Design and Synthesis of 4,5-Diphenyl-4-isoxazolines: Novel Inhibitors of Cyclooxygenase-2 with Analgesic and Antiinflammatory Activity

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4,5-Diphenyl-4-isoxazolines (**13a**−**k**) possessing a variety of substituents (H, F, MeS, $MeSO_2$) at the para position of one of the phenyl rings were synthesized for evaluation as analgesic and selective cyclooxygenase-2 (COX-2) inhibitory antiinflammatory (AI) agents. Although the 4,5-phenyl-4-isoxazolines (**13a**−**d,f**), which do not have a C-3 Me substituent, exhibited potent analgesic and AI activities, those compounds evaluated (**13a**, **13b**, **13h**, and **13k**) were not selective inhibitors of COX-2. In contrast, 2,3-dimethyl-5-(4-methylsulfonylphenyl)-4-phenyl-4-isoxazoline (**13j**) exhibited excellent analgesic and AI activities, and it was a potent and selective COX-2 inhibitor (COX-1, $IC_{50} = 258\ \mu M$; COX-2, $IC_{50} = 0.004\ \mu M$). A related compound **13k** having a F substituent at the para position of the 4-phenyl ring was also a selective (SI = 3162) but less potent ($IC_{50} = 0.0316\ \mu M$) inhibitor of COX-2 than **13j**. A molecular modeling (docking study) for **13j** showed that the S atom of the $MeSO_2$ substituent is positioned about 6.46 Å inside the entrance to the COX-2 secondary pocket (Val^{523}) and that a C-3 Me (**13j**, **13k**) central isoxazoline ring substituent is crucial to selective inhibition of COX-2 for this class of compounds.

Introduction

The use of nonsteroidal antiinflammatory drugs (NSAIDs) for the treatment of arthritic inflammation and pain is often accompanied by adverse gastrointestinal and renal side effects. In this regard, a single cyclooxygenase (COX) enzyme, which catalyzes the bioconversion of arachidonic acid to prostaglandins and thromboxanes, was historically believed to be responsible for both the therapeutic and adverse effects exhibited by NSAIDs prior to the discovery that there are two COX isozymes, COX-1 and COX-2.[1,2] COX-2 is induced by mitogenic and proinflammatory stimuli,[3] implicating its involvement in inflammatory processes.[4] In contrast, the constitutively expressed COX-1 isozyme is believed to play a role in physiological processes such as gastroprotection and vascular homeostasis.[5] Many of the currently available NSAIDs inhibit both COX-1 and COX-2, with a preferential selectivity for COX-1 inhibition.[6] The discovery and characterization of COX-2 suggested that selective inhibition of COX-2 would provide a method to circumvent the side effects associated with NSAID therapy while retaining therapeutic antiinflammatory (AI) efficacy. Following the discovery of the original selective COX-2 inhibitors NS-398 (**1**)[7] and DuP-697 (**2**),[8] many selective COX-2 inhibitors have been subsequently reported that can be placed in three structural classes which include (i) acidic sulfonamides such as NS-398 (**1**), (ii) tricyclic compounds such as DuP-697 (**2**), celecoxib (**3**),[9] SC-588 (**4**), and rofecoxib (**5**),[10] and (iii) modification of classical NSAIDs such as the indomethacin derivative (**6**)[11] as illustrated in Figure **1**. Postmarket clinical studies attest to the efficacy of the selective COX-2 inhibitors celecoxib (**3**) and rofecoxib (**5**).[12,13]

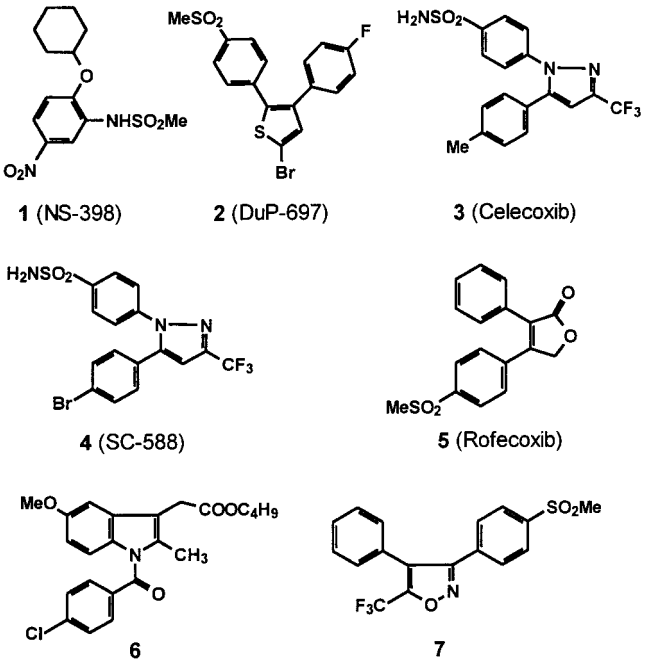

Figure 1. Representative examples of selective COX-2 inhibitors.

Recently, we reported results from a study describing the design, synthesis, and AI properties of a series of diarylisoxazoles.[14] The lead compound (**7**) in this group of compounds exhibited excellent in vitro inhibitory potency against COX-2 ($IC_{50} = 1$ nM) with no inhibition of COX-1 ($IC_{50} > 500\ \mu M$). Many compounds in this group also showed impressive activity in an in vivo model of inflammation. Incorporation of a C-5 CF_3 substituent on the central isoxazole ring enhanced selectivity toward COX-2. It was therefore of interest, as part of our ongoing program to design selective COX-2 inhibitors and acquire structure−activity cor-

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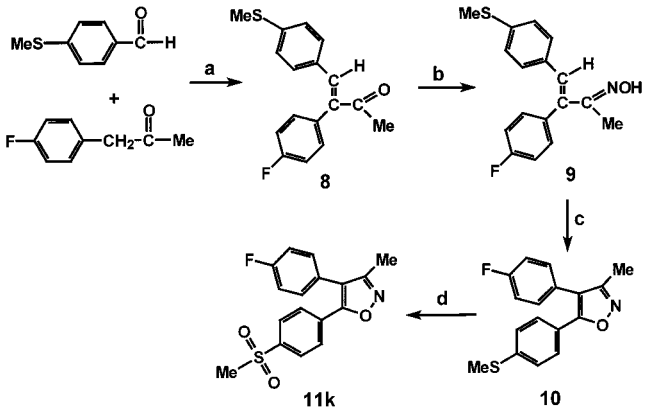
Scheme 1[a]



[a] Reagents and conditions: (a) piperidine, benzene, reflux, 24 h; (b) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, reflux, 5 h; (c) KI, I_2, $NaHCO_3$, THF, H_2O, reflux, 7 h; (d) Oxone, THF, MeOH, 25 °C, 3 h.

