Novel Synthesis of 3,4-Diarylisoxazole Analogues of Valdecoxib: Reversal Cyclooxygenase-2 Selectivity by Sulfonamide Group Removal

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3,4-Diarylisoxazole analogues of valdecoxib [4-(5-methyl-3-phenylisoxazol-4-yl)-benzensulfonamide], a selective cyclooxygenase-2 (COX-2) inhibitor, were synthesized by 1,3-dipolar cycloaddition of arylnitrile oxides to the enolate ion of phenylacetone regioselectively prepared in situ with lithium diisopropylamide at 0 °C. The corresponding 3-aryl-5-methyl-4-phenylisoxazoles were easily generated by a dehydration/aromatization reaction under basic conditions of 3-aryl-5-hydroxy-5-methyl-4-phenyl-2-isoxazolines and further transformed into their benzenesulfonamide derivatives. The biochemical COX-1/COX-2 selectivity was evaluated in vitro by using the human whole blood assays of COX isozyme activity. Three compounds not bearing the sulfonamide group present in valdecoxib were selective COX-1 inhibitors.

Introduction

The enzyme cyclooxygenase (COX) catalyzes the rate-limiting step in the formation of prostanoids from arachidonic acid (AA).[1−5] Two isoforms of the COX enzyme (known as COX-1 and COX-2), encoded by two different genes, have been identified.[6] COX-1 displays the characteristics of a "housekeeping gene" and is constitutively expressed in virtually all tissues. High expression of COX-1 has been detected in platelet and gastric mucosa. Differently, COX-2 is expressed in response to inflammatory and mitogenic stimuli.[7] However, this simplified paradigm of constitutive COX-1 and inducible COX-2 has many exceptions: COX-1 can be regulated during development,[1,8] whereas COX-2 is constitutively expressed in the brain,[9] reproductive tissues,[10] and kidney.[11−13] The inhibition of COX-2 is thought to mediate the therapeutic actions of nonselective nonsteroidal antiinflammatory drugs (NSAIDs), while the inhibition of COX-1 results in unwanted side effects, particularly in the gastrointestinal (GI) tract.[14,15] In fact, selective COX-2 inhibitors (denominated coxibs) are efficacious as nonselective NSAIDs for the treatment of acute pain and chronic inflammatory diseases and show the advantage of reducing the GI toxicity associated with the administration of nonselective NSAIDs by virtue of COX-1 sparing.[14,15] Several selective COX-2 inhibitors such as rofecoxib,[16] celecoxib,[17] and valdecoxib[18] (Figure 1) have been approved for the treatment of rheumatoid arthritis (RA) and osteoarthritis (OA) and for the relief of acute pain associated with dental surgery and primary dysmenorrhea.

These compounds are diaryl heterocyclic derivatives containing a phenylsulfone or a phenylsulfonamide moiety that interact with the COX-2 side pocket through slow, tight-binding kinetics.[19,20] However, selective COX-2 inhibition reduces the biosynthesis of prostacyclin, thus leaving the thromboxane action unopposed. The cardiovascular implications of these effects are currently debated on the basis of the results of the Vioxx Gastrointestinal Outcomes Research (VIGOR) trial showing a statistically significant difference in acute myocardial infarction (MI) rates between rofecoxib and naproxen, a nonselective NSAID (0.4% vs 0.1%, respectively).[21]

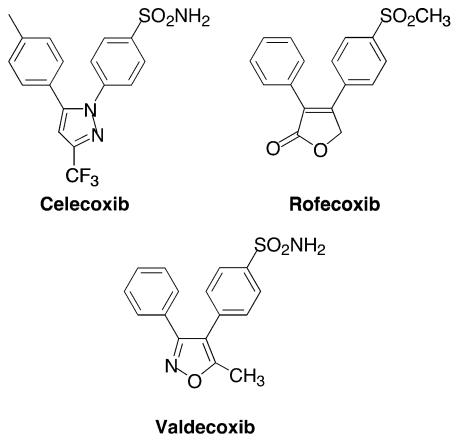

Figure 1.

Recently, experimental and clinical results have suggested a possible involvement of COX-1 in pain and cancer development, thus providing the rationale for the development of selective COX-1 inhibitors.[22−28] In fact, animal studies have demonstrated that COX-1 plays a role in intestinal polyposis and skin carcinogenesis, consistent with epidemiological data demonstrating that regular use of low-dose aspirin, which inhibits only platelet COX-1 activity, can reduce colon cancer incidence and mortality.[29−31] In addition, in ovarian cancer, the COX-1 enzyme is overexpressed and promotes angiogenic growth factor production.[32] Moreover, it has been suggested that COX-1 may play an important role in pain processing and sensitization in spinal cord and gracile nucleus after surgery.[33] Thus, selective COX-1 inhibitors might be useful analgesic and chemopreventive agents.

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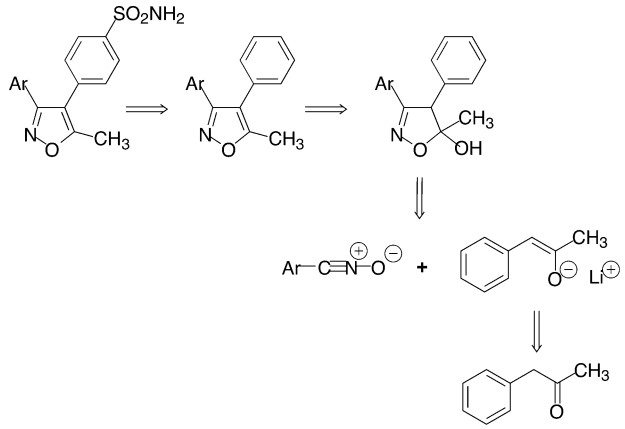
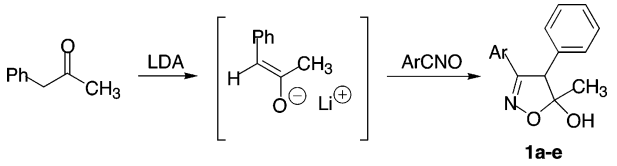


Figure 2.

Scheme 1



In this study, we report a novel synthesis of a series of new 3,4-diarylisoxazole analogues of valdecoxib. Using the well-known human blood assays for COX isozyme selectivity,[34−36] we found that sulfonamide group removal was associated with reversal COX-2 selectivity.

Chemistry

Recently, we found that a number of 5-alkyl-3-arylisoxazoles structurally related to valdecoxib can be prepared by reacting arylnitrile oxides with free enolate ions regioselectively obtained by metallation of various alkyl methyl ketones with lithium diisopropylamide (LDA) at −78 °C, followed by a dehydration/aromatization reaction.[37] The same procedure was successfully applied to the synthesis of valdecoxib with better results compared to other reported valdecoxib syntheses.[38,39] To find more selective and more potent COX-2 inhibitors, we prepared other valdecoxib-like isoxazoles by using the same synthetic approach (Figure 2).

The key step in this methodology is the preparation of the needed enolate from phenylacetone. In principle, the phenylacetone can afford two different enolates in the presence of a base. By use of thermodynamic control conditions, namely, phenylacetone with LDA at 0 °C, the needed enolate was generated. Then, such an enolate was allowed to react with various arylnitrile oxides to afford 3-aryl-5-hydroxy-5-methyl-4-phenyl-2-isoxazolines (**1a**−**e**) (Scheme 1) with fair to good yields and diasteroselectivity ratios (cis/trans) ranging between 70:30 and 85:15 (Table 1).

The diastereoisomeric ratios were determined by ^{1}H NMR spectra analysis of the reaction crudes by attributing the lower (more shielded) δ_{CH3} values to cis **1a**−**e** (major isomers) by analogy with other already known similar systems[40] (see, for example, a comparison of δ values of *cis*-**1a** and *trans*-**1a** with those of *cis*- and *trans*-5-hydroxy-3,5-dimethyl-4-phenyl-2-isoxazoline **A** and **B** in Figure 3). However, both the hydroxyisoxazoline diastereoisomers can be transformed into the same isoxazole by a dehydration/aromatization reaction under basic (or acidic) conditions so that their relative distribution has no synthetic importance with reference to the isoxazole synthesis.

Furthermore, in some cases (reaction of mesitylnitrile oxide and 5-chloro-2-furylnitrile oxide with the enolate ion of phenylacetone and subsequent chromatographic separation), partial or complete dehydration/aromatization of 5-hydroxyisoxazolines to the corresponding isoxazoles can also occur "spontaneously" (Table 2 and footnote c of Table 1).

