4-[5-Methyl-3-phenylisoxazol-4-yl]-benzenesulfonamide, Valdecoxib: A Potent and Selective Inhibitor of COX-2

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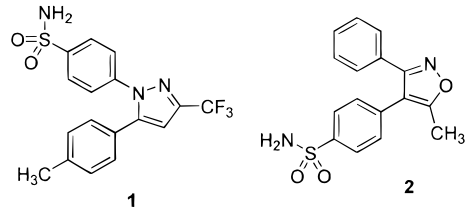
Introduction. Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most widely used prescription and over the counter medications, used primarily for the treatment of pain and inflammation, particularly arthritis. In the 1970s, it was demonstrated that aspirin and other NSAIDs block the formation of prostaglandins (PGs) produced from arachidonic acid by the enzyme cyclooxygenase (COX), sometimes called prostaglandin synthase.[1] PGs are produced by most cells, and their presence in tissues elicits a broad array of biological responses. Most notably, PGs in the gastrointestinal tract are cytoprotective, in the kidney they are responsible for normal renal function, and they allow platelets to aggregate. PGs mediate a number of characteristic features of the body's response to tissue injury or inflammation. Dilation of small blood vessels leading to development of redness and heat is mediated by PGs. They contribute to an increase in vascular permeability leading to the characteristic swelling of tissues. In addition, PGs sensitize peripheral nerve endings to transmit pain signals to the brain and spinal cord. Thus, it is now known that PGs play an important role in pathologic as well as physiologic processes.

Consumption of NSAIDs for inflammatory diseases unavoidably leads to blockade of PGs required for normal physiology. It is now evident that the therapeutic effects and side effects of this class of drug are related to their mechanism of action. The first-line mode of therapeutic intervention of chronic inflammatory diseases such as osteoarthritis and rheumatoid arthritis is NSAIDs. As a consequence, NSAID users suffer from a high incidence of gastrointestinal irritation, including the development of life-threatening gastrointestinal ulcers.[2] Additionally, consumption of NSAIDs can lead to abnormal renal physiology.[3,4] More advanced means of treatment of chronic inflammatory diseases are glucocorticoids and immunosuppressives that possess an even greater array of side effects.[5]

Until the latter part of the 1980s, most researchers in the prostaglandin field felt that the formation of PGs was limited only by the available pool of arachidonic acid. Several observations suggested this view was not entirely accurate. It was found that the amount of COX protein in inflamed tissues was appreciably higher than in normal tissues.[6] The amount of COX could be increased by stimulating cells with selected cytokines such as interleukin-1, TNF-α, or bacterial endotoxin. The increase in COX protein synthesis was found to be blocked by glucocorticoids, whereas the level of free arachidonic acid did not appreciably change.[7,8] These observations led to the hypothesis that a second form of COX existed. In 1991, the inducible form of the enzyme, COX-2, was cloned.[9−11] The cloned COX-2 was the same as a unique form of the enzyme that had previously been reported.[12,13] It is now known that the constitutive enzyme (COX-1) is expressed in essentially all tissues, whereas COX-2 expression is largely absent unless induced by inflammatory stimuli. The discovery of a second enzyme, intimately associated with inflammatory states, led to the hypothesis that selective inhibitors of COX-2 would be antiinflammatory with diminished side effects.

Since the mid-1990s, a number of reports have appeared on the preparation and biological activity of selective COX-2 inhibitors.[14] Recently we detailed the synthesis and biological profile of celecoxib (**1**).[15] As part of our continuing efforts to identify inhibitors with greater potency and high specificity for COX-2, we prepared a series of diarylisoxazole derivatives that possess exceptional antiinflammatory properties. Herein we detail our study on two of the diarylisoxazole COX-2 inhibitors. This research effort culminated in the identification of SC-65872 (valdecoxib, **2**) which is currently in clinical evaluation for the management of pain and inflammation.



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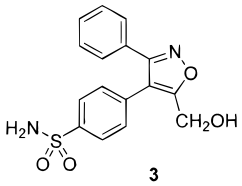

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Chemistry. The synthesis of **2** was very concise and is outlined in Scheme 1. Deoxybenzoin was converted to the corresponding oxime by treatment with hydroxylamine hydrochloride in the presence of sodium acetate.[16] Deprotonation of the oxime with 2 equiv of butyllithium followed by condensation with ethyl acetate afforded isoxazoline **4**.[17] Treatment of **4** with chlorosulfonic acid followed by reaction of the incipient sulfonyl chloride with aqueous ammonia afforded **2** in good yield.

After some experimentation, a workable method for the preparation of **3** was identified, Scheme 2. The route involved condensation of the dianion of deoxybenzoin

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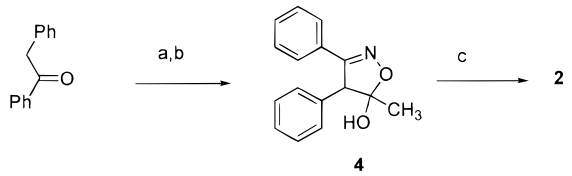
Table 1. In Vitro Activity of **1**−**3**