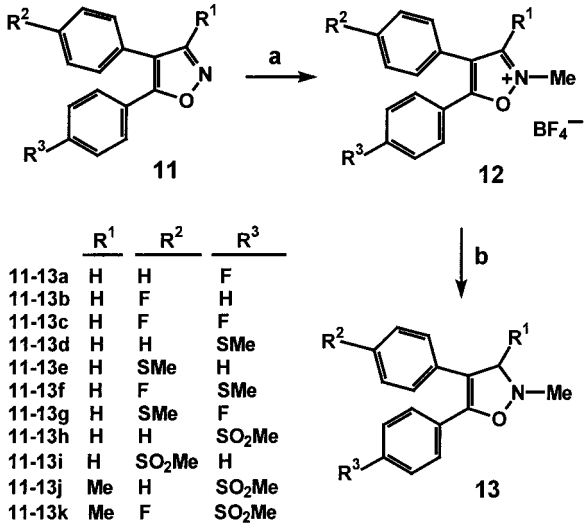
relations, to determine whether replacement of the planar aromatic central 4-isoxazole ring in the class of compounds (**7**) by a less planar nonaromatic central isoxazoline ring would retain COX-2 selectivity and in vivo AI. Accordingly, we now describe the design, synthesis, cyclooxygenase inhibitory activity, and in vivo AI activity for this class of 4,5-diphenyl-4-isoxazolines (**13a**−**k**).

Chemistry

A group of 4,5-diphenyl-2-methyl-4-isoxazolines **13a**−**k** that possess a H, F, SMe, or SO_2Me substituent at the para position of one of the phenyl rings, with or without a 3-methyl substituent, were synthesized from the corresponding isoxazoles **11a**−**k**. All isoxazoles **11** employed were prepared using methods reported previously[14−16] except for the isoxazole **11k**, which was prepared following the reaction sequence shown in Scheme 1. Thus, the α,*β*-unsaturated ketone **8**, prepared by condensation of 4-fluorophenylacetone with 4-methylthiobenzaldehyde in the presence of piperidine, was converted to the α,*β*-unsaturated oxime **9** using hydroxylamine hydrochloride. Subsequent cyclization of the oxime **9** upon treatment with iodine, potassium iodide, and sodium bicarbonate afforded the 5-(4-methylthiophenyl)isoxazole derivative **10**, and then oxidation of the SMe substituent using Oxone afforded the 5-(4-methylsulfonylphenyl)isoxazole derivative **11k**. Quaternization of the isoxazoles **11a**−**k** by reaction with $Me_3O^+\ {}^-BF_4$ yielded the corresponding 2-methylisoxazolium tetrafluoroborate salts **12a**−**k**, which upon reduction with $NaBH_4$ afforded the corresponding 4-isoxazolines **13a**−**k** in good yields (50−75%) as illustrated in Scheme 2.

Isoxazolines **13a**−**i**, having two C-3 hydrogen substituents, showed two broad ^1H NMR resonances in the *δ* 3.85−3.95 and 4.55−4.65 ranges of equal intensity for the C-3 protons that coalesced into a single broad resonance upon heating to 61 °C. Irradiation of either one of these two resonances resulted in complete saturation (disappearance) of the other resonance. In contrast, the isoxazolines **13j** and **13k** possessing a C-3 Me substituent showed a broad multiplet resembling a quartet in the *δ* 4.20−4.30 range, which appeared as sharp quartet ($J_{CH,Me}$ = 7 Hz) upon heating to 61 °C. These ^1H NMR spectral data suggest that the 4-isoxazoline ring of compounds **13** exists as a mixture of two conformations at 25 °C.

Scheme 2[a]



	R^1	R^2	R^3
11-13a	H	H	F
11-13b	H	F	H
11-13c	H	F	F
11-13d	H	H	SMe
11-13e	H	SMe	H
11-13f	H	F	SMe
11-13g	H	SMe	F
11-13h	H	H	SO_2Me
11-13i	H	SO_2Me	H
11-13j	Me	H	SO_2Me
11-13k	Me	F	SO_2Me

[a] Reagents and conditions: (a) $Me_3O^+\ {}^-BF_4$, CH_2Cl_2, N_2 atmosphere, 25 °C, 15 h; (b) $NaBH_4$, EtOH, argon atmosphere, 25 °C, 12 h.

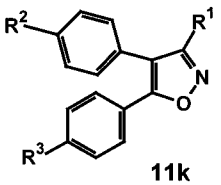
Results and Discussion

A group of 4,5-diphenyl-4-isoxazolines **13a**−**k** and the related isoxazole **11k** were prepared to investigate the effect of a H, F, MeS, or $MeSO_2$ substituent (R^2, R^3) at the para position of one of the pendant phenyl rings, in conjunction with a C-3 substituent (R^1 = H or Me), on AI and analgesic activity and COX-2 selectivity (see Table 1).

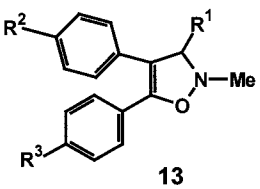
Comparison of the AI activities for **13a**−**i** at 3 h postdrug administration, determined using the carrageenan-induced rat paw edema assay, showed that the nature (H, F, MeS, $MeSO_2$) and regioisomeric location of these substituents on either the C-4 or C-5 phenyl ring (R^2 or R^3) were determinants of activity. In this regard, the relative AI activity profile for compounds having a C-4 phenyl ring (R^2 = H) with respect to the C-5 para phenyl ring subsituent (R^3) was F (**13a**) > SMe (**13d**) > inactive SO_2Me (**13h**). A similar comparison for compounds having a C-5 phenyl substituent (R^3 = H) with respect to the C-4 para phenyl substituent (R^2) showed the relative AI potency order is F (**13b**) > weakly active SMe (**13e**) > inactive SO_2Me (**13i**). The location of specific substituents at the para position of the phenyl ring for C-4 and C-5 regioisomers was also a determinant of AI activity where the relative potency order was **13a** (R^3 = F) > **13b** (R^2 = F), and **13d** (R^3 = SMe) > weakly active **13e** (R^2 = SMe). In contrast, the two regioisomers **13h** (R^2 = H, R^3 = SO_2Me) and **13i** (R^2 = SO_2Me, R^3 = H) were inactive AI agents. When two substituents (F, SMe) other than hydrogen were present at the para position of the phenyl rings, the R^3 SMe regioisomer (**13f**) provided superior AI activity relative to the weakly active R^2 SMe regioisomer (**13g**). These differences in potency for regioisomers are consistent with previous observations that para phenyl substituents in regioisomeric compounds can dramatically alter COX-2 selectivity and inhibitory potency.[17−18]

The relative analgesic activity profile for isoxazolines **13a**−**k** generally followed a potency order similar to that

Table 1. Antiinflammatory and Analgesic Activities of 4-(4-Fluorophenyl)-5-(4-methylsulfonylphenyl)-3-methylisoxazole (**11k**) and 4,5-Diphenyl-2-methyl-4-isoxazolines (**13a**−**k**)