Complete conversion of all 5-hydroxyisoxazolines **1a**−**e** into the corresponding 3-aryl-5-methyl-4-phenyl-isoxazoles **2a**−**e** required, however, a separate treatment under less basic conditions (Scheme 2 and Table 3).

As previously found,[41] optimal conditions for basic dehydration/aromatization of 5-hydroxyisoxazolines

Table 1. Yields and Diastereoisomeric Ratios in 1,3-Dipolar Cycloaddition of Arylnitrile Oxides with "Thermodynamic" Lithium Enolate of Phenylacetone

compd	Ar	yield (%)[a]	dr[b]
1a	phenyl	61	85:15
1b	mesityl	29[c]	70:30
1c	5-chloro-2-furyl	45[c]	80:20
1d	2,4,6-trimethoxyphenyl	52	70:30
1e	3-chloro-2,4,6-trimethoxyphenyl	60	70:30

[a] Yields refer to the recrystallized products. [b] Diastereoisomeric ratios were determined by ^{1}H NMR. [c] The apparent low yields are due to a partial spontaneous dehydration to isoxazoles (see text and Table 2).

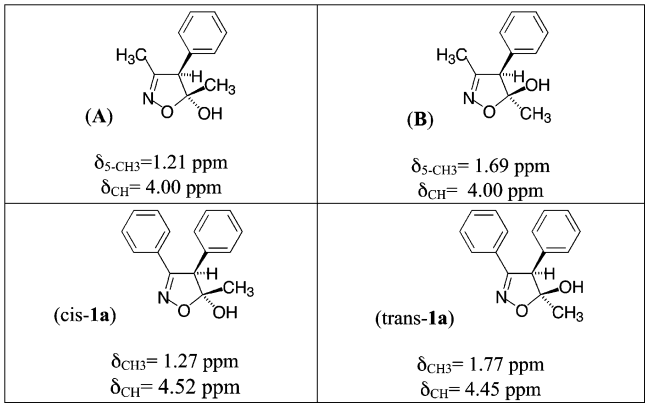


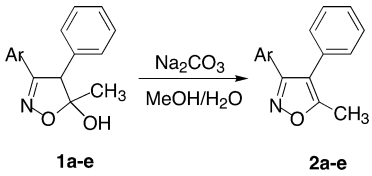
Figure 3.

Table 2. Yields of Isoxazolines (**1b** and **1c**) and Isoxazoles (**2b** and **2c**) Formed in the Reaction of Mesitylnitrile Oxide and 5-Chloro-2-furylnitrile Oxide with Lithium Enolate of Phenylacetone

		yield (%)[a]	
Ar	reaction time (h)	isoxazoline	isoxazole
mesityl-	3	29 (**1b**)	55 (**2b**)
mesityl-	overnight		73 (**2b**)
5-chloro-2-furyl	overnight	45 (**1c**)	13 (**2c**)

[a] Yields refer to the products isolated by chromatography.

Scheme 2



Scheme 3

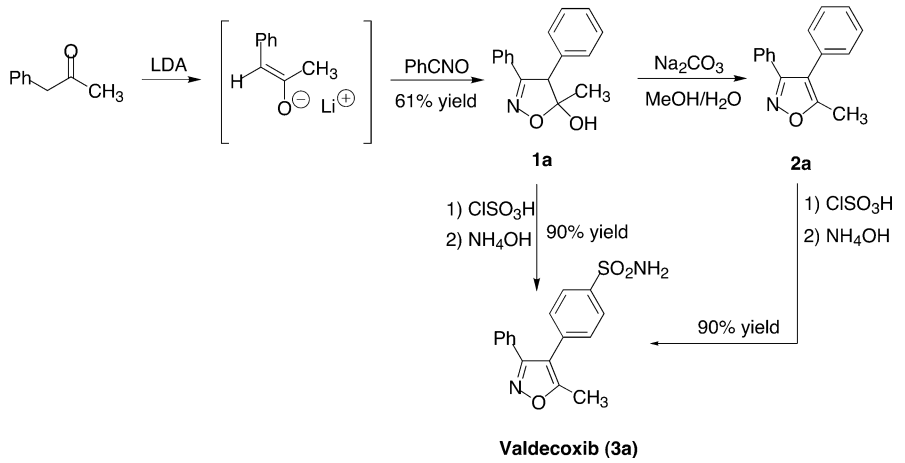


Table 3. Yields of Isoxazoles **2a**−**e** Formed from 5-Hydroxyisoxazolines (Scheme 2)

compd	Ar	reaction time	yield (%)[a]
2a	phenyl	1 h	80
2b	mesityl	15 min	50
2c	5-chloro-2-furyl	1 h	60
2d	2,4,6-trimethoxyphenyl	2 h	74
2e	3-chloro-2,4,6-trimethoxyphenyl	2 h	75

[a] Yields refer to the products actually isolated by chromatography.

should require a not very strong base. This should ensure, in fact, the incomplete conversion into the corresponding alcoholates, thus allowing the existence of the undissociated hydroxy group likely involved in the dehydration reaction (possibly by an E_1cB mechanism).

As mentioned above, we applied such an approach (1,3-dipolar cycloaddition of arylnitrile oxides with lithium enolate of phenylacetone and dehydration of the formed 5-hydroxyisoxazolines) to the synthesis of the potent and selective COX-2 inhibitor 4-(5-methyl-3-phenylisoxazol-4-yl)benzenesulfonamide, well-known as valdecoxib (Scheme 3).[42]

The synthesis was accomplished by subjecting 5-methyl-3,4-diphenylisoxazole **2a** to reaction with $ClSO_3H$/NH_4OH or even directly reacting hydroxyisoxazoline **1a** with $ClSO_3H$/NH_4OH. In the latter case, dehydration to isoxazole occurs by means of $ClSO_3H$ (acid conditions) and the conversion into valdecoxib is performed by a "one-pot" procedure (just like already reported in the previous synthetic approach).[38,42]

The actual overall yields (i.e., after separation of the final product by chromatography and with reference to the initial reagents) resulted in both cases being notably higher (55% yield by performing the last conversion by the "one-pot" procedure) compared to that (32%) reported in the literature.[38,39]

On the other hand, following the same synthetic approach, some valdecoxib analogues were also prepared (Scheme 4). While **3a** (valdecoxib) and **3c** were obtained in high yields (90% and 73% yield, respectively; yields referred to the sole conversion of **2** to **3**), **3e** was instead obtained in only 23% yield (Table 4). In this case, some competition of trimethoxycholorophenyl ring toward the reaction with $ClSO_3H$ is also observed (Scheme 5). This is possibly due to the electron-donating

Scheme 4

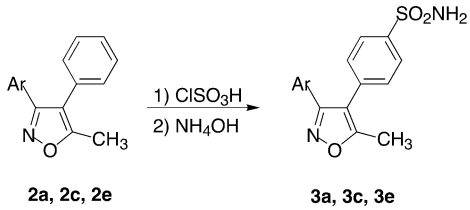


Table 4. Yields of Valdecoxib Analogues from Compounds **2**

compd	Ar	yield (%)[a]
3a	phenyl	90
3c	5-chloro-2-furyl	73
3e	3-chloro-2,4,6-trimethoxyphenyl	23

[a] Yields refer to the products actually isolated by chromatography.

effects of methoxy groups, compensating the deactivation toward the electrophiles associated with both the preexisting chloro atom and the unfavorable position 3 of isoxazole. However, chlorination instead of sulfonation of the ring is unusually observed in this case, which could be explained by assuming that the electrophilic species originated from $ClSO_3H$ still retains Cl (e.g., ClS_2O_6H)[43,44] and, possibly due to the concomitant steric effects of the methoxy groups, preferentially attack in this case using the peripheral Cl rather than an internal S atom.

