Design, Synthesis, and Biological Evaluation of 6-Substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones: A Novel Class of Diarylheterocyclic Selective Cyclooxygenase-2 Inhibitors

P. N. Praveen Rao, Mohsen Amini, Huiying Li, Amgad G. Habeeb, and Edward E. Knaus*

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, T6G 2N8, Canada

Received May 19, 2003

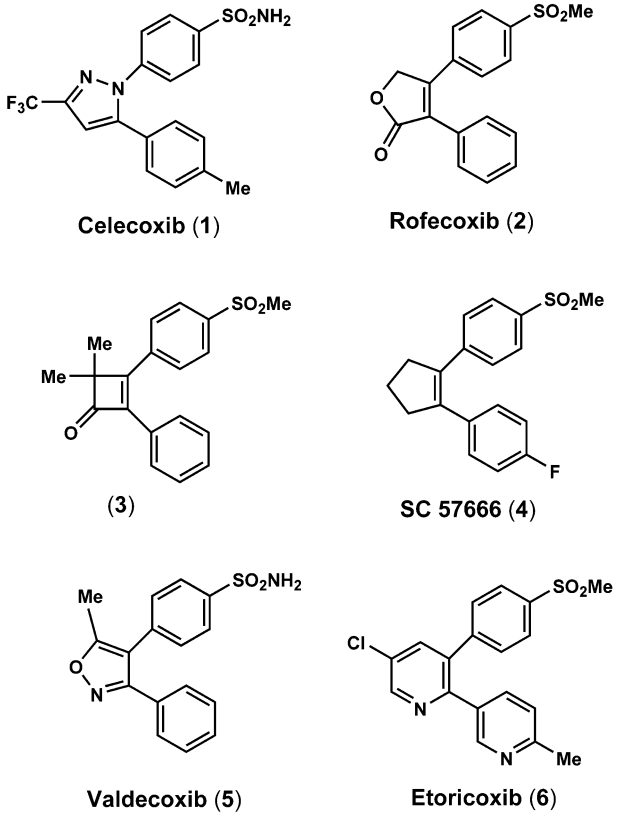
A group of 6-alkyl (alkoxy or alkylthio)-4-aryl-3-(4-methanesulfonylphenyl)pyran-2-ones **(14a**−**v**), possessing either a H or F substituent at the para-position of the C-4 phenyl ring, were designed for evaluation as selective cyclooxygenase-2 (COX-2) inhibitors with in vivo antiinflammatory−analgesic activities. Although 6-ethylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**14s**) exhibited a very high in vitro COX-2 inhibitory potency (IC_{50} = 0.0032 μM) and COX-2 selectivity (SI > 120 000), **14s** exhibited moderate antiinflammatory activity compared to celecoxib in a carrageenan-induced rat paw edema assay. In contrast, the less potent (IC_{50} = 0.10 μM), and less selective (SI = 2880) COX-2 inhibitor 6-ethoxy-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**14i**) exhibited good antiinflammatory activity where a 1 mg/kg oral dose reduced inflammation 32 and 67% at 3 and 5 h postdrug administration relative to the reference drug celecoxib where a 50 mg/kg oral dose reduced inflammation by 79 and 58% at the respective 3 and 5 h time periods. Molecular modeling studies, where **14i** was docked in the active site of both COX-1 and COX-2, reveals that the C-6 ethoxy substituent orients the pyran-2-one ring to position the SO_2Me pharmacophore in the vicinity of the secondary pocket in COX-2. The absence of this COX-2 secondary pocket in the COX-1 binding site is due to the presence of the bulky Ile^{523} in COX-1 such that access to the amino acid residues (Ile^{517}, Phe^{518}, Gln^{192}, and His^{90}), which line the COX-2 secondary pocket with which the SO_2Me pharmacophore could interact, is hindered. The six-membered pyran-2-one ring system is a suitable central template to design selective COX-2 inhibitors.

Introduction

The differential tissue distribution of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) provides a rationale for the development of selective COX-2 inhibitors as antiinflammatory−analgesic agents that lack the GI side effects exhibited by traditional nonsteroidal antiinflammatory drugs (NSAIDs).[1−4] This hypothesis has been applied successfully in the design of two highly selective tricyclic COX-2 inhibitors that possess a diaryl heterocyclic ring template, namely celecoxib (**1**), and rofecoxib (**2**), respectively (Chart 1).[5,6] In addition to its role in rheumatoid arthritis and osteoarthritis, COX-2 is also implicated in colon cancer and angiogenesis.[7−9] Recent studies have shown that the progression of Alzheimer's disease is reduced among some users of NSAIDs. Chronic treatment with selective COX-2 inhibitors may therefore slow the progress of Alzheimer's disease, without causing GI damage.[10]

Diarylheterocycles, and other central ring pharmacophore templates, have been extensively studied as cyclooxygenase inhibitors. All these tricyclic molecules possess 1,2-diaryl substitution on a central four-, five-, or six-membered ring system such as cyclobutenone (**3**), cyclopentene (**4**), isoxazole (**5**), pyrazole (**1**), 2-(5*H*)-furanone (**2**), or pyridine (**6**), respectively (Chart 1).[5,11−15] Structure−activity relationship (SAR) studies have shown that for optimum COX-2 selectivity and inhibi-

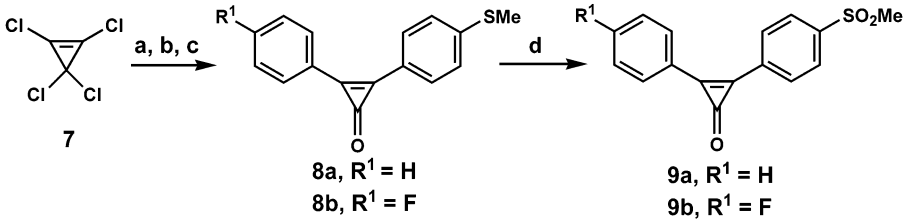
Chart 1. Representative Examples of Selective Tricyclic COX-2 Inhibitors



* To whom correspondence should be addressed. Phone: 780-492-5993. Fax: 780-492-1217. E-mail: eknaus@pharmacy.ualberta.ca.

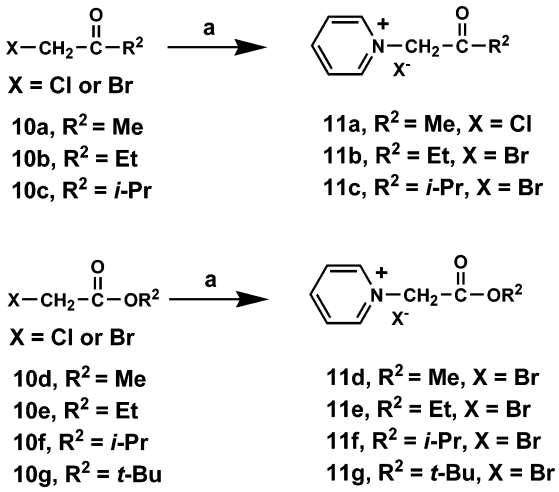
10.1021/jm0302391 CCC: $25.00 © 2003 American Chemical Society
Published on Web 09/30/2003

Scheme 1[a]



[a] Reagents and conditions: (a) dry $AlCl_3$, 1,2-dichloroethane, R^1-C_6H_5 (R^1 = H, F), 25 °C, 24 h; (b) thioanisole, 25 °C, 24 h; (c) H_2O, 25 °C, 10 min; (d) aqueous Oxone, THF, MeOH, 25 °C, 4−5 h.

Scheme 2[a]



[a] Reagents and conditions: (a) pyridine, THF, 25 °C, 6 h.

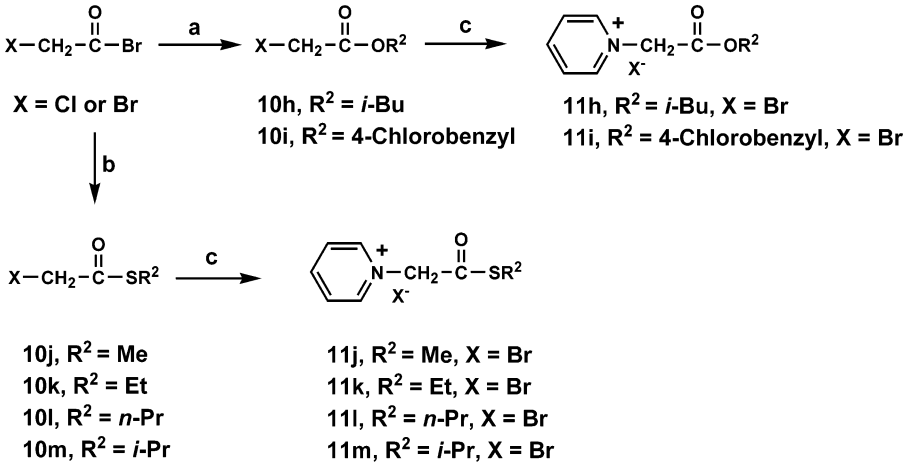
tory potency, a SO_2Me or SO_2NH_2 substituent at the para-position of a phenyl ring, and that the presence of a *p*-F substituent on the nonsulfonyl vicinal phenyl ring improves in vivo activity.[16] In a very recent letter, we reported the design and synthesis of a novel class of diarylheterocycle with a central six-membered lactone (pyran-2-one) ring which exhibited good in vitro COX-2 inhibitory potency and selectivity.[17] The Merck Co. has also developed novel methods to synthesize diarylheterocycles having a central six-membered lactone (pyran-2-one) ring system.[18] We now describe the design, synthesis, and biological evaluation of a diverse group of 6-alkyl-, 6-alkoxy-, or 6-alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones as selective COX-2 inhibitors with antiinflammatory−analgesic activities.

Chemistry

The title 3,4-diphenylpyran-2-ones (**14a**−**v**), possessing a central six-membered lactone ring with either a 6-alkyl, alkoxy, or alkylthio substituent, were prepared by the condensation of a substituted 2,3-diphenylcycloprop-2-en-1-one (**9a** or **9b**) with the respective pyridinium ylide derivative of **11a**−**m** as illustrated in Schemes 1−4. 2-(4-Methanesulfonylphenyl)-3-phenylcycloprop-2-en-1-one (**9a**) and the 3-(4-fluorophenyl) analogue (**9b**) were prepared using a one-pot reaction, starting with tetrachlorocyclopropene **7** according to our previously reported procedure as shown in Scheme 1.[19] The pyridinium salts **11a**−**g** were prepared by the reaction of the respective haloalkyl ketone (**10a**−**c**) or alkyl haloacetate (**10d**−**g**) with pyridine (Scheme 2). The nucleophilic substitution reaction of either chloroacetyl chloride or bromoacetyl bromide with various alcohols or thiols gave the respective alkyl haloacetate (**10h**−**i**) or alkyl halothioacetate (**10j**−**m**), which on subsequent reaction with pyridine afforded the respective *N*-alkoxy-, or alkylthiocarbonylmethylpyridinium halides (**11h**−**m**) in moderate to good yields (33−68%) as shown in Scheme 3.

