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FROM INDOMETHACIN TO A SELECTIVE COX-2 INHIBITOR: DEVELOPMENT OF INDOLALKANOIC ACIDS AS POTENT AND SELECTIVE CYCLOOXYGENASE-2 INHIBITORS

W. C. Black,* C. Bayly, M. Belley, C.-C. Chan, S. Charleson, D. Denis, J. Y. Gauthier, R. Gordon, D. Guay, S. Kargman, C. K. Lau, Y. Leblanc, J. Mancini, M. Ouellet, D. Percival, P. Roy, K. Skorey, P. Tagari, P. Vickers, E. Wong, L. Xu, and P. Prasit

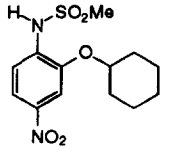
Merck Frosst Centre for Therapeutic Research
P.O. Box 1005, Pointe Claire-Dorval, Québec, Canada H9R 4P8

Abstract: A series of potent and highly selective cyclooxygenase-2 inhibitors have been prepared by replacing the benzoyl group of indomethacin with a 4-bromobenzyl group, and by extending the acetic acid side chain. These compounds show anti-inflammatory activity in rats with no evidence of GI toxicity, even at high doses.

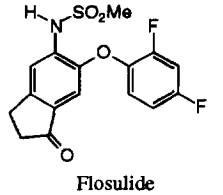
Introduction.

Cyclooxygenase (COX) catalyzes the conversion of arachidonic acid to prostaglandins, and is known to be the principle target of nonsteroidal anti-inflammatory drugs (NSAIDs). Recently, it has been found that there are at least two isoforms of COX, each with a distinct physiological role.[1] One isoform, COX-1, is constitutively produced in a variety of tissues, and appears to be important in the maintenance of normal physiological functions including gastric cytoprotection. The second isoform, COX-2, is induced by a wide variety of inflammatory mediators, and appears to be largely responsible for the high-level production of prostaglandins that results in inflammation.[2] It is likely that a selective COX-2 inhibitor could have useful anti-inflammatory activity without the ulcerogenic side effects associated with currently available NSAIDS, all of which inhibit both COX-1 and COX-2.[3]

DuP 697



NS-398



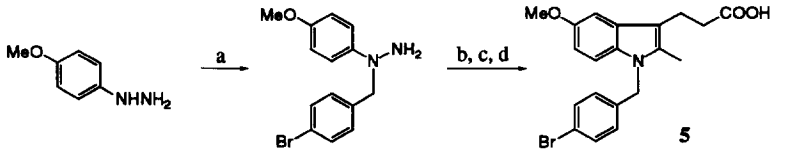
Flosulide

The recent identification of Dup 697,[4] NS-398[5] and Flosulide[6] as anti-inflammatory agents with a high degree of selectivity for COX-2 has led to efforts by several laboratories to design potent and selective inhibitors based on these structures.[7] As a complement to this work, we felt that it should be possible to modify a conventional, non-selective NSAID to obtain COX-2 selectivity, and thus take advantage of a structural class with a well-established safety profile. In this paper, we describe the realization of this goal and present a series of COX-2 selective inhibitors based on the non-selective NSAID indomethacin (**1**).

Chemistry.

The synthesis of indolealkanoic acids has been well studied.[8] The majority of the compounds presented were prepared using standard methods. If the appropriate methyl ketone was readily available, a Fischer indole synthesis strategy was used as illustrated below for compound **5**.

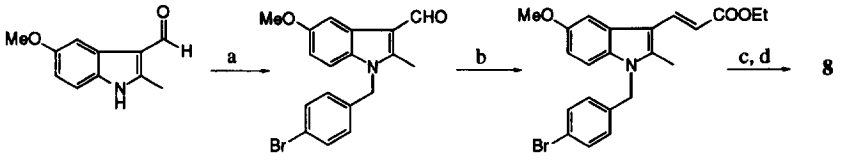
Scheme I



Conditions: (a) 4-Bromobenzyl bromide, K_2CO_3, DMF; (b) Ethyl 4-acetylbutyrate, HOAc; (c) HCl, dioxane, 50 °C; (d) NaOH.

Alternatively, the side chain could be functionalized after the construction of the indole nucleus, as in the synthesis of compound **8**. An enantioselective preparation of compound **11** is provided in the accompanying paper.[9]

Scheme II



Conditions: (a) 4-Bromobenzyl bromide, NaH, DMF; (b) (Carbethoxymethylene)triphenylphosphorane, $PhCH_3$; (c) Me_2CuLi, TMSCl (d) LiOH.

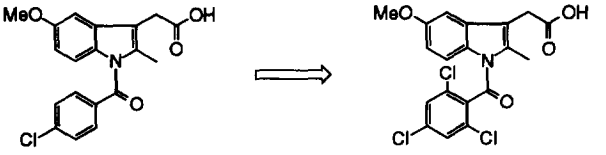
Results and Discussion.