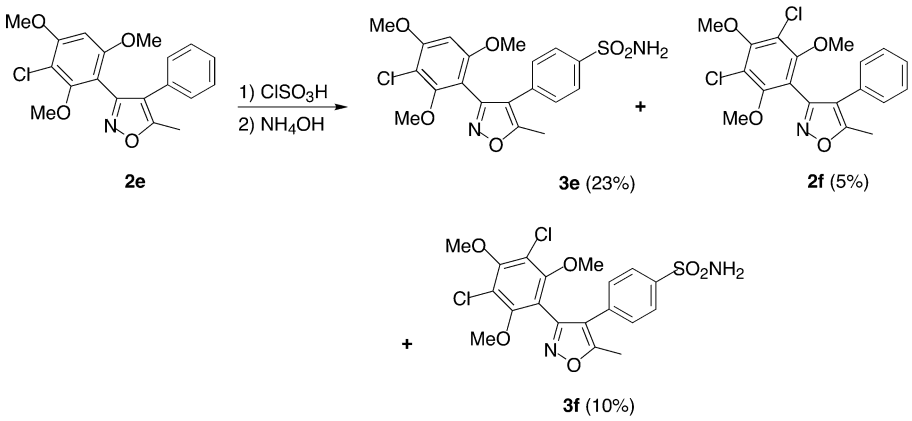
Finally, preferential or exclusive reaction of $ClSO_3H$/NH_4OH at the mesityl ring is observed in the case of 3-mesityl-5-methyl-4-phenylisoxazole **2b**. In this case, in fact, variable amounts of sulfonamides **3b** and **4** were isolated (Scheme 6 and Table 5). And this is once again the result of the activation of such a ring by methyl groups, associated, however, with lower steric effects compared to **2e**, as can be deduced by comparing some reported steric parameters (v or V^a constants) of Me and OMe groups.[45]

Pharmacology

To investigate the effect of valdecoxib molecular structure modification on COX-1/COX-2 inhibitory activity, we tested the synthesized isoxazoles by the human whole blood assays, as summarized in the Table 6.

We assessed the inhibitory effects of test compounds on lipopolysaccharide (LPS) stimulated whole blood

Scheme 5



Scheme 6

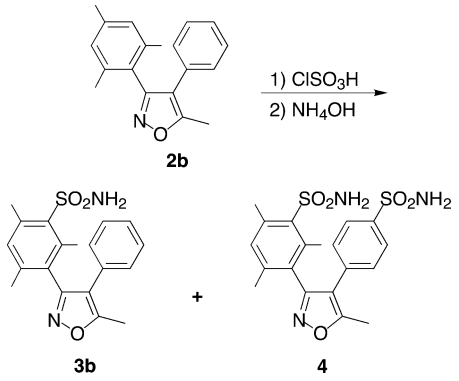


Table 5. Yields of Compounds **3b** and **4** Formed in the Reaction Depicted in the Scheme 6

	yield (%)	
isoxazole/$ClSO_3H$	**3b**	**4**
1:8	44	16
1:11	–	60

[a] Yields refer to the products actually isolated by chromatography.

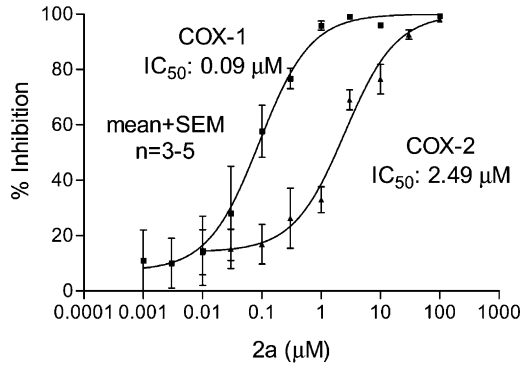


Figure 4. Concentration-response curves for the inhibition of whole blood COX-1 and COX-2 activities by **2a**. Increasing concentrations of **2a** (0.01−100 μM) were incubated with 1 mL of heparinized whole blood samples, drawn from healthy volunteers pretreated with 300 mg of aspirin 48 h before sampling, in the presence of LPS (10 μg/mL) for 24 h, and plasma PGE_2 levels were assayed as a reflection of monocyte COX-2 activity (▲). **2a** (0.001−100 μM) was also incubated with 1 mL of whole blood samples (drawn from healthy subjects when they had not taken any NSAID during the 2 weeks preceding the study) allowed to clot for 1 h, and serum TXB_2 levels were measured as a reflection of platelet COX-1 activity (■). Results are depicted as percentage inhibition from three to five separate experiments.

PGE_2 production and thrombin stimulated whole blood TXB_2 production, indexes of monocyte COX-2 and platelet COX-1 activity, respectively.[34−36] LPS-stimulated whole blood samples, drawn from healthy subjects treated with 300 mg of aspirin 48 h before sampling, produced 33 ± 5.8 ng of PGE_2 per mL of plasma (mean ± SEM, $n = 13$). TXB_2 production in clotting whole blood samples, obtained from the same subjects in aspirin-free periods, averaged 466 ± 53 ng/mL (mean ± SEM, $n = 13$).

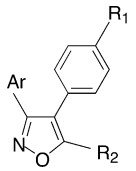
As shown in Figures 4, 5, 6, and 7, **2a**,[46,47] **2c**, **2g**,[46,48] and valdecoxib (**3a**) inhibited LPS-induced monocyte COX-2 and thrombin-stimulated platelet COX-1 activities in a concentration-dependent fashion. The IC_{50} values for inhibition of platelet COX-1 and monocyte COX-2 activities are detailed in Table 6.

Valdecoxib (**3a**) inhibited monocyte COX-2 and platelet COX-1 activities with the following IC_{50} (95% confidence interval) values, i.e., 27 (16.22−44.63) and 0.57 (0.4619−0.7056) μM, respectively, similar to previously published results.[49]

The analysis of the sigmoidal concentration-response curves for inhibition of monocyte COX-2 and platelet COX-1 by **2c** showed that virtually complete suppression (>90%) of platelet COX-1 activity occurred at concentrations that did not affect monocyte COX-2 activity (Figure 5). Similar results were obtained with **2a** and **2g** (Figures 4 and 6). Interestingly, removal of the sulfonamide moiety gave compounds **2g**, **2a**, and **2c**, which inhibited platelet COX-1 activity with IC_{50} values that were 540-, 300-, and 54-fold lower than that of valdecoxib (**3a**) (used as active control), respectively, while inhibiting monocyte COX-2 activity with IC_{50} values that were 2.6-, 4.3-, and >175-fold higher than that of valdecoxib (Table 6); thus, they shared a reversal COX-2 selectivity (**2g**, **2a**, and **2c**: COX-2/COX-1 IC_{50} ratios of 27.7, 30, and >200, respectively). The other synthesized compounds showed IC_{50} values toward monocyte COX-2 and platelet COX-1 activities that were >100 μM.

These results confirm that (a) within the 3,4-diarylisoxazole class (valdecoxib and its analogues) of COX-2 inhibitors, the *p*-sulfamoylphenyl group is essential for good COX-2 inhibitory potency, (b) lacking the sulfonamide moiety reverses the COX-2 selectivity, (c) the reduced side pocket volume of COX-1 is not the only source of diarylheterocycle COX-2 inhibitors selectiv-

Table 6. Inhibitory Activity of Valdecoxib Analogues by Human Whole Blood Assays in Vitro[a]



compd	Ar	R_1	R_2	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)
2a	phenyl	H	CH_3	0.090[b] (0.05−0.144)	2.49[c] (1.577−3.954)
2g	phenyl	H	CH_3CH_2	0.05[b] (0.028−0.097)	1.49[d] (0.812−2.768)
2b	mesityl-	H	CH_3	>100	>100
2c	5-chloro-2-furyl	H	CH_3	0.5[b] (0.2641−0.9248)	>100[b]
2d	2,4,6-trimethoxyphenyl	H	CH_3	>100	>100
2e	3-chloro-2,4,6-trimethoxyphenyl	H	CH_3	>100	>100
valdecoxib **(3a)**	phenyl	SO_2NH_2	CH_3	27[e] (16.22−44.63)	0.57[f] (0.4619−0.7056)
3b	2,4,6-trimethyl-3-benzenesulfonamide	H	CH_3	>100	>100
3c	5-chloro-2-furyl	SO_2NH_2	CH_3	>100	>100
3e	3-chloro-2,4,6-trimethoxyphenyl	SO_2NH_2	CH_3	>100	>100
4	2,4,6-trimethyl-3-benzenesulfonamide	SO_2NH_2	CH_3	>100	>100

[a] Values are the mean of at least two measurements. Values in parentheses are the IC_{50} confidence interval. [b] $n = 3$. [c] $n = 5$. [d] $n = 4$. [e] $n = 11$. [f] $n = 13$.