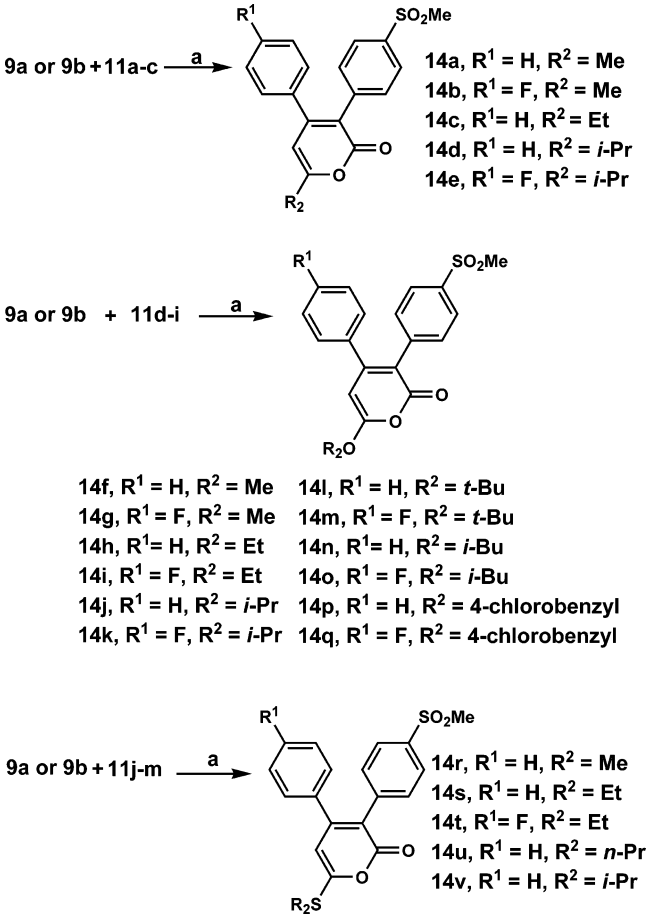
The 3,4-diarylpyran-2-ones (**14a**−**v**) were prepared in low to moderate yields (8−44%) by condensation of a 2-(4-methanesulfonylphenyl)-3-phenylcycloprop-2-en-1-one (**9a** or **9b**) with a pyridinium salt (**11a**−**m**) in the presence of the base triethylamine. The initial ylide compound that is formed in the reaction undergoes a spontaneous ring expansion[20−22] reaction to produce the

Scheme 3[a]



[a] Reagents and conditions: (a) diethyl ether, R^2OH (R^2 = *i*-Bu or 4-Cl−C_6H_4−CH_2), −23 °C → 25 °C, 4 h; (b) diethyl ether, R^2SH or $R^2S^-Na^+$ (R^2 = Me, Et, *n*-Pr or *i*-Pr), −23 °C → 25 °C, 4 h; (c) pyridine, THF, 25 °C, 6 h.

Scheme 4[a]



[a] Reagents and conditions: (a) benzene, Et_3N, 25 °C, 16−18 h.

target 6-alkyl-, 6-alkoxy-, or 6-alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**14a**−**v**) having a central six-membered lactone ring (pyran-2-one) as illustrated in Scheme 4. No other regioisomer was observed using the reaction conditions employed. The structures of compounds **14** were consistent with microanalytical and mass spectral data. ^{1}H NMR nuclear Overhauser enhancement (NOE) studies showed NOE interactions between H-5 and the 6-alkyl (R^2), alkoxy (OR^2), or alkylthio (SR^2) moiety, and between H-5 and the C-4 *o*-phenyl hydrogens, which establishes the regiochemistry of the C-4 and C-5 phenyl rings.

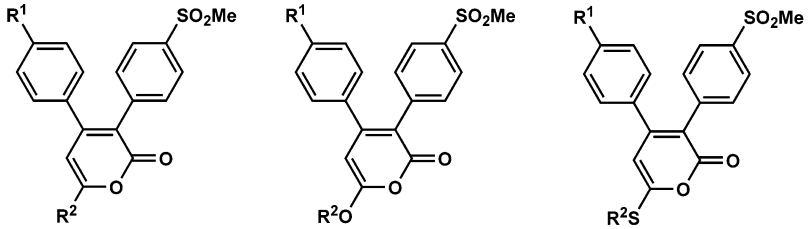
Results and Discussion

The in vitro abilities (IC_{50} values) of the title compounds **(14a**−**v)** to inhibit the isozymes COX-1 and COX-2 were determined by modification of a previously reported procedure (Table 1).[23] The rational design of selective COX-2 inhibitors frequently exploits the difference in the volume of the binding sites for COX-1 and COX-2. Thus, a SO_2Me or a SO_2NH_2 substituent placed at the para-position of one of the phenyl rings can interact with secondary pocket amino acid residues such as His[90], Gln[192], Phe[518], and Arg[513] that are associated with, and accessible from, the primary COX-2 binding site.[24,25] Accordingly, we have designed a novel class of 6-substituted-3,4-diphenylpyran-2-ones having a C-3 4-methanesulfonylphenyl substituent in conjunction with either a C-4 phenyl, or 4-fluorophenyl substituent.

In vitro enzyme inhibition studies for the C-6 alkyl (Me, Et, *i*-Pr) subgroup of compounds (**14a**−**e**) showed weak to good COX-2 inhibitory activity (IC_{50} values in the 0.5−125.3 μM range) with 6-methyl-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**14a**), showing the best combination of COX-2 inhibitory potency and selectivity index (IC_{50} = 0.68 μM; SI = 904). In this C-6 alkyl subgroup (**14a**−**e**) of compounds, a *p*-fluoro substituent on the C-4 phenyl ring decreased both COX-2 selectivity and inhibitory potency but increased COX-1 inhibitory potency (Table 1). In the C-6 alkoxy (OMe, OEt, OPr-*i*, OBu-*t*, OBu-*i*, OCH_2-C_6H_4-4-Cl) subgroup of compounds **14** having a C-4 phenyl substituent, in vitro COX-2 inhibitory activity may not be totally dependent upon steric effects even though the C-6 OEt, *i*-PrO, *t*-BuO, and *i*-BuO analogues were equipotent (IC_{50} values in the 1.3 to 2.7 μM range). Introduction of a C-6 4-chlorobenzyloxy substituent (**14p**) significantly reduced COX-2 inhibitory activity and selectivity (COX-2 IC_{50} = 415.8 μM; COX-1 IC_{50} = 50.7 μM; SI < 0.12) as shown in Table 1. Compounds having a C-4 4-fluorophenyl substituent are generally less potent COX-2 inhibitors than the corresponding C-4 phenyl analogues, except for the C-6 OEt compound **14i** (COX-2 IC_{50} = 0.10 μM; COX-1 IC_{50} = 288 μM; SI = 2880). The C-6 substituent in the thioalkyl (MeS, EtS, *n*-PrS, *i*-PrS) subgroup of compounds had a significant effect on COX-2 inhibitory potency (EtS ≫ MeS > inactive *n*-PrS and *i*-PrS) and COX-2 selectivity. For example, the C-6 thioethyl compound **14s** is a highly potent and selective COX-2 inhibitor (COX-1 IC_{50} = 386.2 μM; COX-2 IC_{50} = 0.0032 μM; SI > 120 000) relative to the reference drugs rofecoxib (COX-2 IC_{50} = 0.43 μM; SI > 1162) and celecoxib (COX-2 IC_{50} = 0.057 μM; SI > 401). The in vitro cyclooxygenase inhibition data for this series (**14a**−**v**) of compounds illustrate the flexibility of the COX-2 active site in accommodating compounds with a range of molecular volumes (293−390 Å^3).

In an earlier communication, we attributed the excellent in vitro COX-2 inhibitory activity exhibited by **14s** to the presence of a weak hydrogen bonding interaction of the sulfur atom of the C-6 thioethyl (SEt) substituent with the O*H* of Ser[530] in the COX-2 binding site.[17] A recent molecular modeling study has confirmed the importance of Ser[530] in the COX-2 inhibitory potency of diarylheterocyclic COX-2 inhibitors.[26] To explain the potent COX-2 (IC_{50} = 0.10 μM) and weak COX-1 inhibitory (IC_{50} = 288 μM) activity of **14i** [6-ethoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one], a molecular modeling study was performed where **14i** was docked in the binding sites of both COX-1 and COX-2. In addition, molecular dynamics (MD) simulations were carried out to assess the stability of the docked ligand−enzyme complexes. Docking **14i** in the COX-2 active site shows that it binds in the primary binding site such that the *p*-SO_2Me substituent orients toward the secondary pocket amino acid residues (His[90], Gln[192], Arg[513], Phe[518], Val[523], and Leu[352]) with one of the *O*-atoms of the SO_2Me substituent forming a hydrogen bond with the amide hydrogen of Phe[518] (2.1 Å) as shown in Figure 1. The C=*O* oxygen atom of the central pyran-2-one ring is oriented toward Tyr[355] which is part of the entrance to the secondary pocket. This C=*O* oxygen atom is positioned about 3.64 Å from the

Table 1. COX-1/COX-2 Inhibitory and Antiinflammatory−Analgesic Activities of 6-Alkyl-, 6-Alkoxy-, and 6-Alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**14a**−**v**)



