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THE DISCOVERY OF ROFECOXIB, [MK 966, VIOXX®, 4-(4'-METHYLSULFONYLPHENYL)-3-PHENYL-2(*5H*)-FURANONE], AN ORALLY ACTIVE CYCLOOXYGENASE-2 INHIBITOR

P. Prasit,* Z. Wang,* C. Brideau, C.-C. Chan, S. Charleson, W. Cromlish, D. Ethier, J.F. Evans, A.W. Ford-Hutchinson, J.Y. Gauthier, R. Gordon, J. Guay, M. Gresser, S. Kargman, B. Kennedy, Y. Leblanc, S. Léger, J. Mancini, G.P. O'Neill, M. Ouellet, M.D. Percival, H. Perrier, D. Riendeau, I. Rodger, P. Tagari, M. Thérien, P. Vickers, E. Wong, L.-J. Xu, R.N. Young, and R. Zamboni

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

S. Boyce and N. Rupniak

Merck Research Laboratories, Harlow, Essex, UK

M. Forrest and D. Visco

Merck Research Laboratories, Rahway, NJ 07065, U.S.A.

D. Patrick

Merck Research Laboratories, West Point, PA 19486, U.S.A.

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Abstract: The development of a COX-2 inhibitor rofecoxib (MK 966, Vioxx®) is described. It is essentially equipotent to indomethacin both in vitro and in vivo but without the ulcerogenic side effect due to COX-1 inhibition.

All currently available nonsteroidal antiinflammatory drugs (NSAIDs) have been characterized as dual COX-1/COX-2 inhibitors.[1] The most notable adverse effect with existing NSAIDs is the increased risk of gastrointestinal ulceration, perforation and hemorrhage, which has been associated with the inhibition of COX-1.[2] It is now generally accepted that a COX-2 inhibitor would have the potential to be a potent antiinflammatory agent without the toxicity associated with nonselective NSAIDs.[3] Free of dose-limiting COX-1-associated toxicity, a COX-2 inhibitor may therefore demonstrate comparable or possibly superior clinical efficacy to existing NSAIDs for a variety of clinical disorders. The improved safety profile of COX-2 inhibitors may allow the use of these new agents for long-term prophylactic use in otherwise healthy individuals with a known genetic susceptibility to certain chronic diseases. Of particular interest is the detection of COX-2 in colorectal carcinoma tissues[4] and the demonstration that COX-2 inhibition reduces the number of polyps in the $Apc^{\Delta 716-/-}$ knockout mice (a model of human familial adenomatous polyposis)[5] and suppresses colonic aberrant crypt foci.[6] This suggests that COX-2 plays a key role in polyp formation and provides the basis for chemopreventive treatment of polyposis and cancer by selective COX-2 inhibitors.[7] The Baltimore Longitudinal Study of Aging, with 1686 participants, reported in 1997 that the risk of developing Alzheimer's disease is reduced among NSAIDs users, especially those who have taken the medications for two years or more.[8] No

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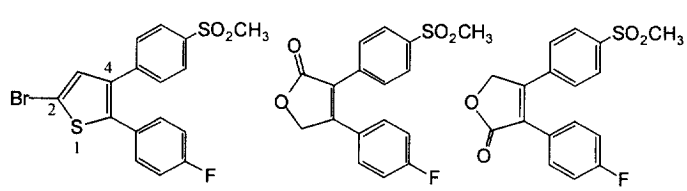
decrease risk was evident with acetominophen or aspirin. In addition, COX-2 has been implicated in angiogenesis[9] and breast cancer.[10] These new potential disease targets will widen the use of selective COX-2 inhibitors from the traditional inflammatory disorders, provided that their safety profile is superior to that of standard NSAIDs.

A number of COX-2 selective inhibitors have been reported by us and others.[3,11] Herein, we describe the discovery and the development of rofecoxib (MK 966, Vioxx®), a COX-2 inhibitor based on the 4-methylsulfonylphenyl series. In the previous papers, we reported the SAR of the tricyclic 4-methylsulfonylphenyl class of compounds.[12] Although the SAR of the progenitor member of the series, DuP 697 (**1**), is very tight, a few interesting features emerged from that study. It was recognized that, in general, the replacement of the methylsulfonyl group by a sulfonamide moiety, although in many instances enhancing the oral bioavailability, resulted in a dramatic increase in COX-1 inhibitory activity thus eroding the COX-1/COX-2 selectivity ratio of the compound. In addition, the presence of a large substituent at C-2, (e.g., a bromine atom or a methyl group) also increases the COX-1 inhibitory activity. A third important feature of this class of compounds is that they tended to have low oral bioavailability.