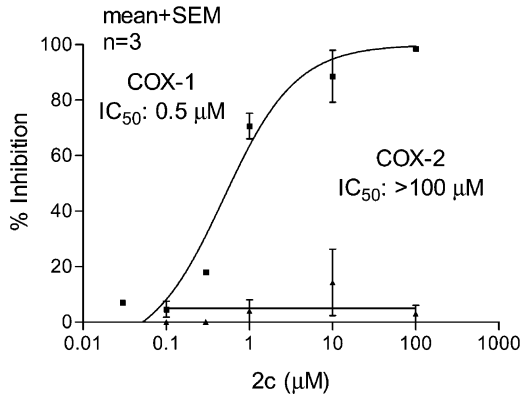


Figure 5. Concentration-response curves for the inhibition of whole blood COX-1 and COX-2 activities by **2c**. Increasing concentrations of **2c** (0.1−100 μM) were incubated with 1 mL of heparinized whole blood samples, drawn from healthy volunteers pretreated with 300 mg of aspirin 48 h before sampling, in the presence of LPS (10 μg/mL) for 24 h, and plasma PGE_2 levels were assayed as a reflection of monocyte COX-2 activity (▲). **2c** (0.03−100 μM) was also incubated with 1 mL of whole blood samples (drawn from healthy subjects when they had not taken any NSAID during the 2 weeks preceding the study) allowed to clot for 1 h, and serum TXB_2 levels were measured as a reflection of platelet COX-1 activity (■). Results are depicted as percentage inhibition from 11 to 13 separate experiments.

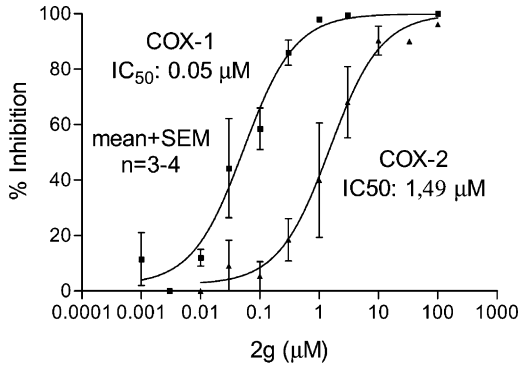


Figure 6. Concentration-response curves for the inhibition of whole blood COX-1 and COX-2 activities by **2g**. Increasing concentrations of **2g** (0.01−100 μM) were incubated with 1 mL of heparinized whole blood samples, drawn from healthy volunteers pretreated with 300 mg of aspirin 48 h before sampling, in the presence of LPS (10 μg/mL) for 24 h, and plasma PGE_2 levels were assayed as a reflection of monocyte COX-2 activity (▲). **2g** (0.001−100 μM) were also incubated with 1 mL of whole blood samples (drawn from healthy subjects when they had not taken any NSAID during the 2 weeks preceding the study) allowed to clot for 1 h, and serum TXB_2 levels were measured as a reflection of platelet COX-1 activity (■). Results are depicted as percentage inhibition from three to four separate experiments.

ity,[50] and (d) the striking feature of **2a**, **2g**, and in particular **2c** is their activity against COX-1.

In conclusion, a series of 3,4-diarylisoxazole analogues of valdecoxib were synthesized in good overall yields. Reversal COX-2 selectivity was obtained in **2a, 2c**, and **2g** compounds by removal of the sulfonamide group. The valdecoxib analogue **2c** characterized by a high selectivity toward COX-1 may represent an adequate tool to study the involvement of COX-1 in cancer development and pain.[32,33,51−55]

Experimental Section

General Methods. Melting points taken on an Electrothermal apparatus were uncorrected. ^1H NMR spectra were recorded on a Mercury 300 MHz spectrometer, and chemical shifts are reported in parts per million (δ). Absolute values of the coupling constant are reported. IR spectra were recorded on a Perkin-Elmer 681 spectrometer. GC analyses were performed by using an HP1 column (methyl siloxane; 30 m × 0.32 mm × 0.25 μm film thickness) on a HP 6890 model, series II. Thin-layer chromatography (TLC) was performed on silica gel sheets with fluorescent indicator, the spots on the TLC were observed under ultraviolet light or were visualized with I_2 vapor. Chromatography was conducted by using silica gel 60 with a particle size distribution of 40−63 μm and 230−400 ASTM. GC−MS analyses were performed on an HP 5995C model, and microanalyses were performed on an elemental analyzer 1106 (Carlo Erba instrument). ESI-MS analyses were performed on an Agilent 1100 LC/MSD trap system VL.

Materials. DMF from a commercial source was purified by distillation from CaH_2 under reduced pressure. Tetrahydrofuran (THF) from a commercial source was purified by

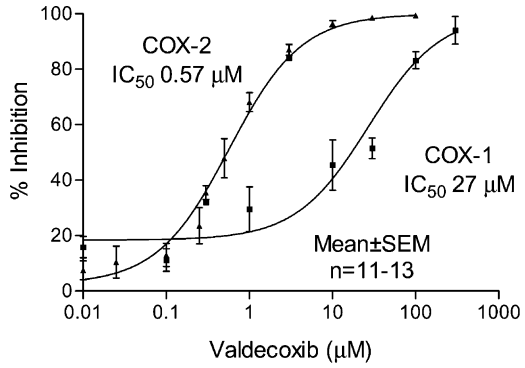


Figure 7. Concentration-response curves for the inhibition of whole blood COX-1 and COX-2 activities by valdecoxib **(3a)**. Increasing concentrations of valdecoxib (**3a**) (0.01−100 μM) were incubated with 1 mL of heparinized whole blood samples, drawn from healthy volunteers pretreated with 300 mg of aspirin 48 h before sampling, in the presence of LPS (10 μg/mL) for 24 h, and plasma PGE_2 levels were assayed as a reflection of monocyte COX-2 activity (▲). Valdecoxib (**3a**) (0.01−300 μM) was also incubated with 1 mL of whole blood samples (drawn from healthy subjects when they had not taken any NSAID during the 2 weeks preceding the study) allowed to clot for 1 h, and serum TXB_2 levels were measured as a reflection of platelet COX-1 activity (■). Results are depicted as percentage inhibition from 11 to 13 separate experiments.

distillation (twice) from sodium wire under nitrogen. Standardized (2.5 M) *n*-butyllithium in hexane was purchased from Aldrich Chemical Co., and its titration was performed by *N*-pivaloyl-*o*-toluidine.[56] Benzonitrile oxide[41] and 5-chloro-2-furylnitrile oxide were prepared from aldehydes through their conversion into the corresponding oximes and then into benzohydroximinoyl chlorides. These were finally converted into nitrile oxides by treatment with NEt_3. All other chemicals and solvents were commercial grade further purified by distillation or crystallization prior to use.

5-Chloro-2-furancarbohydroximoyl Chloride. 2-Furancarbaldehydoxime[57] (0.500 g, 4.505 mmol) dissolved in anhydrous DMF (5 mL), contained in a round-bottom flask equipped with magnetic stirrer, was cooled to 0 °C. NCS (1.203 g, 9.009 mmol) was slowly added, and the obtained suspension was stirred for 5 h to room temperature. Then, Et_2O was added and the solution was washed three times with water to remove DMF. The combined organic extracts were dried over anhydrous Na_2SO_4, and then the solvent was evaporated under vacuum. The residue was dissolved in ethyl acetate. A pale-yellow solid formed in 75% yield by slow addition of petroleum ether (ethyl acetate/petroleum ether = 1:1). Mp 82−83 °C. FT-IR (KBr): 3550−3200, 3130, 2924, 2851, 1622, 1498, 1433, 1353, 1278, 1214, 1154, 1041, 1021, 1000, 939, 874, 799 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 6.27−6.29 (d, 1H, J = 3.50 Hz), 6.82−6.84 (d, 1H, J = 3.50 Hz), 8.40−10.30 (bs, 1H, OH, exchange with D_2O). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 145.03, 139.95, 130.22, 116.07, 108.92.

3-Chloro-2,4,6-trimethoxybenzaldoxime. In a round-bottom flask equipped with magnetic stirrer the 2,4,6-trimethoxybenzaldoxime (0.300 g, 1.422 mmol) was dissolved in anhydrous DMF (5 mL), then the suspension was cooled to 0 °C. NCS (0.189 g, 1.422 mmol) was slowly added, and the suspension was stirred for 3 h to 0 °C. Then, water was added, and the reaction mixture became a limpid yellow solution. The two phases were separated, and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic extracts were washed with water to remove succinimide, then the solution was dried over anhydrous Na_2SO_4. A pale-yellow solid was obtained in 88% yield after evaporation of the solvent under vacuum. Mp 186−190 °C (lit.[58] 192 °C). FT-IR (KBr): 3500−3200, 3020, 2985, 2950, 1607, 1580, 1465, 1450, 1432, 1410, 1335, 1232, 1210, 1165, 1130, 1046, 1021, 948, 910, 812 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 3.84 (s, 3H), 3.92 (s, 3H), 3.94 (s, 3H), 6.36 (s, 1H, aromatic proton), 8.40 (s, 1H), 10.00−10.60 (bs, 1H, OH, exchange with D_2O). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 56.22, 56.58, 61.79, 92.68, 108.73, 109.33, 143.66, 156.99, 157.48, 158.01. GC−MS (70 eV) *m*/*z* (rel int): 247 [$M(^{37}Cl)^+$, 12], 245 [$M(^{35}Cl)^+$, 36], 230 (16), 229 (28), 228 (47), 227 (62), 215 (35), 214 (15), 213 (100), 212 (10), 186 (12), 185 (22), 184 (25), 179 (12), 178 (96), 171 (12), 170 (15), 169 (11), 163 (16), 155 (17), 143 (10), 69 (9).