14a-e **14f-q** **14r-v**

			IC$_{50}$ (μM)[a]			AI activity[c]		analgesic activity[d]		
compd	R^1	R^2	COX-1	COX-2	selectivity[b] index	% inhibition at 3 h	% inhibition at 5 h	% inhibition at 30 min	% inhibition at 60 min.	volume (Å)[e]
14a	H	Me	614.8	0.68	904	12.5 ± 2.2	23.5 ± 3.2	57.0 ± 4.1	60.4 ± 7.3	293.5
14b	F	Me	201.5	125.3	1.6	−	−	−	−	296.6
14c	H	Et	8.0	1.5	5.3	−	−	−	−	310.1
14d	H	*i*-Pr	341.5	0.50	683	09.8 ± 3.1	19.5 ± 5.3	67.8 ± 9.1	56.9 ± 7.1	326.7
14e	F	*i*-Pr	15.7	13.6	1.1	35.8 ± 4.8	69.1 ± 4.9	50.9 ± 4.2	20.7 ± 5.4	329.8
14f	H	Me	14.7	28.3	<0.5	inactive	22.0 ± 5.4	55.2 ± 10.1	83.4 ± 1.9	301.7
14g	F	Me	>1000	59.5	>20	30.6 ± 4.4	30.0 ± 6.5	49.0 ± 10.5	72.6 ± 4.6	305.5
14h	H	Et	281.5	1.3	216	−	−	−	−	318.7
14i	F	Et	288.0	0.10	2880	32.7 ± 5.3	67.8 ± 5.5	42.8 ± 8.2	75.7 ± 4.1	322.3
14j	H	*i*-Pr	4.0	2.0	2.0	−	−	−	−	336.0
14k	F	*i*-Pr	560.0	83.6	6.7	−	−	−	−	338.7
14l	H	*t*-Bu	>100	2.7	>37	29.0 ± 2.8 [f]	30.1 ± 3.0 [f]	73.8 ± 4.0 [g]	80.9 ± 6.2 [g]	351.3
14m	F	*t*-Bu	68.5	20.4	3.3	−	−	−	−	355.6
14n	H	*i*-Bu	3.1	1.8	>1.7	−	−	−	−	351.7
14o	F	*i*-Bu	42.6	35.0	1.2	−	−	−	−	355.5
14p	H	4-Cl−PhCH$_2$	50.7	415.8	< 0.2	−	−	−	−	386.5
14q	F	4-Cl−PhCH$_2$	301.5	53.3	5.6	−	−	−	−	390.8
14r	H	Me	>100	2.8	>35	−	−	−	−	311.5
14s	H	Et	386.2	0.0032	>120 687	09.2 ± 3.0 [f]	19.5 ± 2.4 [f]	81.0 ± 5.4 [g]	77.4 ± 8.1 [g]	328.5
14t	F	Et	>100	1.44	>69	25.8 ± 4.0 [f]	16.8 ± 7.1 [f]	54.3 ± 11.5 [g]	66.0 ± 6.5 [g]	331.2
14u	H	*n*-Pr	>100	>100	−	−	−	−	−	344.6
14v	H	*i*-Pr	>100	>100	−	−	−	−	−	345.6
Ibuprofen						56.2 ± 2.0 [f]	41.5 ± 4.9 [f]	−	−	209.5
Celecoxib			22.9	0.057	>401	79.9 ± 1.9 [f,h]	58.2 ± 1.8 [f]	31.7 ± 9.6 [g]	62.0 ± 7.3 [g]	298.5
Rofecoxib			>500	0.43	>1162	−	−	−	−	267.2

[a] Values are means of two determinations and deviation from the mean is <10% of the mean value. [b] In vitro COX-2 selectivity index (IC$_{50}$ COX-1/IC$_{50}$ COX-2). [c] Inhibitory activity on carrageenan-induced rat paw edema. The results are expressed as mean ± SEM (n = 4−6) following a 1 mg/kg oral dose of the test compound. [d] Inhibitory activity in the rat 4% NaCl-induced abdominal constriction assay. The results are expressed as mean ± SEM (n = 4−6) following a 1 mg/kg intraperitoneal dose of the test compound. [e] The volume of the molecule, after minimization using the PM3 force field, was calculated using the Alchemy 2000 program. [f] 50 mg/kg po dose. [g] 50 mg/kg ip dose. [h] ID$_{50}$ = 10.8 mg/kg po dose.

O*H* of Tyr^{355} and the O-atom of the central pyran-2-one ring is about 4.7 Å from the N*H*$_2$ (guanidino group) of Arg^{120}. The C-4-substituted phenyl ring lies in a hydrophobic cavity lined by Tyr^{385} and Trp^{387} with the *p*-fluoro substituent located about 5.8 Å away from the O*H* of Tyr^{385} and where the distance between the center of the C-4 phenyl ring and the *O*H of Ser^{530} was about 6.9 Å. A recent study has shown the importance of Ser^{530} in the COX-2 inhibitory activity of rofecoxib.[26] Accordingly, the C-6 ethoxy (OEt) substituent of **14i** is located in a hydrophobic region formed by Val^{349}, Ile^{345}, Ser^{530}, Leu^{531}, and Met^{535} with the O-atom of C-6 OEt substituent positioned about 4.9 Å away from the O*H* of Ser^{530} (Figure 1).

Similar docking of **14i** on the COX-1 active site showed the drug−enzyme binding interaction was not dramatically different from that observed in COX-2 (Figure 2). However, closer examination revealed potentially crucial interactions involving the central pyran-2-one C=*O* with the key active site amino acid residue Tyr^{355}, the interaction of the C-3 *p*-SO$_2$Me substituent with Ile/Val^{523} that forms part of the entrance to the secondary pocket and interaction of the C-6 OEt substituent with Ser^{530} that may contribute to the in vitro COX-2 inhibitory activity of **14i**. The importance of Tyr^{355} in the binding affinities of diarylheterocyclic COX-2 inhibitors was shown in a recent study, wherein mutation of Tyr^{355} to Ala in the COX-2 active site led to increased dissociation rates of celecoxib and valdecoxib.[29] Docking **14i** in the COX-1 active site suggests that the C=*O* oxygen atom is separated by a greater distance from the O*H* of Tyr^{355} (5.0 Å) than observed for a similar interaction in COX-2 (3.64 Å), and that insertion of the *p*-SO$_2$Me substituent into the secondary pocket is sterically impeded by the bulky Ile^{523} as shown in Figure 2. In addition, the C-6 ethoxy oxygen atom is further removed (5.5 Å) from the O*H* of Ser^{530} compared to COX-2 (4.9 Å). It has been shown that a single amino acid difference (Ile/Val) at position 523 in the respective active sites of COX-1 and COX-2 can be exploited to design diarylheterocyclic COX-2 inhibitors.[24,25] However, a recent molecular modeling study of the diarylheterocyclic COX-2 inhibitor rofecoxib in the COX-1 active site has shown that steric hindrance due to the substitution of Ile/Val^{523} is not enough to prevent binding of the ligand to COX-1.[27] Recent studies

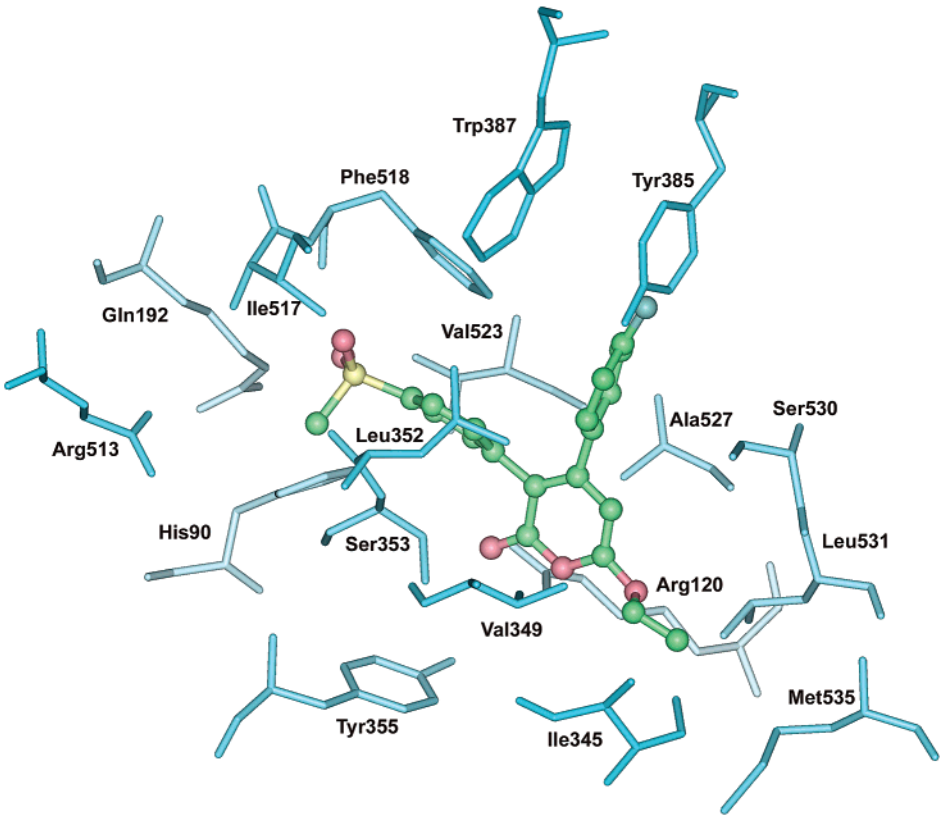


Figure 1. Docking 6-ethoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (**14i**) (ball-and-stick) in the active site of murine COX-2 ($E_{\text{intermolecular}} = -60.7$ kcal/mol). Hydrogens atoms are not shown for clarity.

have also shown that COX-2 inhibitors belonging to the diarylheterocyclic class show time dependent inhibition of COX-2 while exhibiting competitive and reversible inhibition of COX-1.[28,29] Consequently, the higher binding affinity exhibited by **14i** for COX-2 may be due in part to its slow dissociation from COX-2. This possibility is consistent with results from a molecular dynamics simulation of **14i** docked in the active site of COX-2 which showed a lower intermolecular energy ($E_{\text{intermolecular}} = -60.7$ kcal/mol) for this ligand−enzyme complex as compared to **14i** docked in the active site of COX-1 ($E_{\text{intermolecular}} = -49.3$ kcal/mol).

In vivo pharmacological evaluation of some title compounds **14** was carried out to assess their potential antiinflammatory and analgesic activities. Initial compound selection for in vivo screening was based on in vitro COX-1/COX-2 enzyme inhibition data obtained. Qualitative structure−activity relationship data, acquired using the antiinflammatory rat paw edema assay, showed that this group of C-6 alkyl-, alkoxy-, or alkylthio-substituted 3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones exhibit antiinflammatory activity in the inactive to good activity range (inactive to 69% inhibition) (Table 1). In the C-6 alkyl series (**14a**, **14d**, and **14e**), 6-isopropyl-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (**14e**) was the most active antiinflammatory agent (35 and 69% reduction in inflammation at 3 and 5 h postdrug administration, respectively) for a 1 mg/kg oral dose as compared to reference drug celecoxib (79 and 58% reduction in inflammation at 3 and 5 h postdrug administration, respectively) for a 50 mg/kg oral dose.