11k



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				AI activity[a]		analgesic activity[b]					
compd	R^1	R^2	R^3	% inhibition at 3 h	% inhibition at 5 h	% inhibition at 30 min	% inhibition at 60 min.	volume[c] (Å^3)	IC_{50} [d] (μM) COX-1	IC_{50} [d] (μM) COX-2	selectivity index (COX-1/COX-2)
11k	Me	F	SO_2Me	56.4 ± 5.5[e]	16.9 ± 2.4	59.7 ± 9.9	63.8 ± 13.6	270.1	>100	>100	
13a	H	H	F	86.2 ± 4.3	70.3 ± 3.5	72.4 ± 3.0	62.0 ± 2.3	230.4	2.64	4.96	0.53
13b	H	F	H	63.6 ± 6.6	51.2 ± 3.6	70.9 ± 13.5	88.6 ± 4.8	229.8	5.86	5.38	1.08
13c	H	F	F	71.0 ± 5.1	48.7 ± 3.3	69.0 ± 12.7	52.1 ± 2.0	233.8			
13d	H	H	SMe	67.1 ± 1.9	40.5 ± 5.8	36.4 ± 8.5	48.4 ± 7.4	260.7			
13e	H	SMe	H	−3.2 ± 6.3	15.0 ± 5.4	82.2 ± 4.3	92.4 ± 0.6	260.3			
13f	H	F	SMe	53.0 ± 3.4	26.0 ± 19.4	53.0 ± 6.8	45.1 ± 6.4	264.9			
13g	H	SMe	F	−1.5 ± 2.9	12.3 ± 3.3	75.4 ± 7.9	77.9 ± 6.6	264.5			
13h	H	H	SO_2Me	7.5 ± 8.2	8.5 ± 7.8	57.9 ± 13.0	82.2 ± 0.9	271.7	>100	>100	
13i	H	SO_2Me	H	9.9 ± 4.4	02.0 ± 9.0	35.7 ± 6.6	38.8 ± 2.0	271.9	5.86	5.38	1.089
13j	Me	H	SO_2Me	50.4 ± 2.5[f]	48.1 ± 1.5	84.1 ± 1.2	66.9 ± 7.6	287.7	258.11	0.0042	61454
13k	Me	F	SO_2Me	59.6 ± 3.0[g]	69.4 ± 8.9	59.8 ± 3.0	69.4 ± 8.9	291.5	>100	0.0316	3162
ibuprofen				43.8 ± 2.8[h]	51.7 ± 3.6			212.0			
celecoxib				79.9 ± 1.9[i]	58.2 ± 1.8[j]	31.7 ± 9.6	62.0 ± 7.3	298.4	22.9	0.0567	404

[a] Inhibitory activity on carrageenan-induced rat paw edema; the result is the mean value ± SEM using four animals following a 50 mg/kg oral dose of the test compound. [b] Inhibitory activity in the rat 4% NaCl-induced abdominal constriction assay; the result is the mean value ± SEM, using four animals following a 50 mg/kg intraparitoneal dose of the test compound. [c] The volume of the molecule, after minimization using the MM3 force field, was calculated using the Alchemy 2000 program. [d] The result is the mean value of two determinations, and the deviation from the mean is <10% of the mean value. [e] ID_{50} = 49.07 mg/kg po dose. [f] ID_{50} = 44.00 mg/kg po dose. [g] ID_{50}= 41.50 mg/kg po dose. [h] 100 mg/kg po dose. [i] ID_{50} = 10.8 mg/kg po dose. [j] ID_{50} = 40.8 mg/kg po dose.

observed for AI activity with the exception of isoxazolines having a 4-(*p*-methylthiophenyl) (**13e,g**), 4-(*p*-methanesulfonyl (**13i**), or 5-(*p*-methanesulfonyl) (**13h**) substituent, which exhibited significantly more potent analgesic activity. The observation that the isoxazole **11k** is a potent AI and analgesic agent that does not inhibit either COX-1 or COX-2 suggests it acts by a different mechanism of action. Similarly, isoxazoline **13e** may also act by a different mechanism of action.

Although certain isoxazolines (**13a**,**b**) exhibited potent AI activity, no selectivity for inhibition of COX-2 was observed (see Table 1). Despite the similarity between the COX-1 and COX-2 isozyme binding sites that have virtually identical tertiary and quaternary structures, there are small structural differences between the two binding sites that can be exploited in drug design. In this regard, a single amino acid residue lining the channel that differs between COX-1 and COX-2 involves the presence of valine (Val^{523}) in COX-2 versus isoleucine (Ile^{523}) in COX-1. Accordingly, the smaller valine side chain in COX-2 induces a conformational change at Tyr^{355}, thereby forming an additional hydrophobic secondary internal pocket protruding off the primary binding site in COX-2 that is absent in COX-1.[19] Consequently, the total volume of the COX-2 primary binding site and its associated secondary pocket (394 Å^3) is about 25% larger than that of the COX-1 binding site (316 Å^3).[20] Mutagenesis experiments have also indicated that the nature of the amino acid residue at position 523 influences COX-1/COX-2 selectivity.[21]

In the tricyclic class of selective COX-2 inhibitors, an additional central ring substituent may more favorably orient the ligand within the COX-2 binding site to enhance COX-2 selectivity.[14,18] Therefore, the isoxazolines **13j** and **13k**, which possess an additional C-3 Me substituent, were prepared to determine the effect of a C-3 Me substituent on COX-2 selectivity. A previous docking study of SC-588 [14] and the current docking studies of **13h** having a C-3 H substituent and **13j** having a C-3 Me substituent on the binding site of the human COX-2 isozyme showed an intermolecular energy (between the ligand and the enzyme) of −42.44,[14] −35.46 (Figure 2), and −49.26 (Figure 3) kcal/mol, respectively. While the interspacial distance between the C atom of the N−CH_3 substituent and the *O*H group of either Tyr^{355} or Ser^{530} for isoxazolines **13h** and **13j** is similar, the ligand **13h** was inserted more deeply in the COX-2 primary binding site such that **13h** is positioned closer to Ser^{530} than **13j**. The SO_2 group in **13j**, which is inserted more deeply (6.46 Å) in the secondary pocket than the SO_2 group in **13h** (4.69 Å), forms a hydrogen bond with Phe^{518} in the secondary pocket of COX-2. This latter observation could be due to a steric interaction between the C-3 Me substituent and Arg^{120}, which is located near the mouth of the COX-2 channel that is possible for **13j** but not for **13h** having C-3 H-substituents. This explanation is consistent with the potent inhibition of COX-2 (IC_{50} = 0.004 μM) exhibited by **13j** and its high COX-2 selectivity index (SI = 61 454) relative to **13h**, which showed minimal, or no, inhibition of either COX-1 or COX-2 (IC_{50} > 100 μM) (see Table 1). Like **13j**, the C-3 Me analogue **13k** also exhibited potent COX-2 inhibition (IC_{50} = 0.0316 μM) and selectivity (SI = 3162). The effect of individual enantiomers for the racemic C-3 Me analogues **13j**−**k** that possess a chiral center at C-3, and which may therefore exhibit different COX-2 selectivity and/or potency, has not been investigated. The molecular volume of the selective COX-2 inhibitors **13j** (287.5 Å^3) and **13k** (291.5 Å^3),