2,4,6-Trimethoxybenzonitrile Oxide and 3-Chloro-2,4,6-trimethoxybenzonitrile Oxide. To a stirred and cooled to 0 °C solution of 2,4,6-trimethoxybenzaldoxime (0.112 g, 0.53 mmol) dissolved in $CHCl_3$ (5 mL), pyridine (0.027 mL) was added. NCS (0.0706 g, 0.53 mmol) was then slowly added in the round-bottom flask, and the turbid reaction mixture was stirred for 2 h at room temperature. Then, water was added, and the reaction mixture became a limpid yellow solution. The two phases were separated, and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic extracts were washed with water to remove succinimide, then the solution was dried over anhydrous Na_2SO_4, and then the solvent evaporated under vacuum. The reaction crude was crystallized from hexane, affording the product in 70% yield. 3-Chloro-2,4,6-trimethoxybenzonitrile oxide was prepared in quantitative yield from 2,4,6-trimethoxybenzaldoxime by using 2 equiv of NCS.

2,4,6-Trimethoxybenzonitrile Oxide. Quantitative yield. Mp 200−210 °C (hexane; dec 135 °C), yellow powder. FT-IR (KBr): 3060, 2947, 2840, 2306, 1607, 1466, 1334, 1211, 1158, 1129, 1063, 1032, 962, 917, 847, 804, 769, 687 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 3.83 (s, 6H), 3.84 (s, 3H), 6.05 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 55.88, 56.14, 56.21, 84.58, 90.72, 160.25, 162.56, 163.65, 163.97. GC−MS (70 eV) *m*/*z* (rel int): 209 (100), 194 (11), 180 (8), 179(8), 166 (45), 151 (10), 123 (8), 69 (8). All these data are identical to those of the commercially available compound.

3-Chloro-2,4,6-trimethoxybenzonitrile Oxide. Quantitative yield. Mp 124.6−129.8 °C (hexane, dec 117 °C), yellow powder. FT-IR (KBr): 3023, 2986, 2949, 2847, 2305, 1609, 1583, 1468, 1454, 1436, 1414, 1334, 1231, 1210, 1162, 1131, 1043, 1023, 949, 915, 811 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 3.90 (s, 3H), 3.94 (s, 3H), 3.96 (s, 3H), 6.27 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 56.25, 56.55, 61.78, 90.79, 91.69, 108.66, 158.80, 159.51, 161.48. GC−MS (70 eV) *m*/*z* (rel int): 245 [$M(^{37}Cl)^+$, 33], 243 [$M(^{35}Cl)^+$, 100], 228 (19), 202 (14), 200 (40), 187 (10), 185 (29), 69 (5). Anal. ($C_{10}H_{10}ClNO_4$) C, H, N.

General Procedure for the Synthesis of 3-Aryl-5-hydroxy-5-methyl-4-phenyl-2-isoxazolines 1a−e by Reaction of Arylnitrile Oxides with Enolate Ion of Phenylacetone (Table 1). A 2.28 M solution of *n*-butyllithium in hexane (1.86 mL, 4.26 mmol) was added to diisopropylamine (0.596 mL, 4.26 mmol) in THF (20 mL) at 0 °C under nitrogen atmosphere, using a nitrogen-flushed, three-necked flask equipped with a magnetic stirrer, a nitrogen inlet, and two dropping funnels. After the mixture had been stirred for 15 min, phenylacetone (0.518 mL, 3.87 mmol) in THF (20 mL) was dropwise added. The yellow reaction mixture was stirred at 0 °C for 1 h, then a solution of arylnitrile oxide (3.87 mmol) in THF (20 mL) was added. The reaction mixture was allowed to reach room temperature and was stirred overnight, with the exception of **1b** that was stirred for 3 h. The reaction was quenched by adding aqueous NH_4Cl solution. The reaction products were extracted three times with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4 and then was evaporated under vacuum. Column chromatography (silica gel, petroleum ether/ethyl acetate = 8:2; the ratio of the two solvents was 6:4 for **1d** and **1e**) of the residue affords the 3-aryl-5-hydroxy-5-methyl-4-phenyl-2-isoxazolines in 29−61% yield (Table 1).

5-Hydroxy-5-methyl-3,4-diphenyl-2-isoxazoline (1a).[38] 61% yield. Diastereoisomeric ratio cis/trans = 80:20. Mp 163−165 °C, white powder. FT-IR (KBr): 3484 (very strong broad band), 3064, 3029, 3005, 2992, 2941, 1561, 1496, 1444, 1385, 1356, 1235, 1147, 1094, 902, 797, 699 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 1.28 (s, 3H, major isomer), 1.77 (s, 3H, minor stereoisomer),

2.66 (bs, 1H, OH, exchanges with D_2O, minor stereoisomer), 3.26 (bs, 1H, OH, exchanges with D_2O, major stereoisomer), 4.46 (s, 1H, minor stereoisomer), 4.52 (s, 1H, major stereoisomer), 7.14−7.64 (m, 10H, aromatic protons, 5H for each stereoisomer). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 22.38, 63.35, 109.51, 127.26, 127.57, 128.34, 128.57, 128.85, 129.12, 129.46, 130.36, 134.85, 160.90. GC−MS (70 eV) *m/z* (rel int): 253 (M^+, 1), 235 (24), 220 (7), 194 (31), 193 (100), 192 (26), 178 (7), 165 (27), 152 (3), 134 (6), 116 (9), 103 (6), 91 (10), 90 (15), 89 (21), 77 (13), 63 (5), 51(3), 43 (9). Anal. ($C_{16}H_{15}NO_2$) C, H, N.

5-Hydroxy-3-(2,4,6-trimethylphenyl)-5-methyl-4-phenyl-2-isoxazoline (1b). 29% yield. Diastereoisomeric ratio cis/trans = 75:25. Oil. FT-IR (neat): 3383 (very strong broad band), 3020, 2922, 2852, 1612, 1495, 1455, 1383, 1312, 1264, 1216, 1136, 1095, 1034, 931, 851, 813, 758 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 1.45 (s, 3H, major isomer), 1.80 (s, 3H, minor isomer), 2.20 (s, 3H for each stereoisomer), 2.25 (s, 3H for each stereoisomer), 2.32 (s, 3H for each stereoisomer), 2.70−2.80 (bs, 1H, OH, exchanges with D_2O, minor isomer), 3.00−3.10 (bs, 1H, OH, exchanges with D_2O, major isomer), 4.35 (s, 1H, major isomer), 4.65 (s, 1H, minor isomer), 6.77−6.84 (s, 2H aromatic protons for each stereoisomer), 7.10−7.40 (m, 5H aromatic protons for each stereoisomer). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 20.64, 21.12, 21.18, 22.05, 66.87, 108.66, 128.39, 128.64, 128.80, 129.05, 129.24, 129.65, 129.84, 137.56, 138.82, 162.32, 163.23. GC−MS (70 eV) *m/z* (rel int): 295 (M^+, 4), 279 (6), 278 (27), 277 (44), 262 (11), 236 (9), 235 (38), 234 (100), 220 (13), 219 (11), 218 (13), 204 (10), 191 (4), 178 (3), 158 (15), 145 (12), 134 (19), 130 (19), 115 (11), 103 (8), 91 (29), 89 (9), 77 (12), 43 (10). Anal. ($C_{19}H_{21}NO_2$) C, H, N.