The C-6 alkoxy-substituted group of compounds (**14f, 14g, 14i**, and **14l**) reduced inflammation by 0−67% at different time intervals as shown in Table 1. 6-Ethoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (**14i**) was the most potent antiinflammatory agent in this series, producing a 32 and 67% reduction in inflammation at 3 and 5 h postdrug administration, respectively, for a 1 mg/kg oral dose. This in vivo antiinflammatory activity parallels its in vitro COX-2 potency and selectivity (COX-2 $IC_{50} = 0.10$ μM; SI = 2880). In general, the C-6 alkyl- and alkoxy-substituted compounds, which possess a *p*-fluoro substituent on the C-4 phenyl ring, exhibit good antiinflammatory activity (Table 1). In contrast, the C-6 thioethyl compound **14s**, which exhibited excellent in vitro COX-2 potency and selectivity ($IC_{50} = 0.0032$ μM; SI > 120 000), was a weak antiinflammatory agent (9 and 19.5% reduction in inflammation at 3 and 5 h postdrug administration, respectively) for a 50 mg/kg oral dose. When **14s** was administered at lower doses (1, 5, and 10 mg/kg oral dose), it did not exhibit antiinflammatory activity. It is possible that **14s** which has a SEt substitutent at the C-6 position of the pyranone ring may be more susceptible to metabolic inactivation compared to C-6 alkyl or alkoxy analogues when administered by the oral route which may result in a lower amount of the drug localization at the inflammation site. Studies using systemic routes of administration may provide more precise explanations for these observations which are not clear.

It is interesting that the C-6 alkyl (**14a**, **14d**, and **14e**) and the C-6 alkoxy (**14f**, **14g**, and **14i**) compounds increased rat paw edema volume when administered at a higher 5 and 10 mg/kg oral dose although the data obtained are not statistically significant. Over the years numerous studies employing rat acute models of inflam-

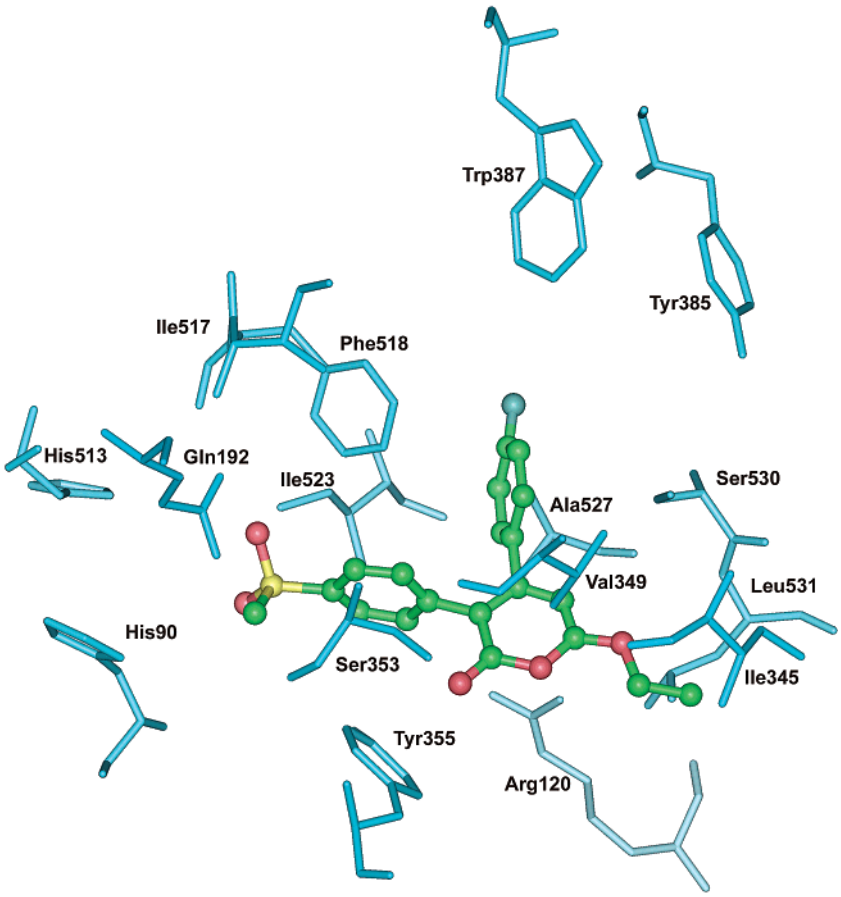


Figure 2. Docking 6-ethoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (**14i**) (ball-and-stick) in the active site of ovine COX-1 ($E_{\text{intermolecular}} = -49.3$ kcal/mol). Hydrogens atoms are not shown for clarity.

mation have not been able to completely assess the contribution of the COX-2 enzyme in the inflammatory process. Recent studies have shown that in a rat carrageenan-induced pleurisy model, rofecoxib increased the levels of proinflammatory cytokine TNF-α in rat paw.[30] In addition, studies have implicated increased levels of inducible nitric oxide synthase in the carrageenan-induced rat paw inflammation assay which was not altered by the administration of COX-2 inhibitors.[31,32] Another recent article has shown the opposite effects of rofecoxib on activating protein (AP-1) and nuclear factor-κB (NF-κB) which may explain the lack of clear dose dependency, influencing both its wanted and unwanted effects.[33] Although the exact mechanisms for these observations are not clear, there are multiple factors that may be responsible for the increase in rat paw volume observed at higher doses for the compounds described in this study.

Analgesic activity was determined using the 4% NaCl-induced abdominal constriction assay. Compounds from the C-6 alkyl (**14a**, **14d**, and **14e**) and C-6 alkoxy (**14f**, **14g**, **14i**, and **14l**) subgroups exhibited a 20−83% inhibition of writhing at different time intervals with **14i** [6-ethoxy-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one] exhibiting 42 and 75% inhibition in writhing at 30 and 60 min, respectively, postdrug administration for a 1 mg/kg intraperitoneal dose (Table 1). 6-Ethylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**14s**) was the most active analgesic agent where writhing was reduced by 81 and 77% inhibition at 30 and 60 min postdrug administration for a 50 mg/kg intraperitoneal dose relative to the reference drug celecoxib (31 and 62% inhibition at 30 and 60 min postdrug administration for a 50 mg/kg intraperitoneal dose). The good analgesic activity exhibited by C-6 alkyl (**14a**, **14d**, and **14e**) and alkoxy (**14f**, **14g,** and **14i**) compounds at the 1 mg/kg intraperitoneal dose suggests roles for both peripheral and central actions of COX-2 inhibitors in alleviating pain in a wide variety of conditions.[34,35]

Conclusions

The qualitative structure−activity relationships acquired for this novel class of 6-alkyl (alkoxy or alkylthio)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones have shown that (i) a six-membered lactone (pyran-2-one) ring serves as a suitable central ring template to design selective COX-2 inhibitors when the C═O oxygen atom is suitably positioned to undergo hydrogen bonding to Tyr^{355} in the COX-2 binding site, (ii) the combined steric and electronic properties of the C-6 central substituted ring modulates COX-2 inhibitory potency and selectivity by orienting the C-3 *p*-SO_2Me phenyl substituent to the vicinity of the secondary pocket in the COX-2 binding site, vis a vis **14i** (C-6 ethoxy) and **14s** (C-6 ethylthio) exhibit excellent COX-2 inhibitory potency and selectivity, and (iii) the moderate to weak COX-1 inhibition exhibited by this class of compounds can be attributed partly to the steric hindrance of Ile^{523} at the entrance to the side pocket which blocks the access of the *p*-SO_2Me substituent, and (iv) the presence of a *p*-fluoro substituent on the C-4 phenyl

ring improves in vivo antiinflammatory activity for this class of compounds.

Experimental Section

General. Melting points were determined using a Buchi capillary apparatus and are uncorrected. Ibuprofen was purchased from Sigma (St. Louis, MO). All other reagents including **10a**−**g** were purchased from Aldrich (Milwaukee, WI). Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70−230 mesh). Infrared (IR) spectra were recorded using a Nicolet 550 Series II Magna FT-IR spectrometer. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were recorded on a Bruker AM-300 spectrometer, and chemical shifts are expressed in parts per million (ppm, δ) relative to tetramethylsilane as internal standard. Spin multiplets are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). Coupling constants (J) are given in hertz (Hz). ^{13}C NMR spectra were acquired using the J modulated spin−echo technique where methyl and methane carbons appear as positive peaks and methylene and quaternary carbon resonances appear as negative peaks. High-resolution mass spectra (HRMS) were acquired using a Kratos MS-50 electron impact (EI) mass spectrometer. Microanalyses were performed for C, H, and N (MicroAnalytical Service Laboratory, Department of Chemistry, University of Alberta) and were within ± 0.4% of the theoretical values. Celecoxib and rofecoxib were synthesized according to the literature procedures.[5,14] Compounds **9a** [2-(4-methanesulfonylphenyl)-3-phenylcycloprop-2-en-1-one], and **9b** [2-(4-methanesulfonylphenyl)-3-(4-fluorophenyl)cycloprop-2-en-1-one], were prepared according to the previously reported method.[19] Male Sprague-Dawley rats, used in the antinflammatory-analgesic screens, were supplied by Animal Health Services, University of Alberta and experiments were carried out using protocols approved by the Animal Welfare Committee, University of Alberta.

General Procedure for the Synthesis of Alkyl Haloacetates (10h−i) and Alkyl Halothioacetates (10j−m). To a stirred solution of either bromoacetyl bromide or chloroacetyl chloride (13.3 mmol) in dry diethyl ether (65 mL) was added 13.3 mmol of the respective alcohol (*i*-BuOH or 4-chlorobenzyl alcohol) or thiol (MeSNa, EtSH, *n*-PrSH or *i*-PrSH) at −23 °C (dry ice/acetone bath) under an argon atmosphere, and the reaction was allowed to proceed at 25 °C for 4 h. The reaction mixture was washed with 10% $NaHCO_3$ solution (30 mL), and the organic phase was separated, and dried (Na_2SO_4). The solvent was removed either in vacuo, or under a stream of argon, to afford the respective alkyl haloacetate (**10h**−**i**) or alkyl halothioacetate (**10j**−**m**) as a pale yellow oil in 59−85% yield. Products **10** were used immediately without further purification for the preparation of compounds **11**.