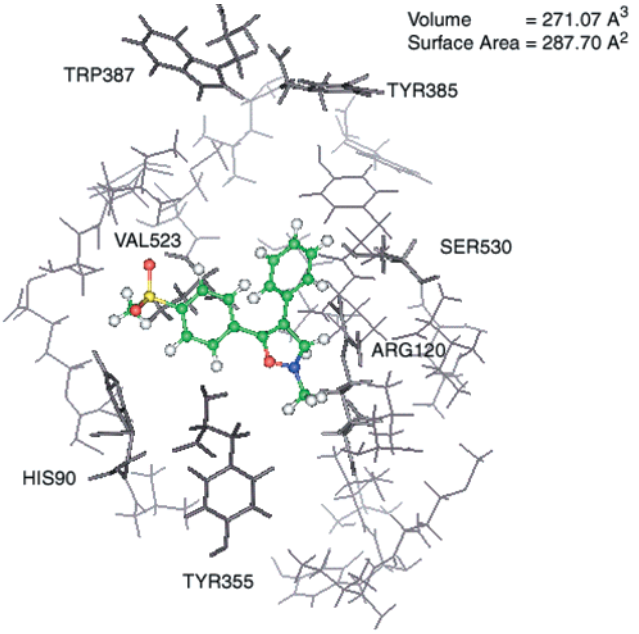

Figure 2. Docking the isoxazoline (**13h**) (ball-and-stick) in the active site of human COX-2 (line and stick) ($E_{intermolecular}$ = −35.46 kcal/mol). The central ring C atom of the N−*C*H$_3$ substituent is 9.55 Å from the phenolic *O*H of Tyr^{355} but removed from the Ser^{530} (*O*H) by 8.31 Å. The S atom of the Me*S*O$_2$ substituent is about 4.69 Å inside the entrance to the secondary pocket (Val^{523}). The center of the 4-phenyl ring is about 5.55 Å from the entrance to the secondary pocket (Val^{523}).

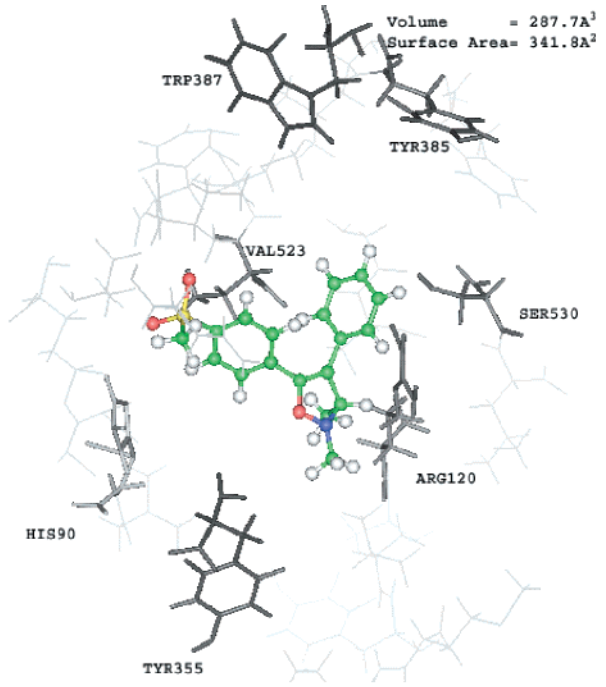

Figure 3. Docking the isoxazoline **13j** (ball-and-stick) in the active site of human COX-2 (line and stick) ($E_{intermolecular}$ = −49.26 kcal/mol). The C atom of the N−*C*H$_3$ substituent is 11.24 Å from the phenolic *O*H of Tyr355 but removed from Ser^{530} (*O*H) by 6.20 Å. The S atom of the Me*S*O$_2$ substituent is about 6.46 Å inside the entrance to the secondary pocket (Val^{523}). The center of the 4-phenyl ring is about 5.81 Å outside the entrance to the secondary pocket (Val^{523}).

which is larger than that for isoxazolines **13a**−**i** not possessing a C-3 Me substituent (230−271 Å^3 range), is similar to that of the selective COX-2 inhibitor celecoxib (298.4 Å^3) but larger than that for ibuprofen (212.0 Å^3). In this regard, differences in ligand volume as small as 15 Å^3 are known to induce significant changes in both COX-2 selectivity and potency.[16] A comparison of COX inhibitory efficacies indicates that **13k** having a C-3 Me substituent attached to an sp^3-hybridized carbon on the less planar (more puckered) 4-isoxazoline central ring is a significantly more potent and selective inhibitor of COX-2 than the structurally related isoxazole analogue **11k** having a C-3 Me substituent attached to an sp^2-hybridized carbon on the planar isoxazole central ring. The distinct difference in COX-2 inhibitory potency and selectivity between **13k** and **11k** could be due to a number of factors that include differences in the conformation of the central heterocyclic ring, the orientation of the C-3 Me substituent, and the smaller molecular volume of **11k** relative to **13k**. Furthermore, the C-3 Me compounds **13j** (ID_{50} = 44 mg/kg po dose) and **13k** (ID_{50} = 41.5 mg/kg po dose) also exhibited good AI activity relative to the selective (SI = 404) COX-2 inhibitor celecoxib (ID_{50} = 10.8 mg/kg po dose at 3 h or ID_{50} = 40.8 mg/kg po dose at 5 h, postdrug administration).

Conclusions

The results of this investigation show that (i) incorporating a central isoxazoline ring C-3 Me substituent provides a useful drug design concept to orient the central isoxazoline ring in the primary COX-2 binding site such that the para SO_2Me on the C-5 phenyl ring is suitably positioned for insertion into the COX-2 secondary pocket, (ii) a larger drug molecular volume (Å^3) closer to the molecular volume of the larger COX-2 binding site (394 Å^3) enhances COX-2 selectivity, (iii) molecular modeling (docking) suitably selected compounds in the active site of the COX-2 enzyme provides a complementary technique to optimize COX-2 selectivity, and (iv) 2,3-dimethyl-5-(4-methylsulfonylphenyl)-4-phenyl-4-isoxazoline (**13j**) is a potent and selective inhibitor of COX-2 that exhibits good AI and analgesic activities.

Experimental Section

General. Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Nicolet 550 series II Magna FT-IR spectrometer. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR, ^{19}F NMR) spectra were recorded on a Bruker AM-300 spectrometer. The assignment of changeable protons (O*H*) was confirmed by the addition of D_2O. NMR multiplicity data are denoted by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). ^{13}C NMR spectra were acquired using the *J* modulated spin-echo technique where methyl and methine carbons appear as positive peaks and methylene and quaternary carbon resonances appear as negative peaks. Microanalyses were within ±0.4% of theoretical values for all elements listed. The volume (Å^3) of compounds was calculated using an IBM computer with the Alchemy 2000 program[22] after minimization using the MM3 force field. Molecular modeling experiments were performed on an Indigo 2 R4400 SGI workstation using the Insight II software[23] modules Builder, Discover, Search & Compare and Affinity. Silica gel column chromatography was performed using Merck 7734 silica gel (70−230 mesh). Dichloromethane was dried with $CaCl_2$ just prior to distillation. Ethanol was dried using magnesium ethoxide. Isoxazoles (**11a**−**i**),[14−15] isoxazole (**11j**),[16] and celecoxib (**3**, Celebrex)[9] were prepared using methods previously reported. All other reagents were purchased from

Aldrich Chemical (Milwaukee, WI). Male Sprague−Dawley rats, used in the antiinflammatory and analgesic screens, were supplied by Animal Health Services, University of Alberta. All experiments involving animals were carried out using protocols approved by the Animal Welfare Committee, University of Alberta.