3-(5-Chlorofuran-2-yl)-5-hydroxy-5-methyl-4-phenyl-2-isoxazoline (1c). 45% yield. Diastereoisomeric ratio cis/trans = 80:20. Mp 160.0−161.6 °C, white powder. FT-IR (KBr): 3495 (strong broad band), 3120, 3085, 3029, 3006, 2960, 1610, 1547, 1489, 1454, 1385, 1377, 1235, 1207, 1152, 1064, 1016, 955, 908, 889, 790, 752, 700 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 1.27 (s, 3H, major isomer), 1.60−1.73 (bs, 1H, OH, exchanges with D_2O, minor isomer), 1.75 (s, 3H, minor isomer), 3.26−3.40 (bs, 1H, OH, exchange with D_2O, major isomer), 4.34 (s, 1H, minor isomer), 4.39 (s, 1H, major isomer), 6.08−6.13 (m, 3H, one furyl proton of the major isomer and two furyl protons of the minor isomer), 6.43−6.44 (d, J = 3.50 Hz, 1H, furyl proton of the major isomer), 7.11−7.41 (m, 10H, five aromatic protons for each stereoisomer). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 22.10, 63.06, 108.69, 109.36, 115.39, 128.42, 128.55, 129.42, 134.35, 139.52, 144.09, 152.57. GC−MS (70 eV) *m/z* (rel int): 279 [$M(^{37}Cl)^+$, 7], 277 [$M(^{35}Cl)^+$, 20], 260 (13), 259 (9), 219 (14), 218 (22), 217 (29), 216 (14), 190 (7), 188 (16), 182 (12), 155 (19), 154 (100), 139 (11), 127 (17), 115 (10), 102 (12), 91 (11), 89 (13), 77 (10), 63 (7), 43 (18). Anal. ($C_{14}H_{12}NO_3Cl$) C, H, N.

5-Hydroxy-3-(2,4,6-trimethoxyphenyl)-5-methyl-4-phenyl-2-isoxazoline (1d). 52% yield. Diastereomeric ratio cis/trans = 70:30. Mp 147−151 °C (dec 143 °C), white solid. FT-IR (KBr): 3461 (strong broad band), 3100, 3084, 2940, 1592, 1435, 1396, 1345, 1213, 1113, 917, 891, 722 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 1.23 (s, 3H, major isomer), 1.76 (s, 3H, minor isomer), 2.90−3.10 (bs, 1H, OH, exchanges with D_2O, minor isomer), 3.50−3.70 (bs, 1H, OH, exchanges with D_2O, major isomer), 3.72−3.75 (m, 18H, OCH_3, nine protons for each stereoisomer), 4.66 (s, 1H, major isomer), 4.74 (s, 1H, minor isomer), 6.01 (s, 2H, aromatic protons, minor isomer), 6.04 (s, 2H, aromatic protons, major isomer), 7.12−7.27 (m, 10H, five aromatic protons for each stereoisomer). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 21.71 (major isomer), 25.46 (minor isomer), 55.51 (minor isomer), 56.17 (major isomer), 64.24 (minor isomer), 66.23 (major isomer), 91.09, 100.49, 106.58, 108.57, 127.83, 128.58, 129.18, 129.47, 133.43, 134.86, 156.96, 157.15, 159.71, 159.88, 162.62. GC−MS (70 eV) *m/z* (rel int): 343 (M^+, 7), 326 (24), 325 (100), 310 (20), 284 (11), 283 (48), 282 (27), 268 (19), 266 (9), 253 (8), 193 (25), 179 (8), 168 (8), 115 (13), 91 (17), 77 (12), 69 (11), 43 (17). Anal. ($C_{19}H_{21}NO_5$) C, H, N.

5-Hydroxy-3-(2,4,6-trimethoxy-3-chlorophenyl)-5-methyl-4-phenyl-2-isoxazoline (1e). 60% yield. Diastereomeric ratio cis/trans = 70:30. Mp 139−147 °C, white solid. FT-IR (KBr): 3466 (strong broad band), 3082, 2940, 2847, 1592, 1453, 1435, 1396, 1345, 1310, 1213, 1113, 917, 891, 807, 731 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 1.25 (s, 3H, major isomer), 1.77 (s, 3H, minor isomer), 2.95−3.08 (bs, 1H, OH, exchanges with D_2O, minor isomer), 3.45−3.55 (bs, 1H, OH, exchanges with D_2O, major isomer), 3.71 (s, 3H, minor isomer), 3.80 (s, 3H, major isomer), 3.83 (s, 3H, minor isomer), 3.84 (s, 3H, major isomer), 3.92 (s, 3H, major isomer), 3.95 (s, 3H, minor isomer), 4.71 (s, 2H, one for each stereoisomer), 6.18 (s, 1H, aromatic proton of the minor isomer), 6.25 (s, 1H, aromatic proton of the major isomer), 7.13−7.28 (m, 10H, five aromatic protons for each stereoisomer). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 22.22 (major isomer), 25.55 (minor isomer), 56.53, 62.79, 64.00 (minor isomer), 65.65 (major isomer), 92.65, 92.91, 107.04, 107.17, 108.72, 109.36, 128.01, 128.13, 128.80 129.12, 129.49, 132.80, 134.53, 156.59, 156.77, 157.47, 157.67, 157.78. GC−MS (70 eV) *m/z* (rel int): 379 [$M(^{37}Cl)^+$, 2], 377 [$M(^{35}Cl)^+$, 6], 361 (36), 360 (24), 359 (100), 346 (12), 344 (19), 319 (19), 318 (17), 317 (53), 316 (18), 304 (14), 302 (35), 287 (18), 272 (8), 227 (12), 178 (11), 134 (9), 115 (12), 103 (11), 91 (17), 77 (9), 43 (18). Anal. ($C_{19}H_{20}NO_5Cl$) C, H, N.

General Procedure for the Synthesis of Isoxazoles 2. A solution of Na_2CO_3 (2.24 mmol) in water (10 mL) was added to 3-aryl-5-hydroxy-5-methyl-4-phenyl-2-isoxazoline (1.12 mmol) in THF (10 mL). The reaction mixture was then heated under reflux (reaction time reported in Table 3). The two phases were separated, and the aqueous layer was extracted three times with ethyl acetate. The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure, affording the isoxazoles in 50−80% yield (Table 3).

5-Methyl-3,4-diphenylisoxazole (2a).[46,47] 80% yield. Mp 97−98 °C (hexane), white crystals. FT-IR (KBr): 3051, 2928, 1619, 1597, 1573, 1497, 1464, 1436, 1414, 1376, 1304, 1239, 1074, 915, 769, 696 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 2.45 (s, 3H), 7.17−7.47 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 11.82, 116.01, 127.90, 128.69, 128.95, 129.38, 129.60, 130.05, 130.61, 161.39, 166.85. GC−MS (70 eV) *m/z* (rel int): 235 (M^+, 100), 220 (28), 194 (14), 193 (90), 192 (37), 165 (28), 103 (10), 90 (12), 89 (62), 78 (10), 77 (24), 63 (23), 51 (48), 43 (70). Anal. ($C_{16}H_{13}NO$) C, H, N.

3-(2,4,6-Trimethylphenyl)-5-methyl-4-phenylisoxazole (2b).[59] 50% yield (Table 3), 73% yield if **2b** is prepared in one step, namely, directly from the mesitylnitrile oxide without the isolation of **1b** (Table 2). Mp 92−93 °C, yellow crystals. FT-IR (KBr): 3061, 3038, 2974, 2919, 2856, 1614, 1495, 1440, 1396, 1235, 1015, 968, 883, 850, 774, 695 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 2.03 (s, 6H), 2.28 (s, 3H), 2.58 (s, 3H), 6.86 (s, 2H), 7.01−7.04 (m, 2H, aromatic protons), 7.23−7.25 (m, 3H, aromatic protons). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.39, 20.30, 21.40, 116.92, 125.88, 127.39, 128.31, 128.53, 128.81, 130.59, 137.48, 138.84, 161.69, 165,76. GC−MS (70 eV) *m/z* (rel int): 277 (M^+, 76), 262 (19), 235 (23), 234 (100), 219 (14), 218 (19), 204 (11), 158 (6), 115 (8), 103 (6), 91 (11), 77 (8), 43 (6). Anal. ($C_{19}H_{19}NO$) C, H, N.

3-(5-Chloro-2-furyl)-5-methyl-4-phenylisoxazole (2c). 60% yield. Mp 71−73 °C, yellow crystals. FT-IR (KBr): 3147, 3051, 2927, 2848, 1633, 1520, 1435, 1412, 1236, 1204, 1134, 1020, 985, 940, 926, 897, 796, 775, 704 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 2.36 (s, 3H), 6.11−6.12 (d, 1H, J = 3.57 Hz), 6.25−6.27 (d, 1H, J = 3.57 Hz), 7.25−7.30 (m, 2H, aromatic protons), 7.40−7.47 (m, 3H, aromatic protons). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 11.41, 108.14, 113.87, 114.99, 128.58, 129.01, 129.63, 130.20, 138.59, 143.76, 152.42, 167.10. GC−MS (70 eV) *m/z* (rel int): 261 [$M(^{37}Cl)^+$, 5], 259 [$M(^{35}Cl)^+$, 15], 219 (11), 217 (36), 154 (17), 127 (10), 115 (5), 102 (5), 89 (14), 77 (9), 63 (10), 51 (12), 43 (100). Anal. ($C_{14}H_{10}NO_2Cl$) C, H, N.