General Procedure for the Synthesis of *N*-Alkyl-, -Alkoxy-, or -Alkylthiocarbonylmethylpyridinium Halides (11a−m). To a stirred solution of dry pyridine (0.45 mL, 5.5 mmol) in dry THF (30 mL) under an argon atmosphere was added either an alkyl ketone (**10a**−**c**), alkyl haloacetate (**10d**−**i**), or alkyl halothioacetate (**10j**−**m**) (5.5 mmol), and the reaction mixture was stirred for 6 h at 25 °C. The solvent was either removed in vacuo, or under a stream of argon gas, and the solid or semisolid product obtained was purified by recrystallization from ethanol-acetone (1:1, v/v) to give the respective product (**11a**−**m**) in 33−88% yield. Some physical and spectroscopic (IR, ^{1}HNMR) data for **11a**−**m** are listed below.

***N*-(Methylcarbonylmethyl)pyridinium Chloride (11a).** This product was obtained as a white solid (0.41 g, 44%) by reaction of pyridine with **10a**: mp 205−206 °C (lit. 202−204 °C[36]); IR (KBr): 1658 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 3H, CH_3), 5.79 (s, 2H, N$^+$CH_2), 8.23 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.68 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.01 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Ethylcarbonylmethyl)pyridinium Bromide (11b).** This product was obtained as a white solid (0.64 g, 50.7%) by reaction of pyridine with **10b**: mp 179−181 °C; IR (KBr): 1660 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 1.01 (t, J = 6.7 Hz, 3H, CH_2CH_3), 2.64 (q, J = 6.7 Hz, 2H, CH_2CH_3), 5.76 (s, 2H, N$^+$CH_2), 8.18 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.64 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 8.84 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Isopropylcarbonylmethyl)pyridinium Bromide (11c).** This product was obtained as a solid (0.69 g, 51.4%) by reaction of pyridine with **10c**: mp 148−150 °C; IR (KBr) 1658 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 1.38 [d, J = 6.7 Hz, 6H, $CH(CH_3)_2$], 3.76−3.83 [m, 1H, CH(CH$_3$)$_2$], 6.00 (s, 2H, N$^+$CH_2), 8.27 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.74 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.08 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Methoxycarbonylmethyl)pyridinium Bromide (11d).** This product was obtained as a white solid (0.41 g, 32.4%) by reaction of pyridine with **10d**: mp 176−178 °C (lit. 174−175 °C[37]); IR (KBr): 1745 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 3.75 (s, 3H, OCH_3), 5.93 (s, 2H, N$^+$CH_2), 8.04 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.54 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.30 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Ethoxycarbonylmethyl)pyridinium Bromide (11e).** This product was obtained as a white solid (0.43 g, 31.8%) by reaction of pyridine with **10e**: mp 132−134 °C (lit. 134−136 °C[37]); IR (KBr): 1750 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 1.23 (t, J = 6.7 Hz, 3H, OCH_2CH_3), 4.20 (q, J = 6.7 Hz, 2H, OCH_2CH_3), 5.65 (s, 2H, N$^+$CH_2), 8.22 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.69 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.04 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Isopropyloxycarbonylmethyl)pyridinium Bromide (11f).** This product was obtained as a white solid (0.92 g, 65%) by reaction of pyridine with **10f**: mp 90−91 °C (lit. 79−81 °C[37]); IR (KBr) 1751 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 1.25 [d, J = 6.7 Hz, 6H, OCH(CH_3)$_2$], 5.01−5.13 [m, 1H, OCH(CH$_3$)$_2$], 5.72 (s, 2H, N$^+$CH_2), 8.27 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.74 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.14 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(*tert*-Butoxycarbonylmethyl)pyridinium Bromide (11g).** This product was obtained as a semisolid (0.70 g, 47%) by reaction of pyridine with **10g**: IR (KBr): 1746 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 1.58 [s, 9H, OC(CH_3)$_3$], 5.83 (s, 2H, N$^+$CH_2), 8.27 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.73 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.14 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Isobutoxycarbonylmethyl)pyridinium Bromide (11h).** This product was obtained as a white solid (1.0 g, 68%) by reaction of pyridine with **10h**: mp 86−88 °C; IR (KBr): 1748 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 0.88 [d, J = 6.7 Hz, 6H, $OCH_2CH(CH_3)_2$], 1.84−1.95 [m, 1H, $OCH_2CH(CH_3)_2$], 3.96 [d, J = 6.7 Hz, 2H, $OCH_2CH(CH_3)_2$], 5.80 (s, 2H, N$^+$CH_2), 8.27 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.74 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.15 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(4-Chlorobenzyloxycarbonylmethyl)pyridinium Bromide (11i).** This product was obtained as a semisolid (0.71 g, 38%) by reaction of pyridine with **10i**: IR (KBr): 1746 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 5.26 (s, 2H, 4-Cl−C$_6$H$_4$−CH_2), 5.79 (s, 2H, N$^+$CH_2), 7.41 (d, J = 8.5 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.45 (d, J = 8.5 Hz, 2H, 4-chlorophenyl H-3, H-5), 8.26 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.73 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.14 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Methylthiocarbonylmethyl)pyridinium Bromide (11j).** This product was obtained as a white solid (0.93 g, 68.3%) by reaction of pyridine with **10j**: mp 225−226 °C; IR (KBr): 1667 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 2.5 (s, 3H, SCH_3), 5.99 (s, 2H, N$^+$CH_2), 8.23 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.66 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.02 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6). Anal. (C$_8$H$_{10}$BrNOS): C, H, N.

***N*-(Ethylthiocarbonylmethyl)pyridinium Bromide (11k).** This product was obtained as a white solid (0.50 g, 33%) by reaction of pyridine with **10k**: mp 173−175 °C; IR (KBr): 1673 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6): δ 1.24 (t, J = 6.7 Hz, 3H, SCH_2CH_3), 3.00 (q, J = 6.7 Hz, 2H, SCH_2CH_3), 5.99 (s, 2H,

N^+CH_2), 8.26 (dd, $J = 7.9$, $J = 5.5$ Hz, 2H, pyridine H-3, H-5), 8.73 (dd, $J = 7.9$, $J = 7.9$ Hz, 1H, pyridine H-4), 9.05 (d, $J = 5.5$ Hz, 2H, pyridine H-2, H-6).

N*-(Propylthiocarbonylmethyl)pyridinium Bromide (11*l*).** This product was obtained as a viscous oil (0.65 g, 43%) by reaction of pyridine with **10*l: IR (KBr): 1675 cm^{-1} (C═O); ^1H NMR (DMSO-d_6): δ 0.90 (t, $J = 7.3$ Hz, 3H, $SCH_2CH_2CH_3$), 1.52−1.55 (m, 2H, $SCH_2CH_2CH_3$), 2.96 (t, 2H, $J = 7.0$ Hz, $SCH_2CH_2CH_3$), 6.00 (s, 2H, N^+CH_2), 8.23 (dd, $J = 7.9$, $J = 5.5$ Hz, 2H, pyridine H-3, H-5), 8.70 (dd, $J = 7.9$, $J = 7.9$ Hz, 1H, pyridine H-4), 9.04 (d, $J = 5.5$ Hz, 2H, pyridine H-2, H-6).

***N*-(Isopropylthiocarbonylmethyl)pyridinium Bromide (11m).** This product was obtained as a semisolid (0.57 g, 38%) by reaction of pyridine with **10m**: IR (KBr): 1672 cm^{-1} (C═O); ^1H NMR (DMSO-d_6): δ 1.38 [d, $J = 6.7$ Hz, 6H, $SCH(CH_3)_2$], 3.76−3.83 [m, 1H, $SCH(CH_3)_2$], 6.00 (s, 2H, N^+CH_2), 8.27 (dd, $J = 7.9$, $J = 5.5$ Hz, 2H, pyridine H-3, H-5), 8.74 (dd, $J = 7.9$, $J = 7.9$ Hz, 1H, pyridine H-4), 9.08 (d, $J = 5.5$ Hz, 2H, pyridine H-2, H-6).

General Procedure for the Synthesis of 6-Alkyl, -alkoxy-, or -alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (14a−v). To a solution of the *N*-alkyl, -alkoxy-, or -alkylthiocarbonylmethylpyridinium salt selected (**11a**−**m**, 2.4 mmol) in dry benzene (60 mL) at 25 °C was added freshly distilled triethylamine (1 mL, 7 mmol). To this reaction mixture was added 2-(4-methanesulfonylphenyl)-3-phenylcycloprop-2-en-1-one (**9a**, 0.29 g, 1 mmol) or 2-(4-methanesulfonylphenyl)-3-(4-fluorophenyl)cycloprop-2-en-1-one (**9b**, 0.31 g, 1 mmol), and the mixture was stirred for 16−18 h at 25 °C. The solvent was evaporated under reduced pressure, and the residue obtained was purified by silica gel column chromatography using hexanes−ethyl acetate (1:2, v/v or 1:3, v/v) as eluent to afford the respective title compound **14a**−**v** in 8−45% yield. Some physical and spectroscopic data for **14a**−**v** are listed below.

6-Methyl-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14a). The product was obtained as a solid by condensation of **9a** with **11a** in the presence of triethylamine (0.03 g, 9%): mp 140−141 °C; IR (KBr): 1710 (C═O), 1306, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3), 3.02 (s, 3H, SO_2CH_3), 6.24 (s, 1H, pyranone H-5), 7.04−7.07 (m, 2H, phenyl H-2, H-6), 7.22−7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.36 (d, $J = 8.5$ Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{19}H_{16}O_4S$): C, H.

6-Methyl-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (14b). The product was obtained as a solid by condensation of **9b** with **11a** in the presence of triethylamine (0.03 g, 8%): mp 156−158 °C; IR (KBr): 1712 (C═O), 1313, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3), 3.03 (s, 3H, SO_2CH_3), 6.20 (s, 1H, pyranone H-5), 6.94 (dd, $J^3_{HH} = 8.4$, $J^3_{FH} = 8.4$ Hz, 2H, 4-fluorophenyl H-3, H-5), 7.05 (dd, $J^3_{HH} = 8.4$, $J^4_{FH} = 5.2$ Hz, 2H, 4-fluorophenyl H-2, H-6), 7.32 (d, $J = 8.2$ Hz, 4-methanesulfonylphenyl H-2, H-6), 7.70 (d, $J = 8.2$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{19}H_{15}FO_4S$): C, H.

6-Ethyl-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14c). The product was obtained as a solid by condensation of **9a** with **11b** in the presence of triethylamine (0.14 g, 38.3%): mp 172−174 °C; IR (KBr): 1709 (C═O), 1313, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.30 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 2.62 (q, $J = 7.3$ Hz, 2H, CH_2CH_3), 3.02 (s, 3H, SO_2CH_3), 6.22 (s, 1H, pyranone H-5), 7.04−7.08 (m, 2H, phenyl H-2, H-6), 7.22−7.33 (m, 3H, phenyl H-3, H-4, H-5), 7.35 (d, $J = 8.5$ Hz, 4-methanesulfonylphenyl H-2, H-6), 7.77 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{20}H_{18}O_4S$): C, H.