4-(4-Methylthiophenyl)-3-(4-fluorophenyl)-3-butene-2-one (8). A solution of 4-fluorophenylacetone (1.25 g, 10 mmol), 4-methylthiobenzaldehyde (1.52 g, 10 mmol), and piperidine (35 mg, 0.4 mmol) in benzene (30 mL) was heated at reflux for 24 h. Removal of the solvent in vacuo gave a residue that was purified by silica gel column chromatography using ether−hexane as eluent to afford **8** (1.77 g, 62%); mp 95−96 °C. IR (KBr): 1662 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.34 (s, 3H, C−CH_3), 2.44 (s, 3H, S−CH_3), 6.93 (d, J = 8 Hz, 2H, H_{arom}), 7.03 (d, J = 8 Hz, 2H, H_{arom}), 7.06−7.18 (m, 4H, H_{arom}), 7.60 (s, 1H, =C*H*). ^{13}C NMR ($CDCl_3$): δ 14.96 (S-*C*H_3), 25.52 (CO*C*H_3), 116.07 (d, J_{CCF} = 22 Hz, *C*CF), 125.32, 131.04, 131.26 (C_{arom}−H), 130.72, 131.02 (C_{arom}−C), 132.69 (=*C*−Ar), 139.11 (=CH−Ar), 141.10 (C_{arom}−*C*S), 162.38 (d, J_{CF} = 247 Hz, *C*−F), 198.46 (*C*O). ^{19}F NMR ($CDCl_3$): δ 47.88 (dddd, J_{FCCH}= 9.6 Hz, J_{FCCCH} = 5.6 Hz, 1F). Anal. ($C_{17}H_{15}FOS$) C, H, N.

3-(4-Fluorophenyl)-4-(4-methylthiophenyl)-3-butene-2-oxime (9). A solution of **8** (1.57 g, 5.5 mmol) in EtOH (15 mL) was added to a solution of NH_2OH·HCl (0.376 g, 5.5 mmol) and NaOAc (0.451 g, 5.5 mmol) in H_2O (7 mL), and the mixture was heated at reflux for 5 h. The reaction mixture was cooled to 25 °C and poured into water (100 mL), and the crude product was filtered. Recrystalization from EtOH−H_2O gave **9** (1.17 g, 71%); mp 197−198 °C. IR (KBr): 1716 (C=NOH) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.08 (s, 3H, C−CH_3), 2.38 (s, 3H, S−CH_3), 6.83 (d, J = 8 Hz, 2H, H_{arom}), 6.97 (d, J = 8 Hz, 2H, H_{arom}), 7.01 (s, 1H, =C*H*), 7.11−7.20 (m, 4H, H_{arom}), 11.20 (s, 1H, C=NO*H*). ^{13}C NMR (DMSO-d_6): δ 10.78 (C−*C*H_3), 14.32 (S−*C*H_3), 115.26 (d, J_{CCF} = 22 Hz, *C*CF), 125.12, 129.50, 129.88 (C_{arom}−H), 131.61 (=*C*H−Ar), 132.52, 134.77 (C_{arom}−C), 137.55 (=*C*−Ar), 138.32 (C_{arom}−*C*S), 156.52 (N=*C*), 161.23 (d, J_{CF} = 242 Hz, *C*−F). ^{19}F NMR (DMSO-d_6): δ 50.97 (dddd, J_{FCCH} = 9.1 Hz, J_{FCCCH} = 5.4 Hz, 1F). Anal. ($C_{17}H_{16}FNOS$) C, H, N.

4-(4-Fluorophenyl)-5-(4-methylthiophenyl)-3-methylisoxazole (10). A solution of KI (1.45 g, 8.75 mmol) and iodine (0.63 g, 2.5 mmol) in H_2O (5 mL) was added to a solution of **9** (0.76, 2.5 mmol) and $NaHCO_3$ (0.84 g, 10 mmol) in THF (20 mL) and water (5 mL) in a reaction flask covered by aluminum foil. The reaction mixture was heated at reflux for 7 h, saturated aqueous sodium bisulfite solution (5 mL) was added, and this mixture was extracted with EtOAc (4 × 50 mL). The combined organic extracts were dried (Na_2SO_4), the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using toluene as eluent to yield **10** (0.48 g, 60%); mp 112−114 °C. IR (KBr): 1630 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.29 (s, 3H, C−CH_3), 2.47 (s, 3H, S−CH_3), 7.08−7.19 (m, 4H, H_{arom}), 7.22−7.31 (m, 2H, H_{arom}), 7.42 (d, J = 8 Hz, 2H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 10.55 (C−*C*H_3), 15.10 (S−*C*H_3), 114.78 (C−4), 116.15 (d, J_{CCF} = 20 Hz, *C*CF), 124.05, 126.57 (C_{arom}−C), 125.78, 126.95, 131.49 (C_{arom}−H), 141.28 (C_{arom}−*C*S), 162.45 (C-3), 161.23 (d, J_{CF} = 242 Hz, *C*−F), 164.13 (C-5). ^{19}F NMR ($CDCl_3$): δ 48.40 (dddd, J_{FCCH} = 9.1 Hz, J_{FCCCH} = 5.5 Hz, 1F). Anal. ($C_{17}H_{14}FNOS$) C, H, N.

4-(4-Fluorophenyl)-5-(4-methylsulfonylphenyl)-3-methylisoxazole (11k). A solution of Oxone (1.6 g) in H_2O (6 mL) and MeOH (4 mL) was added dropwise to a solution of **10** (0.46 g, 1.53 mmol) in THF (10 mL) at 25 °C with stirring. The reaction was allowed to proceed for 3 h prior to addition of H_2O (10 mL) and extraction with CH_2Cl_2 (4 × 30 mL). The organic layer was separated and dried (Na_2SO_4), the solvent was removed in vacuo, and the residue was purified by silica gel column chromatography using CH_2Cl_2 as eluent to yield **11k** (0.41 g, 81%); mp 152−153 °C. IR (KBr): 1610 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.25 (s, 3H, C−CH_3), 3.05 (s, 3H, SO_2CH_3), 7.12−7.28 (m, 4H, H_{arom}), 7.75 (d, J = 8.5 Hz, 2H, H_{arom}), 7.90 (d, J = 8.5 Hz, 2H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 10.5 (C−*C*H_3), 44.34 (SO_2−*C*H_3), 116.55 (d, J_{CCF} = 20 Hz, *C*CF), 117.40 (C-4), 125.52, 132.51 (C_{arom}−C), 127.36, 127.79, 133.46 (C_{arom}−H), 141.19 (C_{arom}−*C*S), 160.35 (C-3), 161.22 (d, J_{CF} = 242 Hz, *C*−F), 164.44 (C-5). ^{19}F NMR ($CDCl_3$): δ 52.40 (dddd, J_{FCCH} = 9.1 Hz, J_{FCCCH} = 5.5 Hz, 1F). Anal. ($C_{17}H_{14}FNO_3S$) C, H, N.

General Method for the Preparation of 4,5-Diphenyl-2-methylisoxazolium Tetrafluoroborate Salts (12a−k). A solution of the isoxazole **11a−k** (2.2 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise to a stirred suspension of trimethyloxonium tetrafluoroborate (0.5 g, 3.3 mmol) in CH_2Cl_2 (20 mL) under a nitrogen atmosphere, and the reaction was allowed to proceed at 25 °C for 15 h. Removal of the solvent in vacuo gave an oil that was triturated with dry Et_2O (2 mL) to yield a yellow powder that was filtered and recrystallized from CH_2Cl_2 to afford the respective salt **12a−k**. Physical and spectral data for **12a−k** are listed below.