Indomethacin is a non-selective cyclooxygenase inhibitor. It has IC_{50}s of 10 nM[10] for COX-2 and 6 nM for COX-1 in our whole cell assays[11] and causes gastrointestinal (GI) toxicity at relatively low doses. This was quantified in rats using a ^{51}Cr excretion assay:[11] a 7-fold increase in fecal ^{51}Cr was observed after a single 10 mg/kg dose, indicating that changes in permeability and possibly lesions in the gastrointestinal tract occurred. Presumably, it is the reduction of cytoprotective prostaglandins in the GI tract via COX-1 inhibition which is responsible for this toxicity.

Initial modification of indomethacin was based on the observation that, after acetylation with aspirin, COX-2 produces a significant amount of 15-HETE upon addition of arachidonic acid whereas COX-1 is essentially inactivated.[12] One interpretation of this data is that acetylation of Ser 529 of COX-1[13] prevents arachidonic acid from reaching the active site while acetylation of Ser 516 of COX-2 (the putative COX-2 aspirin acetylation site[1a]) does not block access to the active site. This suggests that the COX-2 enzyme may have a larger active site than COX-1. Based on this hypothesis, it may be possible to increase the size of the indomethacin nucleus to produce a compound that would still fit into the COX-2 active site but not into the COX-1 active site, thus generating the desired selectivity. The result of this hypothesis was the replacement of the 4-chlorobenzoyl group in indomethacin with a 2,4,6-trichlorobenzoyl group, a modification which forces the phenyl ring to adopt a more sterically demanding conformation perpendicular to the plane of the indole ring (see

Figure). This compound (**2**, L-748,780) showed reasonable COX-2 activity in the whole cell assay with almost complete elimination of the COX-1 activity. In addition, **2** was nearly as potent as indomethacin in the rat paw edema assay, with an ED_{30} of 1.3 mg/kg (Table I).

Table I



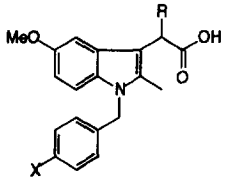
Indomethacin (**1**) **2**

	COX-2 Whole Cell IC_{50} (nM)	COX-1 Whole Cell IC_{50} (nM)	Rat Paw Edema ED_{30} (mg/kg)
Indomethacin	10	6	0.9
2 (L-748,780)	500	>100,000	1.3

A number of analogues were prepared in an attempt to optimize the system. However, increasing the size of the *ortho*-substituents of the benzoyl group resulted in loss of COX-2 potency, while decreasing their bulk resulted in non-selective compounds. Modification of the 4-chloro substituent or the indole nucleus also resulted in loss of COX-2 potency.

L-748,780 clearly indicated that it was possible for a COX-2 selective inhibitor to be derived from indomethacin. To pursue this avenue, a number of indole acetic acid analogs from Merck's sample collection were examined, resulting in the identification of *N*-benzyl indole **3** as a highly selective COX-2 inhibitor. However, despite its good in vitro potency, the compound was only moderately active in vivo with an ED_{30} of 3.0 mg/kg in the rat paw edema assay.

Table II

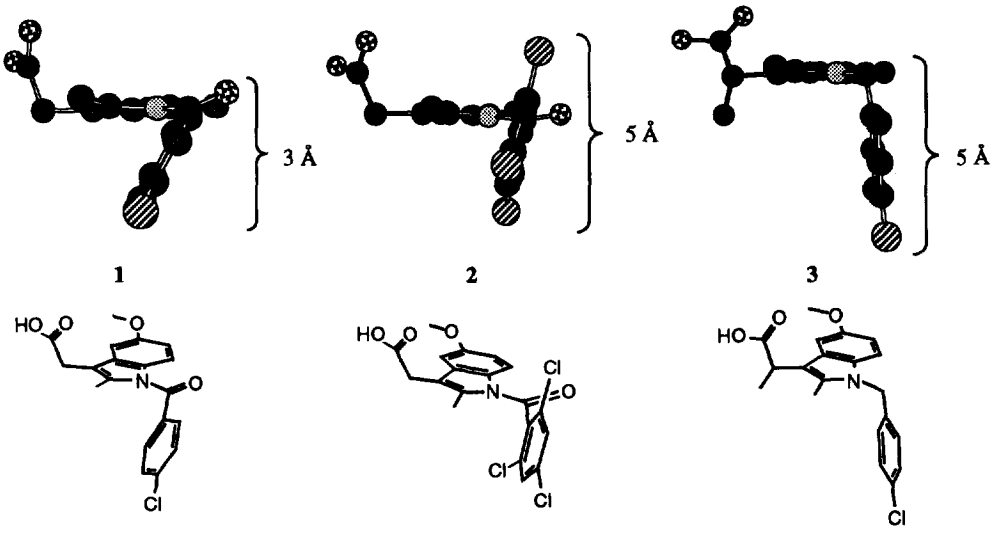


	COX-2 Whole Cell IC_{50} (nM)	COX-1 Whole Cell IC_{50} (nM)	Rat Paw Edema ED_{30} (mg/kg)
3 (R = Me, X=Cl)	20	11,000	3.0
4 (R = H, X=Br)	9	41,000	0.6