6-Isopropyl-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14d). The product was obtained by condensation of **9a** with **11c** in the presence of triethylamine (0.05 g, 14%): mp 174−175 °C; IR (KBr): 1703 (C═O), 1307, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.34 [d, $J = 7.0$ Hz, 6H, $CH(CH_3)_2$], 2.86−2.91 [m, 1H, $CH(CH_3)_2$], 3.03 (s, 3H, SO_2CH_3), 6.21 (s, 1H, pyranone H-5), 7.05−7.08 (m, 2H, phenyl H-2, H-6), 7.23−7.34 (m, 3H, phenyl H-3, H-4, H-5), 7.36 (d, $J = 8.5$ Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{21}H_{20}O_4S$): C, H.

6-Isopropyl-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (14e). The product was obtained by condensation of **9b** with **11c** in the presence of triethylamine (0.06 g, 16%): mp 188−189 °C; IR (KBr): 1710 (C═O), 1312, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.36 [d, $J = 7.0$ Hz, 6H, $CH(CH_3)_2$], 2.87−2.93 [m, 1H, $CH(CH_3)_2$], 3.05 (s, 3H, SO_2CH_3), 6.13 (s, 1H, pyranone H-5), 6.98 (dd, $J^3_{HH} = 8.7$, $J^3_{FH} = 8.7$ Hz, 2H, 4-fluorophenyl H-3, H-5), 7.07 (dd, $J^3_{HH} = 8.7$, $J^4_{FH} = 5.2$ Hz, 2H, 4-fluorophenyl H-2, H-6), 7.38 (d, $J = 8.2$ Hz, 4-methanesulfonylphenyl H-2, H-6), 7.83 (d, $J = 8.2$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5. Anal. ($C_{21}H_{19}FO_4S$): C, H.

6-Methoxy-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14f). The product was obtained as a solid by condensation of **9a** with **11d** in the presence of triethylamine (0.04 g, 12%): mp 110−112 °C; IR (KBr): 1719 (C═O), 1310, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.02 (s, 3H, SO_2CH_3), 4.03 (s, 3H, OCH_3), 5.59 (s, 1H, pyranone H-5), 7.06−7.09 (m, 2H, phenyl H-2, H-6), 7.21−7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.33 (d, $J = 8.5$ Hz, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{19}H_{16}O_5S$): C, H.

6-Methoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (14g). The product was obtained as a solid by condensation of **9b** with **11d** in the presence of triethylamine (0.05 g, 14%): mp 148−149 °C; IR (KBr): 1720 (C═O), 1315, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.03 (s, 3H, SO_2CH_3), 4.04 (s, 3H, OCH_3), 5.56 (s, 1H, pyranone H-5), 6.93 (dd, $J^3_{HH} = 8.4$, $J^3_{FH} = 8.4$ Hz, 2H, 4-fluorophenyl H-3, H-5), 7.07 (dd, $J^3_{HH} = 8.4$, $J^4_{FH} = 5.2$ Hz, 2H, 4-fluorophenyl H-2, H-6), 7.33 (d, $J = 8.2$ Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, $J = 8.2$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{19}H_{15}FO_5S$): C, H.

6-Ethoxy-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14h). The product was obtained as a solid by condensation of **9a** with **11e** in the presence of triethylamine (0.06 g, 16%): mp 174−176 °C; IR (KBr): 1719 (C═O), 1310, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.46 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 3.02 (s, 3H, SO_2CH_3), 4.32 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3), 5.57 (s, 1H, pyranone H-5), 7.07−7.08 (m, 2H, phenyl H-2, H-6), 7.18−7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.35 (d, $J = 8.5$ Hz, 4-methanesulfonylphenyl H-2, H-6), 7.74 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 14.68 (OCH_2CH_3), 44.51 (SO_2CH_3), 65.73 (OCH_2CH_3), 107.24 (pyranone C-5), 112.71 (pyranone C-3), 126.80 (phenyl C-3, C-5), 128.39 and 128.67 (phenyl C-2, C-6; 4-methanesulfonylphenyl C-2, C-6), 129.21 (phenyl C-4), 132.09 (4-methanesulfonylphenyl C-3, C-5), 137.42 (phenyl C-1), 138.68 (4-methanesulfonylphenyl C-1), 140.45 (4-methanesulfonylphenyl C-4), 159.01 (pyranone C-4), 160.57 (pyranone C-6), 162.75 (pyranone C-2); HRMS *m*/*z* calcd for $C_{20}H_{18}O_5S$, 370.08749, found 370.08729. Anal. ($C_{20}H_{18}O_5S$): C, H.

6-Ethoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (14i). The product was obtained as a solid by condensation of **9b** with **11e** in the presence of triethylamine (0.08 g, 20%): mp 154−156 °C; IR (KBr): 1719 (C═O), 1312, 1146 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.49 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 3.03 (s, 3H, SO_2CH_3), 4.38 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3), 5.55 (s, 1H, pyranone H-5), 6.95 (dd, $J^3_{HH} = 8.3$, $J^3_{FH} = 8.3$ Hz, 2H, 4-fluorophenyl H-3, H-5), 7.08 (dd, $J^3_{HH} = 8.3$, $J^4_{FH} = 5.2$ Hz, 2H, 4-fluorophenyl H-2, H-6), 7.33 (d, $J = 8.5$ Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{20}H_{17}FO_5S$): C, H.

6-Isopropyloxy-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14j). The product was obtained as a solid by condensation of **9a** with **11f** in the presence of triethylamine (0.10 g, 24%): mp 185−187 °C; IR (KBr): 1720 (C═O), 1319, 1140 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.46 [d, $J = 7.0$ Hz, 6H, $OCH(CH_3)_2$], 3.02 (s, 3H, SO_2CH_3), 5.06−5.13 [m, 1H, OC*H*-

$(CH_3)_2$], 5.58 (s, 1H, pyranone H-5), 7.04−7.08 (m, 2H, phenyl H-2, H-6), 7.20−7.32 (m, 3H, phenyl H-3, H-4, H-5), 7.32 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{21}H_{20}O_5S$): C, H.

6-Isopropyloxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (14k). The product was obtained as a solid by condensation of **9b** with **11f** in the presence of triethylamine (0.09 g, 21%): mp 180−181 °C; IR (KBr): 1722 (C═O), 1310, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.46 [d, J = 6.1 Hz, 6H, $OCH(CH_3)_2$], 3.03 (s, 3H, SO_2CH_3), 5.06−5.13 [m, 1H, $OCH(CH_3)_2$], 5.55 (s, 1H, pyranone H-5), 6.94 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.06 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.33 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{21}H_{19}FO_5S$): C, H.

6-*tert*-Butoxy-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14l). The product was obtained as a solid by condensation of **9a** with **11g** in the presence of triethylamine (0.10 g, 25%): mp 129−131 °C; IR (KBr): 1719 (C═O), 1310, 1146 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.63 [s, 9H, $OC(CH_3)_3$], 3.02 (s, 3H, SO_2CH_3), 5.68 (s, 1H, pyranone H-5), 7.04−7.07 (m, 2H, phenyl H-2, H-6), 7.21−7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.38 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{22}H_{22}O_5S$): C, H.

6-*tert*-Butoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (14m). The product was obtained as a solid by condensation of **9b** with **11g** in the presence of triethylamine (0.09 g, 18%): mp 132−133 °C; IR (KBr): 1722 (C═O), 1310, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.55 [s, 9H, $OC(CH_3)_3$], 3.01 (s, 3H, SO_2CH_3), 5.56 (s, 1H, pyranone H-5), 6.95 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.07 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.25 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.70 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{22}H_{21}FO_5S$): C, H.

6-Isobutoxy-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14n). The product was obtained as a solid by condensation of **9a** with **11h** in the presence of triethylamine (0.09 g, 22%): mp 156−158 °C; IR (KBr): 1720 (C═O), 1311, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.05 [d, J = 6.7 Hz, 6H, $OCH_2CH(CH_3)_2$], 2.11−2.19 [m, 1H, $OCH_2CH(CH_3)_2$], 3.01 (s, 3H, SO_2CH_3), 4.03 [d, J = 6.7 Hz, 2H, $OCH_2CH(CH_3)_2$], 5.58 (s, 1H, pyranone H-5), 7.05−7.09 (m, 2H, phenyl H-2, H-6), 7.20−7.30 (m, 3H, phenyl H-3, H-4, H-5), 7.34 (d, J = 8.0 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.0 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{22}H_{22}O_5S$): C, H.

6-Isobutoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (14o). The product was obtained as a solid by condensation of **9b** with **11h** in the presence of triethylamine (0.10 g, 24%): mp 138−139 °C; IR (KBr): 1719 (C═O), 1310, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.06 [d, J = 6.7 Hz, 6H, $OCH_2CH(CH_3)_2$], 2.12−2.18 [m, 1H, $OCH_2CH(CH_3)_2$], 3.03 (s, 3H, SO_2CH_3), 4.06 [d, J = 6.7 Hz, 2H, $OCH_2CH(CH_3)_2$], 5.55 (s, 1H, pyranone H-5), 6.95 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.07 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.32 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.79 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{22}H_{21}FO_5S$): C, H.

6-(4-Chlorobenzyloxy)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14p). The product was obtained as a solid by condensation of **9a** with **11i** in the presence of triethylamine (0.08 g, 16%): mp 157−159 °C; IR (KBr): 1724 (C═O), 1310, 1146 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.02 (s, 3H, SO_2CH_3), 5.32 (s, 2H, 4-Cl−C_6H_4−CH_2O), 5.66 (s, 1H, pyranone H-5), 7.03 (m, 2H, phenyl H-2, H-6), 7.13−7.24 (m, 3H, phenyl H-3, H-4, H-5), 7.27 (d, 2H, J = 8.4 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.31 (d, J = 8.5 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.37 (d, J = 8.5 Hz, 2H, 4-chlorophenyl H-3, H-5), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{25}H_{19}ClO_5S$): C, H.

6-(4-Chlorobenzyloxy)-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (14q). The product was obtained as a solid by condensation of **9b** with **11i** in the presence of triethylamine (0.07 g, 14%): mp 173−174 °C; IR (KBr): 1722 (C═O), 1310, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.03 (s, 3H, SO_2CH_3), 5.32 (s, 2H, 4-Cl-C_6H_4-CH_2O), 5.63 (s, 1H, pyranone H-5), 6.94 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.05 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.32 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.41 (d, J = 8.5 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.44 (d, J = 8.5 Hz, 2H, 4-chlorophenyl, H-3, H-5), 7.79 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{25}H_{18}ClFO_5S$): C, H.