5-(4-Fluorophenyl)-2-methyl-4-phenylisoxazolium Tetrafluoroborate (12a). Yield, 98%; mp 131−132 °C. IR (KBr): 1645 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.47 (s, 3H, 2-CH_3), 7.49−7.55 (m, 7H, H_{arom}), 7.73−8.10 (m, 2H, H_{arom}), 10.00 (s, 1H, H-3). Anal. ($C_{16}H_{13}BF_5NO$) C, H, N.

4-(4-Fluorophenyl)-2-methyl-5-phenylisoxazolium Tetrafluoroborate (12b). Yield, 80%; oil. IR (neat): 1640 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.43 (s, 3H, 2-CH_3), 7.01−7.07 (m, 2H, H_{arom}), 7.26−7.46 (m, 4H, H_{arom}), 7.53−7.60 (m, 3H, H_{arom}), 9.28 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ 41.09 (2-*C*H_3), 116.50 (d, J_{CCF} = 22 Hz, *C*CF), 119.09 (C-4), 121.34, 122.93 (C_{arom}−*C*), 128.29, 129.37, 131.17, 133.31 (C_{arom}−H), 149.50 (C-3), 163.34 (d, $J_{C,F}$ = 249 Hz, *C*-F), 167.29 (*C*-5). ^{19}F NMR ($CDCl_3$): δ 10.37 (s, 4F, BF_4), 51.57 (dddd, J_{FCCH} = 9.1, J_{FCCCH} = 5.1 Hz, 1F, C_{arom}−*F*). Anal. ($C_{16}H_{13}BF_5NO$) C, H, N.

4,5-Bis(4-fluorophenyl)-2-methylisoxazolium Tetrafluoroborate (12c). Yield, 88%; mp 152−153 °C. IR (KBr): 1635 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.48 (s, 3H, 2-CH_3), 7.40−7.59 (m, 6H, H_{arom}), 7.70−7.76 (m, 2H, H_{arom}), 9.90 (s, 1H, H-3). Anal. ($C_{16}H_{12}BF_6NO$) C, H, N.

5-(4-Methylthiophenyl)-2-methyl-4-phenylisoxazolium Tetrafluoroborate (12d). Yield, 55%; mp 150−153 °C. IR (KBr): 1650 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.69 (s, 3H, S−CH_3), 4.42 (s, 3H, 2-CH_3), 7.16−7.20 (m, 2H, H_{arom}), 7.30−7.46 (m, 7H, H_{arom}), 9.28 (s, 1H, H-3). Anal. ($C_{17}H_{16}BF_4NOS$) C, H,N.

4-(4-Methylthiophenyl)-2-methyl-5-phenylisoxazolium Tetrafluoroborate (12e). Yield, 65%; mp 233−235 °C. IR (KBr): 1651 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.50 (s, 3H, SCH_3), 4.49 (s, 3H, 2-CH_3), 7.50−7.70 (m, 5H, H_{arom}), 7.72 (d, J = 8 Hz, 2H, H_{arom}), 8.19 (d, J = 8 Hz, 2H, H_{arom}), 10.03 (s, 1H, H-3). Anal. ($C_{17}H_{16}BF_4NOS$) C, H, N.

4-(4-Fluorophenyl)-5-(4-methylthiophenyl)-2-methylisoxazolium Tetrafluoroborate (12f). Yield, 72%; mp 144−146 °C. IR (KBr): 1650 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.50 (s, 3H, SCH_3), 4.50 (s, 3H, 2-CH_3), 7.07−7.13 (m, 2H, H_{arom}), 7.20−7.30 (m, 2H, H_{arom}), 7.45−7.53 (m, 4H, H_{arom}), 9.43 (s, 1H, C-3). ^{13}C NMR ($CDCl_3$): δ 14.62 (S-*C*H_3), 41.30 (N-*C*H_3), 116.70 (d, J_{CCF} = 22 Hz, *C*CF), 118.58 (C-4), 121.30, 121.40 (C_{arom}−*C*), 125.74, 128.30, 131.16 (C_{arom}−H), 147.43 (C_{arom}−*C*S), 150.01 (C-3), 163.57 (d, $J_{C,F}$ = 252 Hz, *C*−F), 167.28 (*C*-5). ^{19}F NMR ($CDCl_3$): δ 10.06 (s, 4F, BF_4), 52.06 (dddd, J_{FCCH} = 9.1, J_{FCCCH} = 5.1 Hz, 1F, C_{arom}−*F*). Anal. ($C_{17}H_{15}BF_5NOS$) C, H, N.

5-(4-Fluorophenyl)-4-(4-methylthiophenyl)-2-methylisoxazolium Tetrafluoroborate (12g). Yield, 75%; mp 235−237 °C. IR (KBr): 1648 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.50 (s, 3H, SCH_3), 4.50 (s, 3H, 2-CH_3), 7.45−7.55 (m, 2H, H_{arom}), 7.67−7.85 (m, 4H, H_{arom}), 8.20−8.27 (m, 2H, H_{arom}), 10.1 (s, 1H, C-3). Anal. ($C_{17}H_{15}BF_5NOS$) C, H, N.

5-(4-Methylsulfonylphenyl)-2-methyl-4-phenylisoxazolium Tetrafluoroborate (12h). Yield, 71%; mp 148−151 °C. IR (KBr): 1650 (C=N) cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.28 (s, 3H, SO_2CH_3), 4.45 (s, 3H, 2-CH_3), 7.51−7.59 (m, 5H, H_{arom}), 7.90 (d, J = 8 Hz, 2H, H_{arom}), 8.14 (d, J = 8 Hz, 2H, H_{arom}), 10.11 (s, 1H, H-3). Anal. ($C_{17}H_{16}BF_4NO_3S$) C, H, N.

4-(4-Methylsulfonylphenyl)-2-methyl-5-phenylisoxazolium Tetrafluoroborate (12i). Yield, 68%; mp 220−221 °C.

IR (KBr): 1650 (C═N) cm^{-1}. ^1H NMR ($CDCl_3$ + CD_3OD): δ 3.28 (s, 3H, SO_2CH_3), 4.45 (s, 3H, 2-CH_3), 7.50−7.75 (m, 5H, H_{arom}), 7.80 (d, J = 8 Hz, 2H, H_{arom}), 8.17 (d, J = 8 Hz, 2H, H_{arom}), 9.94 (s, 1H, H-3). Anal. ($C_{17}H_{16}BF_4NO_3S$) C, H, N.

2,3-Dimethyl-5-(4-methylsulfonylphenyl)-4-phenylisoxazolium Tetrafluoroborate (12j). Yield, 69%; mp 179−181 °C. IR (KBr): 1650 (C═N) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.50 (s, 3H, 3-CH_3), 3.28 (s, 3H, SO_2CH_3), 4.45 (s, 3H, 2-CH_3), 7.46−7.49 (m, 2H, H_{arom}), 7.61−7.63 (m, 3H, H_{arom}), 7.78 (d, J = 8 Hz, 2H, H_{arom}), 8.10 (d, J = 8 Hz, 2H, H_{arom}). Anal. ($C_{18}H_{18}BF_4NO_3S$) C, H, N.