We propose that the high selectivity of **3** is due to the conformation of the benzyl group which protrudes below the indole plane and gives the molecule greater steric bulk analogous to the trichlorobenzoyl derivative **2** (see Figure). The optimal benzyl substituent was found to be the 4-bromo group, while removal of the methyl group α to the acid provided an increase in selectivity. The resulting compound **4** was more potent in vivo with an ED_{30} of 0.6 mg/kg in the rat paw edema assay. Modifications of the 2-methyl substituent and

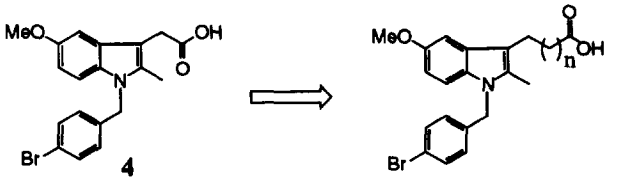
the benzylic methylene were not tolerated, while replacement of the 5-methoxy group with a halogen gave an active, but less selective compound.

Figure. Comparison of the phenyl ring orientation in compounds **1**, **2**, and **3**, and their sizes in the dimension perpendicular to the indole ring.[14]



We have found that extension of the carboxylic acid side chain of these molecules gives highly selective inhibitors. Both propanoic acid (**5**) and butanoic acid (**6**) side chains showed good COX-2 potency yet were inactive in the COX-1 whole cell assay. The pentanoic acid side chain was inactive against both enzymes.

Table III



4

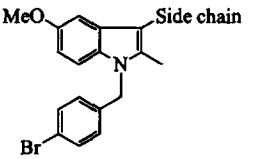
	COX-2 Whole Cell IC_{50} (nM)	COX-1 Whole Cell IC_{50} (nM)	Rat Paw Edema ED_{30} (mg/kg)
4	9	41,000	0.6
5 (n=1)	3	>50,000	>3
6 (n=2)	6	>50,000	1.2

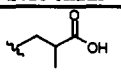
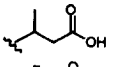
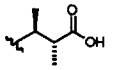
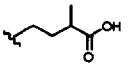
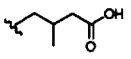
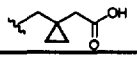
The low in vivo activity of propanoic acid derivative **5** (ED_{30} >3 mg/kg) is likely due to its poor pharmacokinetics. The compound is cleared rapidly, presumably due to β-oxidative metabolism of the acid side chain. By placing an alkyl substituent on the side chain, this metabolism can be blocked, resulting in active and selective compounds with improved pharmacokinetics and good in vivo potencies. Several alkyl-substituted

propanoic acids were prepared, and compound **8**, with a β-methyl substituent was found to be the most promising. It has an IC_{50} = 4 nM against COX-2 and >50 μM against COX-1 with an ED_{30} in the rat paw edema assay of 0.6 mg/kg. Similar results were obtained in the butanoic acid series. Alkyl substituents are well tolerated on the α- and β-positions but less well in the γ-position. The results are summarized in Table **IV**.

Among these compounds, the β-methyl substituted butanoic acid **11** (L-761,000) has the best in vitro and in vivo profiles. It has an IC_{50} of 2 nM against COX-2, is inactive against COX-1 at 50 μM, and is active in the rat paw edema assay with an ED_{30} of 0.4 mg/kg. The compound also shows good bioavailability, giving 15 μM plasma levels in rats when dosed at 5 mg/kg. To test the hypothesis that a COX-2 selective inhibitor would be GI sparing, rats were dosed with L-761,000 for 5 days at 100 mg/kg BID. No evidence of increased ^{51}Cr excretion in the feces was observed under these conditions, indicating a high level of GI tolerance.

Table IV



	Side chain	COX-2 Whole Cell IC_{50} (nM)	COX-1 Whole Cell IC_{50} (nM)	Rat Paw Edema ED_{30} (mg/kg)
7		3	>50,000	2.6
8		4	>50,000	0.6
9		60	>50,000	0.6
10		4	>10,000[a]	1.6
11		2	>10,000[a]	0.4
12		16	>10,000[a]	0.5

Note: (a) interference with the method of detection prevented testing of these compounds at concentrations above 10,000 nM.

This study represents the first example of the conversion of a non-selective NSAID into a highly selective COX-2 inhibitor. We have identified *N*-benzyl indolealkanoic acids to be potent anti-inflammatory agents with a much improved GI safety profile over conventional NSAIDs. The data obtained for L-761,000 provide further support for the hypothesis that COX-1 activity plays a key role in the GI toxicity of known NSAIDs and show that a potent anti-inflammatory agent can be prepared which exhibits a high degree of GI tolerance.

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