6-Methylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14r). The product was obtained as a solid by condensation of **9a** with **11j** in the presence of triethylamine (0.14 g, 34.3%): mp 207−208 °C; IR (KBr): 1725 (C═O), 1308, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.6 (s, 3H, SCH_3), 3.02 (s, 3H, SO_2CH_3), 6.29 (s, 1H, pyranone H-5), 7.05−7.07 (m, 2H, phenyl H-2, H-6), 7.22−7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.33 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.76 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{19}H_{16}O_4S_2 \cdot 1/2H_2O$): C, H.

6-Ethylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14s). The product was obtained as a solid by condensation of **9a** with **11k** in the presence of triethylamine (0.06 g, 15%): mp 175−176 °C; IR (KBr): 1718 (C═O), 1314, 1153 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.46 (t, J = 7.3 Hz, 3H, SCH_2CH_3), 3.03 (s, 3H, SO_2CH_3), 3.15 (q, J = 7.3 Hz, 2H, SCH_2CH_3), 6.35 (s, 1H, pyranone H-5), 7.04−7.07 (m, 2H, phenyl H-2, H-6), 7.21−7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.35 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 14.75 (SCH_2CH_3), 25.78 (SCH_2CH_3), 44.51 (SO_2CH_3), 107.34 (pyranone C-5), 117.56 (pyranone C-3), 126.89 (phenyl C-3, C-5), 128.40 and 128.65 (phenyl C-2, C-6; 4-methanesulfonylphenyl C-2, C-6), 129.34 (phenyl C-4), 131.93 (4-methanesulfonylphenyl C-3, C-5), 136.50 (phenyl C-1), 139.18 (4-methanesulfonylphenyl C-1), 139.89 (4-methanesulfonylphenyl C-4), 154.26 (pyranone C-4), 161.69 (pyranone C-6), 162.20 (pyranone C-2). Anal. ($C_{20}H_{18}O_4S_2$): C, H.

6-Ethylthio-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (14t). The product was obtained as a solid by condensation of **9b** with **11k** in the presence of triethylamine (0.07 g, 17%): mp 171−172 °C; IR (KBr): 1716 (C═O), 1313, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.46 (t, J = 7.3 Hz, 3H, SCH_2CH_3), 3.04 (s, 3H, SO_2CH_3), 3.15 (q, J = 7.3 Hz, 2H, SCH_2CH_3), 6.32 (s, 1H, pyranone H-5), 6.96 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.07 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.35 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.80 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{20}H_{17}FO_4S_2$): C, H.

6-*n*-Propylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14u). The product was obtained as a solid by condensation of **9a** with **11l** in the presence of triethylamine (0.14 g, 32%): mp 158−160 °C; IR (KBr): 1727 (C═O), 1308, 1157 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.06 (t, J = 7.3 Hz, 3H, $SCH_2CH_2CH_3$), 1.56−1.87 (m, 2H, $SCH_2CH_2CH_3$), 3.02 (s, 3H, SO_2CH_3), 3.09 (t, J = 7.3 Hz, 2H, $SCH_2CH_2CH_3$), 6.35 (s, 1H, pyranone H-5), 7.04−7.06 (m, 2H, phenyl H-2, H-6), 7.22−7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.34 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.76 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 13.28 ($SCH_2CH_2CH_3$), 22.86 ($SCH_2CH_2CH_3$), 33.24 ($SCH_2CH_2CH_3$), 44.51 (SO_2CH_3), 107.24 (pyranone C-5), 117.40 (pyranone C-3), 126.93 (phenyl C-3, C-5), 128.40 and 128.80 ((phenyl C-2, C-6; 4-methanesulfonylphenyl C-2, C-6), 129.35 (phenyl C-4), 131.94 (4-methanesulfonylphenyl C-3, C-5), 136.44 (phenyl C-1), 139.02 (4-methanesulfonylphenyl C-1), 139.91 (4-methanesulfonylphenyl C-4), 154.32 (pyranone C-4), 161.92 (pyranone C-6), 162.28 (pyranone C-2). Anal. ($C_{21}H_{20}O_4S_2$): C, H.

6-Isopropylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14v). The product was obtained by condensation of **9a** with **11m** in the presence of triethylamine (0.19 g, 44.5%): mp 164−166 °C; IR (KBr): 1721 (C═O), 1310, 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.42 [d, J = 6.7 Hz, 6H, SCH(CH_3)$_2$], 3.01 (s, 3H, SO_2CH_3), 3.83−3.92 [m, 1H, SCH(CH_3)$_2$], 6.39 (s, 1H, pyranone H-5), 7.02−7.05 (m, 2H, phenyl H-2, H-6), 7.20−7.29 (m, 3H, phenyl H-3, H-4, H-5), 7.33 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{21}H_{20}O_4S_2$): C, H.

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, Ann Arbor, MI) according to the manufacturer's instructions. 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). Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (960 μL, 0.1 M Tris-HCl pH 8.0 containing 5 mM EDTA and 2 mM phenol) with either COX-1 or COX-2 (10 μL) enzyme in the presence of heme (10 μL) was added 10 μL of various concentrations of test drug solutions (0.001, 0.01, 0.1, 1, 10, 100, and 500 μM in a final volume of 1 mL). These solution were incubated for a period of 2 min at 37 °C after which 10 μL of AA (100 μM) was added, and the COX reaction was stopped by the addition of 50 μL of 1 M HCl after 2 min. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, was measured by enzyme immunoassay. 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 since the concentration of the PG tracer is held constant while the concentration of PGs varies. This antibody−PG complex binds to a mouse anti-rabbit monoclonal antibody that had 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 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.[38,39]

Analgesic Assay. Analgesic activity was determined using the 4% sodium chloride-induced writhing (abdominal constriction) assay as described previously. [40,41]

Molecular Modeling (Docking) Studies. Docking experiments were performed using Insight II software Version 2000.1 (Accelrys Inc.) running on a Silicon Graphics Octane 2 R14000A workstation. The coordinates for the X-ray crystal structures of the enzymes COX-1 and COX-2 were obtained from the RCSB Protein Data Bank and hydrogens were added. The ligand molecules were constructed using the Builder module and then energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The docking experiment on COX-2 was carried out by superimposing the energy minimized ligand on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. In the case of COX-1 (PDB file 1prh), the ligand was suitably positioned in the active site while carefully monitoring nonbonded interactions of the ligand−enzyme assembly and any side chain bumps. The resulting ligand−enzyme complex was subjected to docking using the Affinity command in the Docking module of Insight II after defining subsets of the enzyme such that residues within 10 Å of the ligand were allowed to relax, while the remainder of the enzyme residues were fixed. The consistent valence force field (CVFF) was employed for all docking purposes. The optimal binding orientation of the ligand−enzyme assembly obtained after docking was further minimized for 1000 iterations using the conjugate gradient method until a convergence of 0.001 kcal/mol Å was reached. The ligand−enzyme assembly was then subjected to a molecular dynamics (MD) simulation using the Discover module Version 2.98 at a constant temperature of 300 K with a 200-step equilibration for over 5000 iterations and a time step of 1 fs using a distance dependent dielectric constant 4r.

Acknowledgment. We are grateful to the Canadian Institutes of Health Research (MOP-14712) for financial support of this research and to Rx & D-HRF/CIHR for a graduate scholarship (to P.R.) The technical assistance of C.-A. McEwen in performing the antiinflammatory and analgesic assays is gratefully acknowledged.

References

(1) Masferrer, J. L.; Zweifel, B. S.; Manning, P. T.; Hauser, S. D.; Leahy, K. M.; Smith, W. G.; Isakson, P. C.; Seibert, K. Selective inhibition of inducible cyclooxygenase-2 in vivo is antiinflammatory and nonulcerogenic. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3228−3232.

(2) Chan, C. C.; Boyce, S.; Brideau, C.; Ford-Hutchinson, A. W.; Gordon, R.; Guay, D.; Hill, R. G.; Li, C. S.; Mancini, J.; Penneton, M.; Prasit, P.; Rasori, R.; Riendeau, D.; Roy, P.; Tagari, P.; Vickers, P.; Wong, E.; Rodger, I. W. Pharmacology of a selective cyclooxygenase-2 inhibitor, L-745,337: a novel nonsteroidal antiinflammatory agent with an ulcerogenic sparing effect in rat and nonhuman primate stomach. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 1531−1537.

(3) Silverstein, F. E.; Faich, G.; Goldstein, J. L.; Simon, L. S.; Pincus, T.; Whelton, A.; Makuch, R.; Eisen, G.; Agrawal, N. M.; Stenson, W. F.; Burr, A. M.; Zhao, W. W.; Kent, J. D.; Lefkowith, J. B.; Verburg, K. M.; Geis, G. S. Gastrointestinal toxicity with celecoxib vs nonsteroidal antiinflammatory drugs for osteoarthritis and rheumatoid rrthrits. The class study: a randomized controlled trial. *JAMA* **2000**, *284*, 1247−1255.

(4) Hawkey, C.; Laine, L.; Simon, T.; Beaulieu, A.; Maldonado, C. J.; Acevedo, E.; Shahane, A.; Quan, H.; Bolognese, J.; Mortensen, E. Comparison of the effect of rofecoxib (a cyclooxygenase-2 inhibitor), ibuprofen and placebo on the gastroduodenal mucosa of patients with osteoarthritis: a randomized, double-blind, placebo-controlled trial. The rofecoxib osteoarthritis endoscopy multinational study group. *Arthritis Rheum.* **2000**, *43*, 370−377.

(5) Penning, T.; Talley, J. J.; Bertenshaw, S.; Carter, J.; Collins, P.; Docter, S.; Graneto, M.; Lee, L.; Malecha, J.; Miyashiro, J.; Rogers, R.; Rogier, D.; Yu, S.; Anderson, G.; Burton, E.; Cogburn, J.; Gregory, S.; Koboldt, C.; Perkins, W.; Seibert, K.; Veenhuizen, A.; Zhang, Y.; Isakson, P. Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl)] benzenesulfonamide (SC-58635, Celecoxib). *J. Med. Chem.* **1997**, *40*, 1347−1365.

