd, J_1 = J_2 = 6.3 Hz, 2H, ArH), 5.20 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.75–3.73 (m, 2H, CH_2), 3.16–3.10 (m, 2H, CH_2),2.30 (s, 3H, CH_3); ESI-CID 557 ($M-H^+$).

4.1.36. *N*-{2-[1-(4-bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethyl}-4-fluorobenzamide (43). 2-[1-(4-Bromobenzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethylamine (42 mg, 0.11 mmol), EDCI (25 mg, 0.13 mmol), DIPEA (23 μL, 0.13 mmol), *p*-fluorobenzoic acid (18 mg, 0.13 mmol), and HOBt (18 mg, 0.13 mmol) were dissolved in DMF (dry, 5 mL) and allowed to stir for 18 h at room temperature. The reaction was quenched with saturated sodium bicarbonate (10 mL) and extracted with ethyl acetate (2 × 20 mL). The combined organic solution was washed with water (20 mL), dried with sodium sulfate, concentrated, and purified on silica (ethyl acetate 50% in hexane) to give a yellow solid (30 mg, 56%) ^{1}H NMR ($CDCl_3$) 8.11–8.08 (m, 1H, ArH), 7.61–7.56 (m, 2H, ArH), 7.34 (d, J = 8.4 Hz, ArH), 7.16–6.98 (m, 5H, ArH), 6.77 (d, J = 8.5 Hz, 2H, ArH), 5.19 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.69-3.67 (m, 2H, CH_2), 3.06–3.01 (m, 2H, CH_2), 2.23 (s, 3H, CH_3); ESI-CID 496 ($M-H^+$).

4.1.37. *N*-2-[1-(4-Bromobenzyl)-3-butyl-5-methoxy-2-methyl]-1*H*-indole (47). A suspension containing 4-meth-

oxyphenylhydrazine·HCl salt (**44**, 5 g, 28.63 mmol) and 2-heptanone (**45**, 3.4 g, 30 mmol) in 50 mL of glacial acetic acid was stirred at rt for 30 min, heated at 60 °C for 30 min and then at 120 °C for 20 h. The dark solution was cooled and diluted with 20 mL heptane and concentrated in vacuo. The residue was partioned between EtOAc and water. The organic layer was washed with brine, water, dried ($MgSO_4$), filtered, and the solvent concentrated in vacuo. Silica gel chromatography (EtOAc:hexanes; 10:90) gave the desired indole as a pale yellow oil **46**, which eventually solidified upon cooling (2.1 g, 33%). The crude indole **46** was alkylated with 4-bromobenzyl bromide as described in the preparation of **32**. Following chromatography on silica gel (EtOAc:hexanes; 5:95), **47** was obtained as a gray solid in 26% yield. mp = 89–90 °C; ^{1}H NMR ($CDCl_3$) δ 7.34–7.38 (m, 2H, ArH), 7.00–7.03 (m, 2H, ArH), 6.72–6.82 (m, 3H, ArH), 5.19 (s, 2H, CH_2), 3.83 (s, 3H, CH_3), 2.67-2.72 (t, 2H, J = 7.5 Hz, CH_2), 2.24 (s, 3H, CH_3), 1.54–1.64 (m, 2H, CH_2), 1.33-1.43 (m, 2H, CH_2), 0.91–0.95 (t, 3H, J = 7.2 Hz, CH_3).

4.2. 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 μM O_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 from the pVL1393 expression vector (Pharmingen), and purified by ion-exchange and gel filtration chromatography.[38] All of the purified proteins were shown by densitometric scanning of a 7.5% SDS–PAGE gel to be >80% pure.

4.2.1. Time- and concentration-dependent inhibition of ovine COX-1 and human COX-2 using the thin layer chromatography assay. Cyclooxygenase activity of ovine COX-1 (44 nM) or human COX-2 (88 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 (88 nM) was preincubated at rt for 20 min with varying inhibitor concentrations in DMSO followed by the addition of [1-^{14}C]arachidonic acid (50 μM) for either 30 s or 10 min 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, DC). 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.

4.2.2. Inhibition of COX-2 activity in activated RAW264.7 cells. Low passage number murine RAW264.7 cells were grown in DMEM containing 10% heat-inactivated FBS. Cells (6.2×10^6 cells/T25 flask) were activated with 500 ng/mL LPS and 10 U/mL IFN-γ in serum-free DMEM for 7 h. Vehicle (DMSO) or inhibitor in DMSO (0–1 μM) was added for 30 min at 37 °C. Inhibition of exogenous arachidonic acid metabolism or inhibition of PGD_2 synthesis was determined by incubating the cells with 20 μM [1-^{14}C]arachidonic acid, respectively, for 15 min at 25 °C. Aliquots (200 μL) were removed into termination solution and total products were quantitated by the TLC assay as described earlier.[29]

Acknowledgment

This study was supported by a grant from the National Institutes of Health (CA89450).

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