3-(2,4,6-Trimethoxyphenyl)-5-methyl-4-phenylisoxazole (2d). 74% yield. Mp 210−217 °C (dec 137 °C), yellow powder. FT-IR (KBr): 3060, 2923, 2851, 1610, 1590, 1461, 1413, 1342, 1230, 1207, 1132, 893, 812, 779, 701 cm^{-1}. ^{1}H NMR ($CDCl_3$, δ): 2.51 (s, 3H), 3.56 (s, 6H), 3.81 (s, 3H), 6.09 (s, 2H), 7.06−7.30 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.15, 55.55, 55.95, 90.99, 100.06, 118.08, 126.99, 128.41, 128.46, 131.57,

157.00, 159.76, 162.66, 164.93. GC−MS (70 eV) *m*/*z* (int rel): 325 (M^+, 100), 310 (27), 284 (5), 283 (29), 282 (32), 268 (14), 266 (11), 253 (6), 115 (10), 103 (4), 89 (5), 77 (4), 69 (5), 43 (5). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

3-(2,4,6-Trimethoxy-3-chlorophenyl)-5-methyl-4-phenylisoxazole (2e). 75% yield. Mp 147−151 °C (dec 143 °C), yellow powder. FT-IR (KBr): 3061, 3006, 2943, 2862, 1599, 1455, 1388, 1350, 1297, 1215, 1112, 1017, 920, 895, 785, 749, 703 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 2.52 (s, 3H), 3.52 (s, 3H), 3.75 (s, 3H), 3.90 (s, 3H), 6.26 (s, 1H), 7.08−7.30 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.11, 56.10, 56.56, 62.39, 92.65, 106.93, 109.02, 118.06, 127.34, 128.57, 128.63, 130.88, 156.62, 157.72, 165.34. GC−MS (70 eV) *m*/*z* (rel int): 361 [$M(^{37}Cl)^+$, 40], 359 [$M(^{35}Cl)^+$, 100], 344 (23), 318 (10), 317 (24), 316 (18), 302 (21), 287 (11), 115 (11), 77 (5), 43 (8). Anal. ($C_{19}H_{18}NO_4Cl$) C, H, N.

3-(3,5-Dichloro-2,4,6-trimethoxyphenyl)-5-methyl-4-phenylisoxazole (2f). 5% yield. Mp 104−106 °C (dec 81 °C), yellow powder. FT-IR (KBr): 3060, 2945, 2868, 1601, 1455, 1380, 1297, 1215, 1112, 1017, 920, 894, 783, 747 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 2.56 (s, 3H), 3.67 (s, 6H), 3.93 (s, 3H), 7.13−7.34 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): 12.12, 61.11, 62.32, 117.37, 117.94, 119.83, 127.77, 128.78, 128.94, 130.01, 154.82, 155.19, 156.14, 165.93. GC−MS (70 eV) *m*/*z* (rel int): 397 [$M(^{37}Cl_2)^+$, 12], 395 [$M(^{37}Cl)^+$, 66], 393 [$M(^{35}Cl)^+$, 100], 380 (12), 378 (18), 353 (10), 351 (15), 350 (10), 338 (15), 336 (26), 321 (14), 115 (17), 103 (11), 89 (9), 77 (7), 43 (19). Anal. ($C_{19}H_{17}NO_4Cl_2$) C, H, N.

5-Ethyl-3,4-diphenylisoxazole (2g).[46,48] *n*-Butyllithium in hexane (2.19 M, 0.213 mL, 0.4675 mmol) was added to 5-methyl-3,4-diphenylisoxazole (0.100 g, 0.425 mmol) in THF (5 mL), and the mixture was kept at −78 °C under nitrogen using a nitrogen-flushed, three-necked flask equipped with a magnetic stirrer, a nitrogen inlet, and two dropping funnels. The obtained red reaction mixture was stirred for 1 h at −78 °C before addition of CH_3I (4.25 mmol). The reaction mixture was allowed to reach room temperature, and then the reaction was quenched by adding aqueous NH_4Cl solution. The two phases were separated, and the aqueous layer was extracted three times with ethyl acetate. The combined organic extracts were dried over anhydrous Na_2SO_4, and then the solvent was evaporated under vacuum. Column chromatography (silica gel, petroleum ether/ethyl acetate = 10:1) of the residue afforded the 3,4-diphenyl-5-ethylisoxazole in 75% yield. Mp 85−87 °C (hexane), white crystals. FT-IR (KBr): 3029, 3005, 2923, 2848, 1625, 1596, 1493, 1467, 1437, 1410, 1327, 1282, 1210, 1011, 905, 771, 702 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$, δ): 1.29 (t, 3H, J = 7.60 Hz), 2.78 (q, 2H, J = 7.60 Hz), 7.12−7.43 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.52, 19.67, 115.20, 127.90, 128.67, 128.93, 129.41, 129.53, 130.16, 130.66, 161.34, 171.43. GC−MS (70 eV) *m*/*z* (rel int): 249 [M^+, 100], 234 (6), 221 (18), 220 (99), 194 (9), 193 (61), 192 (46), 165 (17), 115 (7), 103 (8), 89 (53), 77 (15), 63 (10), 51 (10). Anal. ($C_{17}H_{15}NO$) C, H, N.

General Procedure for the Synthesis of Benzenesulfonamides 3. To the isoxazole cooled to 0 °C in a round-bottom flask equipped with magnetic stirrer chlorosulfonic acid (1:11) was added. The brown reaction mixture was stirred at 0 °C for 2 h and then dropwise added to a stirring suspension of ice (10 mL) and dichloromethane (10 mL). Then, the layers were separated and the organic phase was added directly to a solution of ammonium hydroxide (28% NH_3 in water) kept at 0 °C. This biphasic mixture was vigorously stirred at 0 °C for 2 h. The two phases were separated, and the aqueous layer was extracted three times with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and then evaporated under vacuum. Column chromatography (silica gel, petroleum ether/ethyl acetate = 6:4) of the residue afforded the product in the 10−90% yield (Tables 4 and 5). Analytical and spectroscopic data of valdecoxib (**3a**) were identical to those reported.[38]

2,4,6-Trimethyl-3-(5-methyl-4-phenylisoxazol-3-yl)benzenesulfonamide (3b). 44% yield. Mp 187−189 °C; white crystals. FT-IR (KBr): 3341, 3236, 3066, 2921, 2853, 1617, 1595, 1546, 1488, 1448, 1424, 1373, 1337, 1234, 1176, 1118, 1019, 886, 875, 782, 699, 665 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 2.01 (s, 3H), 2.42 (s, 3H), 2.58 (s, 3H), 2.65 (s, 3H), 4.93 (bs, 2H, NH_2, exchange with D_2O), 6.97−7.00 (m, 3H), 7.23−7.28 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, δ) 12.37, 19.61, 21.27, 23.67, 117.08, 127.85, 128.24, 129.07, 129.76, 129.85, 132.94, 137.74, 138.11, 139.44, 142.14, 161.15, 166.59. GC−MS (70 eV) *m*/*z* (rel int): 356 (M^+, 100), 341 (4), 313 (10), 275 (20), 274 (10), 260 (13), 248 (10), 234 (15), 232 (70), 231 (13), 230 (10), 218 (21), 217 (19), 216 (9), 204 (5), 189 (6), 115 (13), 103 (8), 91 (10), 77 (9), 43 (10). MS-ESI *m*/*z* (%): 355 $[M - H]^-$ (100). Anal. ($C_{19}H_{20}N_2O_3S$) C, H, N, S.