(6) Chan, C. C.; Boyce, S.; Brideau, C.; Charleson, S.; Cromlish, W.; Ethier, D.; Evans, J.; Ford-Hutchinson, A. W.; Forrest, M. J.; Gauthier, J. Y.; Gordon, R.; Gresser, M.; Guay, J.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Leger, S.; Mancini, J.; O'Neill, G. P.; Ouellet, M.; Patrick, D.; Percival, M. D.; Perrier, H.; Prasit, P.; Rodger, I. Rofecoxib [Vioxx, MK-0966; 4-(4′-methylsulfonylphenyl)-3-phenyl-2-(5*H*)-furanone]: a potent and orally active cyclooxygenase-2 inhibitor. Pharmacological and biochemical profiles. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 551−560.

(7) Kawamori, T.; Rao, C. V.; Seibert, K.; Reddy, B. S. Chemopreventive activity of celecoxib, a specific cylooxygenase-2 inhibitor, against colon carcinogenesis. *Cancer. Res.* **1998**, *58*, 409−412.

(8) Ghosh, A. J.; Hirasawa, N.; Niki, H.; Ohuchi, K. Cyclooxygenase-2 mediated Angiogenesis in carrageenan-induced granulation tissue in rats. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 802−809.

(9) Katori, M.; Majima, M. Cyclooxygenase-2: its rich diversity of roles and possible application of its selective inhibitors. *Inflamm. Res.* **2000**, *49*, 367−392.

(10) Vane, J. R.; Botting, R. M. Antiinflammatory drugs and their mechanism of action. *Inflamm. Res.* **1998**, *47*, S78−S87.

(11) Friesen, R. W.; Dube, D.; Fortin, R.; Frenette, R.; Prescott, S.; Cromlish, W.; Greig, G. M.; Kargman, S.; Wong, E.; Chan, C. C.; Gordon, R.; Xu, L. J. Novel 1,2-diarylcyclobutenes: Selective and orally active COX-2 inhibitors. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2677−2682.

(12) Li, J. J.; Anderson, G. D.; Gregory, S. A.; Huang, H. C.; Isakson, P. C.; Koboldt, C. M.; Logusch, E. W.; Norton, M. B.; Perkins, W. E.; Reinhard, E. J.; Seibert, K.; Veenhuizen, A. W. 1,2-Diarylcyclopentenes as selective cyclooxygenase-2 inhibitors and orally active antiinflammatory agents. *J. Med. Chem.* **1995**, *38*, 4570−4578.

(13) Talley, J. J.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. 4-[5-Methyl-3-phenylisoxazol-4-yl]-benzenesulfonamide, valdecoxib: a potent and selective inhibitor of COX-2. *J. Med. Chem.* **2000**, *43*, 775−777.

(14) Prasit, P.; Wang, Z.; Brideau, C.; Chan, C. C.; Charleson, S.; Cromlish, W.; Ethier, D.; Evans, J. F.; Ford-Hutchinson, A. W.; Gauthier, J. Y.; Gordon, R.; Guay, J.; Gresser, M.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Leger, S.; Mancini, J.; O′Neill, G. P.; Quellet, M.; Percival, M. D.; Perrier, H.; Riendeau, D.; Rodger, I.; Tagari, P.; Therien, M.; Vickers, P.; Wong, E.; Xu, L. J.; Young, R. N.; Zamboni, R.; Boyce, S.; Rupniak, N.; Forrest, M.; Visco, D.; Patrick, D.; The discovery of rofecoxib, [MK 966, Vioxx, 4-(4′-methylsulfonylphenyl)-3-phenyl-2(*5H*)-furanone], an orally active cyclooxygenase-2 inhibitor. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1773−1778.

(15) Riendeau, D.; Percival, M. D.; Brideau, C.; Dube, C. S.; Ethier, D.; Falgueyret, J. P.; Friesen, R. W.; Gordon, R.; Greig, G.; Guay, J.; Mancini, J.; Ouellet, M.; Wong, E.; Xu, L.; Boyce, S.; Visco, D.; Girard, Y.; Prasit, P.; Zamboni, R.; Rodger, I. W.; Gresser, M.; Ford-Hutchinson, A. W.; Young, R. N.; Chan, C. C. Etoricoxib (MK-0663): preclinical profile and comparison with other agents that selectively inhibit cyclooxygenase-2. *J. Pharmacol. Exp. Ther.* **2002**, *296*, 558−566.

(16) Talley, J. J. Selective inhibitors of cyclooxygenase-2 (COX-2). *Progr. Med. Chem.* **1999**, *36*, 201−233.

(17) Rao, P. N. P.; Amini, M.; Li, H.; Habeeb, A. G.; Knaus, E. E. 6-Alkyl, alkoxy, or alkylthio-substituted 3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones: a novel class of diarylheterocyclic selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2205−2209.

(18) Li, C. S.; Lau, C. K.; Therien, M.; Prasit, P. Pyrones as inhibitors of cyclooxygenase-2. World Patent WO 02/02547, 10 January 10, 2002; *Chem. Abstr. 136*, 85758.

(19) Li, H.; Rao, P. N. P.; Habeeb, A. G.; Knaus, E. E. Design, syntheses, and evaluation of 2,3-diphenylcycloprop-2-en-1-ones and oxime derivatives as potential cyclooxygenase-2 (COX-2) inhibitors with analgesic-antinflammatory activity. *Drug Dev. Res.* **2002**, *57*, 6−17.

(20) Hayashi, Y.; Nozaki, H. Reaction of sulphonium ylides with diphenylcyclopropenone. A new synthesis of 2-pyrones. *Tetrahedron* **1971**, *27*, 3085−3093.

(21) Sasaki, T.; Kanematsu, K.; Kakehi, A. Formation of 1,3-oxazine and 2-pyrone derivatives from the reaction of pyridinium ylides with diphenylcyclopropenone. *J. Org. Chem.* **1971**, *36*, 2451−2453.

(22) Henry, W. P.; Hughes, R. P. Organic synthesis using carbon monoxide. Regiospecific cobalt-mediated synthesis of 2*H*-pyran-2-ones. *J. Am. Chem. Soc.* **1986**, *108*, 7862−7864.

(23) Habeeb, A. G.; Rao, P. N. P.; Knaus, E. E. Design and synthesis of 4,5-diphenyl-4-isoxazolines: novel inhibitors of cyclooxygenase-2 with analgesic and antiinflammatory activity. *J. Med. Chem.* **2001**, *44*, 2921−2927.

(24) Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Pak, J. Y.; Gildehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K.; Isakson, P. C.; Stallings, W. C. Structural basis for selective inhibition of cyclooxygenase-2 by antiinflammatory agents. *Nature* **1996**, *384*, 644−648.

(25) Gierse, J. K.; McDonald, J. J.; Hauser, S. D.; Rangwala, S. H.; Koboldt, C. M.; Seibert, K. A single amino acid difference between cyclooxygenase-1 (COX-1) and −2 (COX-2) reverses the selectivity of COX-2 specific inhibitors. *J. Biol. Chem.* **1996**, *271*, 15810−15814.

(26) Soliva, R.; Almansa, C.; Kalko, S. G.; Luque, F. J.; Orozco, M. Theoretical studies on the inhibition mechanism of cyclooxygenase-2. is there a unique recognition site? *J. Med. Chem.* **2003**, *46*, 1372−1382.

(27) Llorens, O.; Perez, J. J.; Palomer, A.; Mauleon, D. Differential binding mode of diverse cyclooxygenase inhibitors. *J. Mol. Graphics Modell.* **2002**, *20*, 359−371.

(28) Wong, E.; Bayly, C.; Waterman, H. L.; Riendeau, D.; Mancini, J. A. Conversion of prostaglandin G/H synthase-1 into an enzyme sensitive to PGHS-2-selective inhibitors by a double His^{513}→Arg and Ile^{523}→Val mutation. *J. Biol. Chem.* **1997**, *272*, 9280−9286.

(29) Hood, W. F.; Gierse, J. K.; Isakson, P. C.; Kiefer, J. R.; Kurumbail, R. G.; Seibert, K.; Monahan, J. B. Characterization of celecoxib and valdecoxib binding to cyclooxygenase. *Mol. Pharmacol.* **2003**, *63*, 870−877.

(30) Pinheiro, R. M.; Calixto, J. B. Effect of the selective COX-2 inhibitors, celecoxib and rofecoxib in rat acute models of inflammation. *Inflamm. Res.* **2002**, *51*, 603−610.

(31) Salvemini, D.; Wang, Z. Q.; Wyatt, P. S.; Bourdon, D. M.; Marino, M. H.; Manning, P. T.; Currie, M. G. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. *Br. J. Pharmacol.* **1996**, *118*, 829−838.

(32) Ahmad, N.; Chen, L. C.; Gordon, M. A.; Laskin, J. D.; Laskin, D. L. Regulation of cyclooxygenase-2 by nitric oxide in activated hepatic macrophages during acute endotoxemia. *J. Leukoc. Biol.* **2002**, *71*, 1005−1011.

(33) Niederberger, E.; Tegeder, I.; Schafer, C.; Seegel, M.; Grosch, S.; Geisslinger, G. Opposite effects of rofecoxib on nuclear factor-κB and activating protein-1 activation. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 1153−1160.

(34) Svensson, C. I.; Yaksh, T. L. The spinal phospholipase-cyclooxygenase-prostanoid cascade in nociceptive processing. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 553−583.

(35) Dannhardt, G. K. W. COX-2 inhibition and control of pain. *Curr. Opin. Investig. Drugs* **2002**, *3*, 1348−1358.

(36) Markovac, A.; Patel, A. R.; LaMontagne, M. P.; Ash, A. B. 4-(3-Nitrophenyl)-2-picoline. *J. Heterocycl. Chem.* **1977**, *14*, 147−148.

(37) Banks, R. E.; Flowers, W. T.; Khaffaf, S. N. Fluorocarbon derivatives of nitrogen. part 19. synthesis and mass spectrometer analysis of some pyridinium (tetrafluoro-4-pyridyl)-methylides. *J. Fluorine Chem.* **1991**, *53*, 127−142.

(38) Winter, C. A.; Risley, E. A.; Nuss, G. W.; Carrageenan-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544−552.

(39) Kumar, P.; Knaus, E. E. Synthesis and antiinflammatory activity of N-substituted dihydropyridylacetic acids, esters and amides. *Drug Design Delivery* **1987**, *2*, 145−149.

(40) Fukawa, K.; Kawano, O.; Hibi, M.; Misaka, N.; Ohba, S.; Hatanaka, Y. Method for evaluating analgesic agents in rats. *J. Pharmacol. Methods* **1980**, *4*, 251−259.

(41) Buolamwini, J. K.; Knaus, E. E. Synthesis and antinociceptive activity of 4-pyridyl and dihydropyridyl analogues of meperidine and ketobemidone. *Drug Des. Delivery* **1990**, *7*, 19−31.

JM0302391