2,3-Dimethyl-4-(4-fluorophenyl)-5-(4-methylsulfonylphenyl)isoxazolium Tetrafluoroborate (12k). Yield, 91%; mp 233−235 °C. IR (KBr): 1650 (C═N) cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.57 (s, 3H, 3-CH_3), 3.29 (s, 3H, SO_2CH_3), 4.46 (s, 3H, 2-CH_3), 7.44−7.57 (m, 4H, H_{arom}), 7.78 (d, J = 8 Hz, 2H, H_{arom}), 8.00 (d, J = 8 Hz, 2H, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 11.28 (3-CH_3), 38.88 (2-CH_3), 42.92 (SO_2CH_3), 116.66 (d, J_{CCF} = 22 Hz, CCF), 120.01 (C-4), 121.38, 127.27 (C_{arom}−C), 128.00, 128.69, 132.15 (C_{arom}−H), 144.09 (C−SO_2), 160.15 (C-3), 161.86 (d, J_{CF} = 250 Hz, CF), 164.60 (C-5). ^{19}F NMR (DMSO-d_6): δ 18.09 (s, 4F, BF_4), 56.20 (dddd, J_{FCCH} = 9.1, J_{FCCCH} = 5.1 Hz, 1F). Anal. ($C_{18}H_{17}BF_5NO_3S$) C, H, N.

General Method for the Preparation of 4,5-Diphenyl-2-methyl-4-isoxazolines (13a−k). A solution of the isoxazolium salt **12a−k** (0.33 mmol) in dry EtOH (5−10 mL) was added to a stirred suspension of $NaBH_4$ (110 mg, 2.9 mmol) in dry EtOH (15 mL) under an argon atmosphere at 25 °C, and the reaction was allowed to proceed for 8−12 h. The reaction was quenched by addition of a saturated aqueous NH_4Cl solution (10 mL), and the mixture was extracted with CH_2Cl_2 (5 × 20 mL). The combined organic extracts were washed with water (10 mL) and dried (Na_2SO_4), and the solvent from the organic fraction was removed in vacuo to give a brown oil that was purified by flash silica gel column chromatography using hexane−CH_2Cl_2 (1:1, v/v) as eluent to afford the respective isoxazolines **13a−k**. Physical and spectral data for **13a−k** are listed below.

5-(4-Fluorophenyl)-2-methyl-4-phenyl-4-isoxazoline (13a). Yield, 50%; oil. IR (neat) 1660 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.90 (s, 3H, 2-CH_3), 3.90−4.0 (br m, 1H total, H-3), 4.70−4.80 (br m, 1H total, H-3), 6.90−7.03 (m, 2H, H_{arom}), 7.16−7.27 (m, 5H, H_{arom}), 7.43−7.49 (m, 2H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 47.43 (2-CH_3), 65.65 (CH_2), 105.8 (C-4), 115.35 (d, J_{CCF} = 22 Hz, CCF), 126.45, 126.83, 128.41, 130.06 (C_{arom}−H), 128.80, 133.60 (C_{arom}−C), 148.10 (C-5), 164.58 (d, $J_{C,F}$ = 251 Hz, CF). ^{19}F NMR ($CDCl_3$): δ 50.81 (dddd, J_{FCCH} = 9.1, J_{FCCCH} = 5.1 Hz, 1F). Anal. ($C_{16}H_{14}FNO$) C, H, N.

4-(4-Fluorophenyl)-2-methyl-5-phenyl-4-isoxazoline (13b). Yield, 25%; oil. IR (neat): 1660 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.92 (s, 3H, 2-CH_3), 3.85−3.95 (br m, 1H total, H-3), 4.65−4.75 (br m, 1H total, H-3), 6.91−6.98 (m, 2H, H_{arom}), 7.12−7.19 (m, 2H, H_{arom}), 7.31−7.38 (m, 3H, H_{arom}), 7.43−7.50 (m, 2H, H_{arom}). Anal. ($C_{16}H_{14}FNO$) C, H, N.

4,5-Bis(4-fluorophenyl)-2-methyl-4-isoxazoline (13c). Yield, 60%; oil. IR (neat): 1660 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.92 (s, 3H, 2-CH_3), 3.85−3.95 (br m, 1H total, H-3), 4.65−4.75 (br m, 1H total, H-3), 6.91−6.97 (m, 2H, H_{arom}), 7.13−7.18 (m, 2H, H_{arom}), 7.31−7.34 (m, 2H, H_{arom}), 7.45−7.49 (m, 2H, H_{arom}). Anal. ($C_{16}H_{13}F_2NO$) C, H, N.

5-(4-Methylthiophenyl)-2-methyl-4-phenyl-4-isoxazoline (13d). Yield, 50%; oil. IR (neat): 1659 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.47 (s, 3H, S−CH_3), 2.91 (s, 3H, 2-CH_3), 3.85−3.95 (br m, 1H total, H-3), 4.65−4.75 (br m, 1H total, H-3), 7.16−7.29 (m, 7H, H_{arom}), 7.42 (d, J = 8.5 Hz, 2H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 15.27 (S-CH_3), 47.42 (2-CH_3), 65.72 (CH_2), 105.61 (C-4), 125.10, 133.53 (C_{arom}−C), 125.72, 126.38, 126.85, 128.38, 128.51 (C_{arom}−H), 140.03 (C_{arom}−CS), 147.10 (C-5). Anal. ($C_{17}H_{17}NOS$) C, H, N.

4-(4-Methylthiophenyl)-2-methyl-5-phenyl-4-isoxazoline (13e). Yield, 52%; oil. IR (neat): 1660 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.47 (s, 3H, S−CH_3), 2.91 (s, 3H, 2-CH_3), 3.85−3.95 (br m, 1H total, H-3), 4.55−4.65 (br m, 1H total, H-3), 7.13−7.35 (m, 7H, H_{arom}), 7.48−7.50 (m, 2H, H_{arom}). Anal. ($C_{17}H_{17}NOS$) C, H, N.

4-(4-Fluorophenyl)-5-(4-methylthiophenyl)-2-methyl-4-isoxazoline (13f). Yield, 50%; oil. IR (neat): 1661 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.47 (s, 3H, S−CH_3), 2.89 (s, 3H, 2-CH_3), 3.60−3.70 (br m, 1H total, H-3), 4.40−4.50 (br m, 1H total, H-3), 6.91−6.98 (m, 2H, H_{arom}), 7.13−7.18 (m, 4H, H_{arom}), 7.35 (d, J = 8.5 Hz, 2H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 15.30 (S−CH_3), 47.50 (2-CH_3), 65.86 (CH_2), 104.67 (C-4), 115.35 (d, J_{CCF} = 22 Hz, CCF), 125.83, 128.36, 128.61 (C_{arom}−H), 125.59, 129.58 (C_{arom}−C), 140.26 (C_{arom}−CS), 146.70 (C-5), 161.30 (d, $J_{C,F}$ = 251 Hz, C−F). ^{19}F NMR ($CDCl_3$): δ 46.53 (dddd, J_{FCCH} = 9.1, J_{FCCCH} = 5.1 Hz, 1F). Anal. ($C_{17}H_{16}FNOS$) C, H, N.