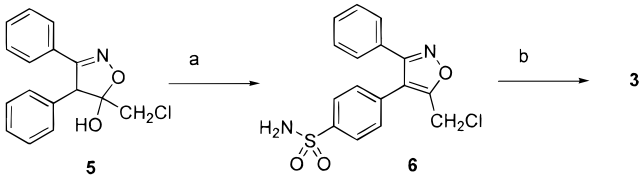
	IC_{50}, μM (n)			
	human recombinant enzymes		human ex vivo whole blood	
no.	COX-1	COX-2	serum TXB_2 COX-1 assay	LPS-induced PGE_2 COX-2 assay
1	15 ± 3.4 (7)	0.04 ± 0.01 (7)	6.67 ± 1.32 (3)	0.164 ± 0.065 (7)
2	140 ± 19 (10)	0.005 ± 0.001 (10)	25.4 ± 1.2 (3)	0.89 ± 0.033 (6)
3	1120 ± 198 (6)	0.18 ± 0.04 (7)	>50 (4)	0.329 ± 0.101 (4)

Scheme 1[a]



[a] (a) NH_2OH·HCl, NaOAc, aq EtOH; (b) 1. 2 equiv nBuLi, 2. EtOAc; (c) 1. $ClSO_3H$, 2. NH_4OH.

Scheme 2[a]



[a] (a) 1. $ClSO_3H$, 2. NH_4OH; (b) 1. HCO_2H, Et_3N, 2. aq NaOH.

Table 2. In Vivo Activity of **1**−**3**

	EC_{50}, mg/kg (n)		
no.	rat air pouch	rat adjuvant arthritis[a]	rat carrageenan edema[b]
1	0.33 ± 0.08 (2)	0.373 ± 0.163 (3)	7.13 ± 0.79 (2)
2	0.05 ± 0.02 (2)	0.032 ± 0.002 (2)	10.2 ± 1.4 (2)
3	0.81 ± 0.19 (2)	1.49 ± 0.37 (3)	1.06 ± 0.01 (3)

[a] ED_{50} values were determined using a minimum of four dose points, 8−10 animals/group. [b] ED_{50} values were determined using a minimum of four dose points, 5 animals/group.

with methyl chloroacetate to provide isoxazoline **5**. Chlorosulfonation and treatment of the corresponding sulfonyl chloride with aqueous ammonia afforded sulfonamide **6**. Treatment of **6** with formic acid in the presence of triethylamine provided the formate ester which was hydrolyzed with aqueous sodium hydroxide to afford **3** in excellent yield.[18]

Results and Discussion. As an initial screen for activity, **2** was evaluated against human recombinant COX-1 and COX-2 enzymes.[19] Against COX-1 **2** showed weak inhibitory activity with an $IC_{50} = 140 \pm 19$ μM (n = 10), whereas the compound showed potent activity against COX-2 with an $IC_{50} = 0.005 \pm 0.001$ μM (n = 10), Table 1. Cellular in vitro activity and selectivity was evaluated in human whole blood.[20] For assessment of the extent of COX-2 inhibition, human heparinized whole blood was cultured with lipopolysaccharide (LPS) overnight in the presence of inhibitor, and plasma was assayed for PGE_2 production as a function of COX-2 inhibition. For COX-1 activity, inhibitors were added to heparinized whole blood for 15 min prior to the addition of calcium ionophore, A-23187. After 10 min, plasma was collected for analysis of TxB_2 as a function of COX-1 activity, Table 1. As summarized in Table 2, **2** showed potent oral activity in an acute antiinflammatory assay [rat carrageenan foot pad edema;[21] $ED_{50} = 10.2 \pm 1.4$ mg/kg (n = 2)]. Chronic antiinflammatory activity was achieved with **2** in the rat adjuvant arthritis model[22] [$ED_{50} = 0.032 \pm 0.002$ mg/kg/day (n = 2)], a potency exceeding that obtained with the most potent NSAIDs. Blockade of prostaglandin production at the inflammatory site was demonstrated in the rat carrageenan air pouch model[23] [$ED_{50} = 0.05 \pm 0.02$ mg/kg (n = 2)]. These data indicate that **2** is a highly potent and selective inhibitor of COX-2 that possesses excellent oral activity as an antiinflammatory in animal models typically used to develop NSAIDs.

Additionally, **2** is converted in vivo in rodents and dogs, and in very low abundance in humans, to an active metabolite **3**. In vitro **3** showed an $IC_{50} = 1120 \pm 198$ μM (n = 6) against COX-1 and an $IC_{50} = 0.18 \pm 0.04$ μM (n = 7) against COX-2, Table 1. Pharmacological evaluation of independently synthesized **3** showed it possessed oral activity in the acute antiinflammatory assay [carrageenan paw edema; $ED_{50} = 1.06 \pm 0.01$ mg/kg (n = 3)]. Chronic antiinflammatory activity was achieved with **3** in the rat adjuvant arthritis model [$ED_{50} = 1.49 \pm 0.37$ mg/kg/day (n = 3)]. Compound **3**, while more potent in the carrageenan paw edema assay than **2**, was less potent against the isolated enzyme and in the air pouch or adjuvant arthritis assays. These seemingly incongruous data likely reflect the different pharmacokinetics and tissue distribution of these two compounds in the different animal models.

Conclusion. We have shown that **2** is a highly selective and potent inhibitor of COX-2 in human whole blood and against the recombinant human enzyme. In animal models of acute and chronic inflammation, **2** showed exceptional potency after oral administration. An active metabolite, **3**, formed in rodents and dogs was also found to be a COX-2-selective inhibitor. Valdecoxib (**2**) is currently in clinical evaluation for the treatment of arthritis and pain.

Supporting Information Available: Biological procedures, synthetic procedures, and spectral data for compounds **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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