With these three key pieces of information in hand, we decided to incorporate the following features into the design of our COX-2 inhibitors so as not to compromise the selectivity ratio while at the same time improving its oral absorption: (1) use of the methylsulfone instead of the sulfonamide moiety to maximize COX-2 selectivity, (2) removal of the C-2 substituent (DuP 697 numbering) to enhance COX-2 selectivity, and (3) addressing the oral absorption issue by replacing the central thiophene template with a new heterocycle and/or use a prodrug approach to enhance absorption. Based on these criteria, it was envisaged that the thiophene ring could be replaced by a lactone moiety. The choice of the lactone as the 5-membered ring replacement has the potential of satisfying all of the criteria set out. Thus the methylsulfone moiety is retained, maximizing the COX-2 selectivity. The "C-2 substituent" is now small, in fact it is merely lone pairs of electrons on the oxygen atom. The two isomeric lactones **2** and **3** were prepared[13] and were tested in the cyclooxygenase assays. The lactone **2** was found to be essentially inactive, only **3** was found to have significant inhibitory activity against COX-2. The lactone **3** was found to be essentially equipotent to indomethacin in both the COX-2 whole cell and the COX-2 whole blood assays (see data in Table 1). However, in contrast to indomethacin, **3** was a relatively poor inhibitor of COX-1 in both whole cell and whole blood assays.



1 **2** **3**

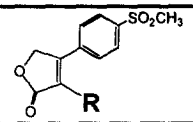
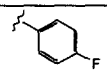
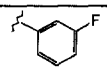
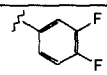
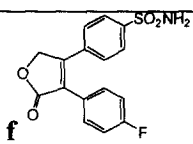
Table 1. Cyclooxygenase Inhibitory Activities of Lactone 3 and indomethacin,1 IC_{50} (μM)

	COX-2 WHOLE BLOOD	COX-1 WHOLE BLOOD	RATIO	COX-2 WHOLE CELL	COX-1 WHOLE CELL	RATIO
INDOMETHACIN	0.5 μM	0.2 μM	**0.4**	0.03 μM	0.02 μM	**0.7**
3	0.6 μM	10 μM	**17**	0.01 μM	4.7 μM	**470**

Thus compound **3** is a potent and selective inhibitor of COX-2 and satisfies two of the three criteria set out earlier. The critical question remained as to whether **3** was orally bioavailable. Fortunately, in contrast to some other heterocycles that had been examined earlier, it was found that as a class, the lactones were very bioavailable. Upon dosing **3** at 20 mg/kg in rats, plasma levels of **3** with a C_{max} as high as 39 μM were achieved. Not surprisingly, the lactone **3,** with such an in vitro potency and absorption, demonstrated significant antiinflammatory and antipyretic activities. In the rat paw edema model,[15] **3** has an ED_{50} of 2.0 mg/kg (5% tween 80 as vehicle) and an ED_{50} of 1.3 mg/kg in the rat pyresis model,[15] which compares favorably with indomethacin (see Table 3 for indomethacin data).

Although **3** is a relatively selective and orally active COX-2 inhibitor, it was felt desirable to enhance its COX-2 selectivity further. As with other COX-2 inhibitors of the tricyclic class, it was found that having a substituent at the 4-position on the lower phenyl ring increases COX-1 activity and thus is detrimental to the selectivity. For example, **3,** (X = 4-fluorine, entry **a**, Table 2), is more potent in the COX-1 assay than entry **b** (X = H). This is generally true for other para-substituents such as halogens, methyl, methoxy and others (data not shown). It is worth noting that these changes do not greatly affect their inhibitory activities against COX-2. Moreover, by moving these substituents from the 4 to the 3- position (entry **c**) or by adding substituents at both the 3 and 4-positions (entry **d**), the COX-1 inhibitory activity is reduced, again without dramatically affecting the inhibitory activity against COX-2. This trend is consistent with the fact that the COX-1 active site is sterically more demanding than that of COX-2[14] making it more sensitive to changes to the substitution pattern. Sulfonamido derivatives were also briefly investigated, but as expected (entry **f**), these compounds in this series led a higher level of COX-1 inhibitory activity than the corresponding methylsulfone derivatives. Some heterocycles can be used to replace the lower phenyl ring of the lactone (entries **e**) but in general, these compounds demonstrate a decrease in COX-2 inhibitory potency.