5-(4-Fluorophenyl)-4-(4-methylthiophenyl)-2-methyl-4-isoxazoline (13g). Yield, 52%; oil. IR (neat): 1660 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.48 (s, 3H, S−CH_3), 2.91 (s, 3H, 2-CH_3), 3.85−3.95 (br m, 1H total, H-3), 4.55−4.65 (br m, 1H total, H-3), 6.90−6.98 (m, 2H, H_{arom}), 7.13−7.18 (m, 4H, H_{arom}), 7.31−7.38 (m, 2H, H_{arom}). Anal. ($C_{17}H_{16}FNOS$) C, H, N.

5-(4-Methylsulfonylphenyl)-2-methyl-4-phenyl-4-isoxazoline (13h). Yield, 55%; oil. IR (neat): 1655 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.94 (s, 3H, 2-CH_3), 3.06 (s, 3H, SO_2CH_3), 3.95−4.10 (br m, 1H total, H-3), 4.5−4.57 (br m, 1H total, H-3), 7.19−7.33 (m, 5H, H_{arom}), 7.66 (d, J = 8 Hz, 2H, H_{arom}), 7.85 (d, J = 8 Hz, 2H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 44.33 (SO_2CH_3), 47.49 (2-CH_3), 66.17 (CH_2), 109.50 (C-4), 127.22, 127.26, 127.30, 128.53, 128.72 (C_{arom}−H), 132.54, 134.74 (C_{arom}−C), 140.42 (C_{arom}−CS) 144.77 (C-5). Anal. ($C_{17}H_{17}NO_3S\cdot H_2O$) C, H, N.

4-(4-Methylsulfonylphenyl)-2-methyl-5-phenyl-4-isoxazoline (13i). Yield, 50%; oil. IR (neat): 1658 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.82 (s, 3H, 2-CH_3), 2.95 (s, 3H, SO_2CH_3), 3.90−4.0 (br m, 1H total, H-3), 4.6−4.7 (br m, 1H total, H-3), 7.10−7.30 (m, 5H, H_{arom}), 7.57 (d, J = 8 Hz, 2H, H_{arom}), 7.76 (d, J = 8 Hz, 2H, H_{arom}). Anal. ($C_{17}H_{17}NO_3S\cdot H_2O$) C, H, N.

2,3-Dimethyl-5-(4-methylsulfonylphenyl)-4-phenyl-4-isoxazoline (13j). Yield, 76%; oil. IR (neat): 1660 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.35 (d, $J_{CH,Me}$= 7 Hz, 3H, CHCH_3), 2.92 (s, 3H, 2-CH_3), 3.02 (s, 3H, SO_2CH_3), 4.22−4.30 (br q, $J_{CH,Me}$ = 7 Hz, 1H, CHCH$_3$), 7.20−7.34 (m, 5H, H_{arom}), 7.57 (d, J = 8 Hz, 2H, H_{arom}), 7.80 (d, J = 8 Hz, 2H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 19.79 (3-CH_3), 44.33 (SO_2CH_3), 46.32 (2-CH_3), 72.67 (CH_3CH), 115.68 (C-4), 127.12, 127.49, 128.10, 128.36, 128.89 (C_{arom}−H), 132.69, 134.88 (C_{arom}−C), 140.07 (C_{arom}−CS), 144.10 (C-5). Anal. ($C_{18}H_{19}NO_3S$) C, H, N.

2,3-Dimethyl-4-(4-fluorophenyl)-5-(4-methylsulfonylphenyl)-4-isoxazoline (13k). Yield, 71%; mp 116−120 °C. IR (KBr): 1660 (C═C) cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.33 (d, $J_{CH,Me}$ = 6 Hz, 3H, CHCH_3), 2.92 (s, 3H, 2-CH_3), 3.01 (s, 3H, SO_2CH_3), 4.20−4.30 (br q, J = 6 Hz, 1H, CHMe), 7.04−7.10 (m, 2H, H_{arom}), 7.15−7.20 (m, 2H, H_{arom}), 7.54 (d, J = 8 Hz, 2H, H_{arom}), 7.82 (d, J = 8 Hz, 2H, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 19.79 (3-CH_3), 44.40 (SO_2CH_3), 46.26 (2-CH_3), 72.74 (CHMe), 114.62 (C-4), 116.10 (d, J_{CCF} = 22 Hz, CCF), 126.70, 127.95, 130.12 (C_{arom}−H), 128.70, 134.70 (C_{arom}−C), 140.07 (C_{arom}−CS), 148.68 (C-5), 161.30 (d, $J_{C,F}$ = 251 Hz, C-F). ^{19}F NMR ($CDCl_3$): δ 48.17 (dddd, J_{FCCH} = 9.1, J_{FCCCH} = 5.1 Hz, 1F). Anal. ($C_{18}H_{18}FNO_3S$) C, H, N.

Cyclooxygenase Inhibition Studies. All compounds described herein were tested for their ability to inhibit COX-1 and COX-2 using a COX-(ovine) inhibitor screening kit (Catalog No. 560101, Cayman Chemical, AnnArbor, MI). Briefly, cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid (AA) to PGH_2. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, is measured by enzyme immunoassay (ACE competitive EIA). This assay is based on the competition between PGs and a PG−acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells because the concentration of the PG tracer is held constant while the concentration of PGs varies. This antibody−PG complex binds to a mouse antirabbit monoclonal antibody that has been previously attached to the well. The plate is

washed to remove any unbound reagents, and then Ellman's reagent, which contains the substrate to acetylcholinesterase, is added to the well. The product of this enzymatic reaction produces a distinct yellow color that absorbs at 405 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of PGs present in the well during the incubation: absorbance ∝ [bound PG tracer] ∝ 1/PGs. Percent inhibition was calculated by comparison of the compound treated to various control incubations. The concentration of the test compound causing 50% inhibition (IC_{50}, μM) was calculated from the concentration−inhibition response curve (duplicate determinations).

Antiinflammatory Assay. The test compounds were evaluated using the in vivo rat carrageenan-induced foot paw edema model reported previously.[24]

Analgesic Assay. Analgesic activity was determined using the 4% sodium chloride-induced writhing (abdominal constriction) assay[25] as described previously.[26]

Molecular Modeling (Docking) Study. The coordinates from the X-ray crystal structure of human COX-2 used in this simulation were obtained from the Protein Data Bank (PDB file 1CX2), where the active site is bound to the selective COX-2 inhibitor SC-588 (**4**). The MM3 optimized structures for isoxazolines **13h** and **13j** were subjected to dynamics optimization using the ALCHEMY 2000[22] program at 300 K over a 0.001 ps time step for 1 ps. The lowest energy conformation obtained in this way was superimposed on SC-588 (**4**) in the PDB file 1CX2 using the Insight II program,[23] after which SC-588 was deleted. To relieve any unfavorable side chain overlaps, the Measure/Bump command was used. Subsets of the enzyme were defined allowing residues within 10 Å of the ligand to relax, whereas all other enzyme residues were fixed. The Affinity command in the Docking module was used to complete the docking experiment. Minimization of the ligand-active site assembly was performed over 20 000 steps, reaching a convergence of 0.01 kcal mol^{-1} $Å^{-1}$ using the steepest descent method followed by the conjugate gradient method to reach a final convergence of 0.001 kcal mol^{-1} $Å^{-1}$. The CVFF force field was used in the docking experiment. The intermolecular energy of the drug-active site (assembly) interaction was used to evaluate the quality of the docking experiment.

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