4-[3-(5-Chlorofuran-2-yl)-5-methylisoxazol-4-yl]benzensulfonamide (3c). 73% yield. Mp 145−147 °C (dec 114 °C), yellow crystals. FT-IR (KBr): 3330, 3258, 3005, 2924, 2848, 1632, 1564, 1520, 1442, 1336, 1165, 1020, 987, 896, 794, 572 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 2.40 (s, 3H), 5.25 (bs, 2H, NH_2, exchange with D_2O), 6.17−6.18 (d, 1H, J = 3.57 Hz), 6.40−6,42 (d, 1H, J = 3.57 Hz), 7.44−7.47 (m, 2H), 8.00−8.02 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 11.62, 108.36, 113.73, 114.08, 127.09, 130.89, 134.62, 138.99, 142.02, 143.09, 152.14, 167.83. GC−MS (70 eV) *m*/*z* (rel int): 340 [$M(^{37}Cl)^+$, 3], 338 [$M(^{35}Cl)^+$, 8], 296 (18), 215 (9), 152 (4), 89 (6), 43 (100). Anal. ($C_{14}H_{11}N_2O_4SCl$) C, H, N, S.

4-[3-(3-Chloro-2,4,6-trimethoxyphenyl)-5-methylisoxazol-4-yl]benzensulfonamide (3e). 23% yield. Mp 130 °C dec, white powder. FT-IR (KBr): 3406, 3295, 3003, 2942, 2850, 1606, 1574, 1523, 1497, 1457, 1436, 1388, 1339, 1217, 1190, 1168, 1110, 1015, 920, 892, 839, 815, 778, 748, 616, 565 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 2.54 (s, 3H), 3.55 (s, 3H), 3.76 (s, 3H), 3.91 (s, 3H), 5.01 (s, 2H, NH_2, exchange with D_2O), 6.27 (s, 1H), 7.22−7.26 (m, 2H), 7.78−7.81 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 12.22, 56.12, 56.60, 62.56, 92.68, 106.03, 109.13, 116.86, 126.85, 129.06, 135.84, 140.68, 156.34, 156.50, 157.54, 158.06, 166.23. GC−MS (70 eV) *m*/*z* (rel int): 440 (M^+ + 2, 37), 438 (M^+, 100), 423 (13), 396 (19), 395 (8), 301 (11), 103 (10), 77 (6), 43 (19). MS-ESI *m*/*z* (%): 437 $[M - H]^-$ (100). Anal. ($C_{19}H_{19}N_2O_6SCl$) C, H, N, S.

4-[3,5-Dichloro-2,4,6-trimethoxyphenyl)-5-methylisoxazol-4-yl]benzensulfonamide (3f). 10% yield. Mp 125 °C dec, yellow powder. FT-IR (KBr): 3405, 3265, 3005, 2918, 2850, 1581, 1451, 1388, 1341, 1293, 1261, 1236, 1165, 1095, 996, 932, 798, 750, 658.1, 612, 562 cm^{-1}. ^1H NMR ($CDCl_3$, δ): 2.57 (s, 3H), 3.70 (s, 6H), 3.94 (s, 3H), 4.84 (s, 2H, NH_2, exchange with D_2O), 7.28−7.31 (d, 2H, J = 8.32 Hz), 7.83−7.86 (d, 2H, J = 8.32 Hz). ^{13}C NMR ($CDCl_3$, δ): 12.18, 61.21, 62.51, 116.78, 120.15, 127.17, 129.40, 134.96, 141.10, 154.67, 155.52, 155.87, 166.79. GC−MS (70 eV) *m*/*z* (rel int): 476 (M^+ + 4, 15), 475 (M^+ + 3, 16), 474 (M^+ + 2, 69), 473 (M^+ + 1, 23), 472 (M^+, 100), 459 (9), 457 (12), 432 (11), 430 (15), 415 (12), 335 (15), 334 (10), 320 (13), 77 (5), 43 (31). Anal. ($C_{19}H_{18}N_2O_6Cl_2S$) C, H, N, S.

2,4,6-Trimethyl-3-[5-methyl-4-(4-sulfamoylphenyl)isoxazol-3-yl]benzenesulfonamide (4). 16% yield. Mp 123−125 °C, yellow crystal. FT-IR (KBr): 3369, 3263, 3095, 2980, 2926, 1622, 1594, 1556, 1429, 1371, 1330, 1239, 1163, 1098, 900, 841, 759, 615, 561 cm^{-1}. ^1H NMR (CD_3CN, δ): 2.04 (s, 3H), 2.39 (s, 3H), 2.60 (s, 3H), 2.65 (s, 3H), 5.69 (bs, 4H, NH_2, exchange with D_2O), 7.17 (s, 1H), 7.25−7.28 (d, 2H, J = 8.27 Hz), 7.77−7.80 (d, 2H, J = 8.27 Hz). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 11.73, 19.01, 19.74, 22.89, 115.69, 126.64, 129.01, 129.37, 132.91, 134.26, 137.68, 138.97, 139.56, 141.70, 142.48, 161.06, 168.29. MS-ESI *m*/*z* (%): 434 $[M - H]^-$ (100). Anal. ($C_{19}H_{21}N_3O_5S_2$) C, H, N, S.

Pharmacological Methods. We tested the synthesized isoxazoles by the human whole blood assays in vitro[34−36] to investigate the effect of substituents on COX-1/COX-2 inhibitory activity with respect to valdecoxib.

Human Whole Blood Assays. Subjects. Eleven healthy volunteers (six females aged 25−29 years) were enrolled to participate in the study after its approval by the ethical committee of the University "G. D'Annunzio" in Chieti. Informed consent was obtained from each subject. The same healthy volunteers were studied on different occasions.

COX-2 Assay. One milliliter aliquots of peripheral venous blood samples containing 10 IU of sodium heparin were incubated in the presence of LPS (10 μg/mL) or saline for 24 h at 37 °C as previously described. The contribution of platelet COX-1 activity was suppressed by pretreating the subjects with 300 mg of aspirin 48 h before sampling. Plasma was separated by centrifugation (10 min at 2000 rpm) and kept at −70 °C until assayed for prostaglandin (PG) E_2 as an index of LPS-induced monocyte COX-2 activity.[34]

COX-1 Assay. Peripheral venous blood samples were drawn from the same donors when they had not taken any NSAID during the 2 weeks preceding the study. One milliliter aliquots of whole blood were immediately transferred into glass tubes and allowed to clot at 37 °C for 1 h. Serum was separated by centrifugation (10 min at 3000 rpm) and kept at −70 °C until assayed for thromboxane (TX) B_2. Whole blood TXB_2 production was measured as a reflection of maximally stimulated platelet COX-1 activity in response to endogenously formed thrombin.[35,36]

Effects of the Test Compounds on Whole Blood COX-2 and COX-1 Activities. Compounds (0.005−150 mM) were dissolved in DMSO, and 2 μL aliquots of the solutions were pipetted directly into test tubes to give final concentrations of 0.01−300 μM in blood. Four to nine different concentrations of each compound were incubated with heparinized whole blood samples in the presence of LPS (10 μg/mL) for 24 h or with whole blood samples allowed to clot at 37 °C for 1 h to examine the concentration dependence of COX-2 vs COX-1 inhibition.[34,35] The actual concentrations of the compounds used for each assay are reported in the figure captions.

Analyses of PGE_2 and TXB_2. PGE_2 and TXB_2 concentrations were measured by previously described and validated radioimmunoassays.[36,60] Unextracted plasma and serum samples were diluted in the standard diluent of the assay (0.02 M phosphate buffer, pH 7.4) and assayed in a volume of 1.5 mL at a final dilution of 1:50 to 1:30000. We used 4000 dpm of [^{3}H]PGE_2 or [^{3}H]TXB_2 and specific anti-PGE_2 and anti-TXB_2 sera diluted 1:100000 and 1:120000, respectively. The least detectable concentration was 1−2 pg/mL for both prostanoids.

Materials. [^{3}H]PGE_2 and [^{3}H]TXB_2 (specific activity, >100 Ci/mmol) were from Perkin-Elmer Life Science Products (Brussels, Belgium). Authentic PGE_2 and TXB_2 were from Cayman Chemical Company (Ann Arbor, MI). Anti-PGE_2 and anti-TXB_2 sera were obtained in our laboratory, and their characteristics have been described previously. Heparin, LPS derived from *Escherichia coli* 026:B6, and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Company (St. Louis, MO).

Statistical Analysis. For each experiment, the amount of PGE_2 produced in LPS-stimulated whole blood in the presence of an inhibitor was subtracted from that produced in the presence of saline and DMSO. The effects of the test compounds were calculated and represented as percent inhibition of prostanoid production assessed in the absence of the test compounds (control). Concentration-response curves were fitted, and IC_{50} values were analyzed with PRISM (GraphPad, San Diego, CA). The IC_{50} values were reported as mean values, and 95% confidence intervals were calculated.

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Supporting Information Available: Elemental analysis data of the compounds in the manuscript. This material is available free of charge via the Internet at http://pubs.acs.org.

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