Table 2. In Vitro Data of Representative Cyclooxygenase-2 Inhibitors, IC_{50} (μM)

	**Human Whole Blood COX-2**	**Human Whole Blood COX-1**	**Whole Cells (CHO) COX-2**	**Whole Cells (CHO) COX-1**
 **a**	0.6	10	0.01	4.7
 **b**	0.5	19	0.02	>15
 **c**	1.8	86	0.02	>50
 **d**	0.9	13	0.03	>50
 **e**	>33	nd	>5.0	nd
 **f**	0.8	5.8	nd	nd
Indomethacin	0.4	0.2	0.03	0.02

Of the large number of compounds prepared and examined, the compound with the best overall profile proved to be entry **b** (now designated as rofecoxib, MK 966 or Vioxx®). The compound has progressed into clinical trials and is currently awaiting FDA approval. Its in vivo activities in animal models in comparison to indomethacin are summarized in Table 3. It is essentially equipotent to indomethacin in most models of pain and inflammation. However, unlike indomethacin, it is non-ulcerogenic (see below). A detailed discussion on the biological and pharmacological properties of rofecoxib will be described elsewhere.[15]

Table 3. Comparison of Rofecoxib and Indomethacin in Various in vivo Assays

ED_{50}, mg/kg	Rofecoxib	Indomethacin
Rat Paw Edema	1.5	2.0
Rat Pyresis	0.2	1.1
Rat Paw Hyperalgesia	1.0	1.5
Adjuvant Arthritis	0.7 (bid)	0.2

A comparison of the in vitro activities, performed under the same conditions, of rofecoxib and other COX-2 inhibitors that have progressed beyond the pre-clinical stage are summarized in Table 4. Rofecoxib exhibited superior COX-2 inhibitory activity and selectivity as compared to celecoxib[16] and JTE 522.[17] The latter two compounds possess a sulfonamido as opposed to the methylsulfonyl moiety as is found in rofecoxcib. Meloxicam and diclofenac are also included in the table as examples of the more selective standard NSAIDs as measured by whole blood assays. However, both meloxicam and diclofenac inhibited COX-1 at therapeutic doses. On the other hand, it has been shown in humans that the oral administration of 1 g of rofecoxib, or approximately 40 times the therapeutic dose, did not affect the thromboxane production ex vivo in the COX-1 human whole blood asssay.[18]

Table 4. Comparison Of Rofecoxib and Other Inhibitors In Human Whole Blood Assays, IC_{50} (μM)

	Rofecoxib	Celecoxib	JTE 522	Meloxicam	Diclofenac
COX-2	0.5	1.0	42% @ 33 μM	0.7	0.05
COX-1	19	6.3	46% @ 100 μM	1.4	0.15
Ratio	38	6.3		2	3

The lack of the gastrointestinal effect of rofecoxib, consistent with its poor COX-1 inhibitory activity, was demonstrated in a number of ways. Using a highly stringent model of GI integrity, i.e., the use of ^{51}Cr salts to probe the intestinal permeability,[19] rofecoxib did not show any ^{51}Cr leakage at a daily oral dose of 200 mg/kg (100 mg/kg, bid), for 5 days in either rats or squirrel monkeys. In contrast, a single dose of a nonselective inhibitor such as diclofenac or indomethacin at 10 mg/kg caused a significant increase in ^{51}Cr excretion. In a pilot safety study, an oral dosing of rofecoxib to rats at 300 mg/kg for 14 days did not produce GI lesions whereas a single dose of indomethacin at 3 mg/kg produced clearly visible gastric lesions.

Given that the ED_{50} of rofecoxib is approximately 1 mg/kg in a number of animal models, this translates into an unprecedented therapeutic index of >300. Moreover, the compound exhibits excellent pharmacokinetics and dose proportionality in a number of species including rats, dogs, mice and squirrel monkeys.

In summary, a potent, selective and orally active COX-2 inhibitor, rofecoxib has been discovered. It possesses an unprecedented in vivo therapeutic window of >300-fold in animal models which is consistent with the in vitro data. It also demonstrates that the selective inhibition of COX-2, and not both isoforms, is sufficient for the reduction of pain, inflammation and fever in animal models. The improved gastric safety profile of rofecoxib over standard NSAIDs should allow for long-term prophylactic use in otherwise healthy individuals with a known genetic susceptibility to certain chronic diseases such as Alzheimer's disease and colon cancer.

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