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6-Alkyl, Alkoxy, or Alkylthio-Substituted 3-(4-Methanesulfonylphenyl)-4-phenylpyran-2-ones: A Novel Class of Diarylheterocyclic Selective Cyclooxygenase-2 Inhibitors

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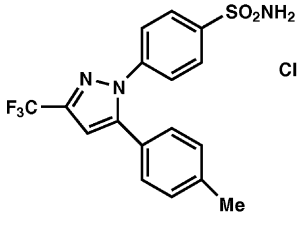
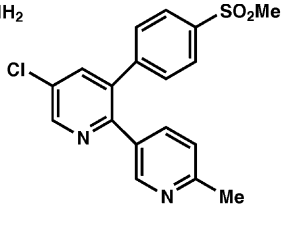
Abstract—A novel class of 3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones possessing a central six-membered lactone (pyran-2-one) ring system, in conjunction with C-6 alkyl (Me, Et or *i*-Pr), alkoxy (OMe, OEt or O-*i*-Pr), and alkylthio (SMe, SEt or S-*i*-Pr) substituents, were designed for evaluation as selective COX-2 inhibitors.

Selective cyclooxygenase-2 (COX-2) inhibitors currently provide effective treatment of inflammatory disease states such as rheumatoid arthritis and osteoarthritis.[1] Recent studies have shown that selective COX-2 inhibitors can also induce apoptosis in colon, stomach, prostate and breast cancer cell lines.[2] Selective COX-2 inhibitors offer potential for the prophylactic prevention of inflammatory neurodegenerative disorders such as Alzheimer's disease.[3]

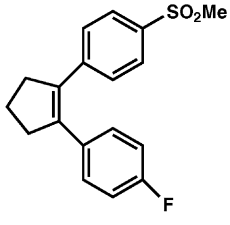
Diarylheterocycles constitute a major class of selective COX-2 inhibitors. In this regard, celecoxib (**1**) possesses a central five-membered pyrazole ring, whereas etoricoxib (**2**) has a central six-membered pyridine ring.[4] Extensive structural–activity relationship (SAR) studies for the diarylheterocycle class have shown that a SO_2NH_2 or SO_2Me and F substituents at the *para*-position of one of the aryl rings often provides optimum COX-2 selectivity and potency.[5] Thus, the selective COX-2 inhibitor SC 57666 (**3**) has a sulfonylmethyl group at the *para*-position of one phenyl ring along with a fluorine atom at the *para*-position on the other phenyl ring.[6] The highly selective COX-2 inhibitor rofecoxib (**4**) belongs to a diarylheterocyclic class that possesses a central five-membered lactone, [2(5*H*)furanone], ring system.[7] We describe herein the design, synthesis and biological evaluation of a novel class of diarylheterocyclic, 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones, that possesses a central six-membered lactone (pyran-2-one) ring.



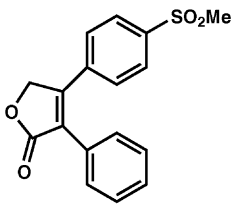
Celecoxib (**1**)



Etoricoxib (**2**)



SC 57666 (**3**)



Rofecoxib (**4**)

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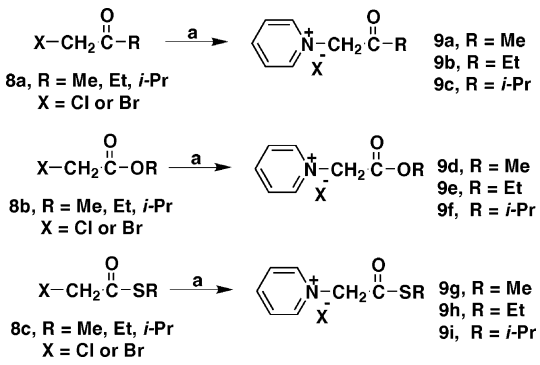
The synthetic reactions used for the synthesis of 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**10a–c**, **11a–c**, and **12a–c**) are outlined in Schemes 1–3. The 2,3-diphenylcyclopropenone (**6**) with a thiomethyl substituent at the *para*-position of one of the phenyl rings was prepared in moderate yields (22–33%) using a one-pot reaction starting with tetrachlorocyclopropene (**5**). The sequential arylation of **5** with benzene and methylthiobenzene, followed by hydration with ice-water yielded 2-(4-methylthiophenyl)-3-phenylcycloprop-2-en-1-one (**6**) as the major product along with the 2-(2-methylthiophenyl)-3-phenylcycloprop-2-en-1-one regioisomer as a minor roduct (ratio 4:1) which could not be purified by column chromatography. Subsequent oxidation of **6** using aqueous Oxone® solution afforded the methane sulfonyl product **7** as shown in Scheme 1.[8] The *N*-alkyl, alkoxy or alkylthiocarbonylmethyl pyridinium chlorides or bromides (**9a–i**, 33–68%) were prepared by the reaction of the respective alkyl, alkoxy or alkylthio-substituted chloro or bromoacetate derivatives (**8a**, **8b**, or **8c**) with pyridine as illustrated in Scheme 2.

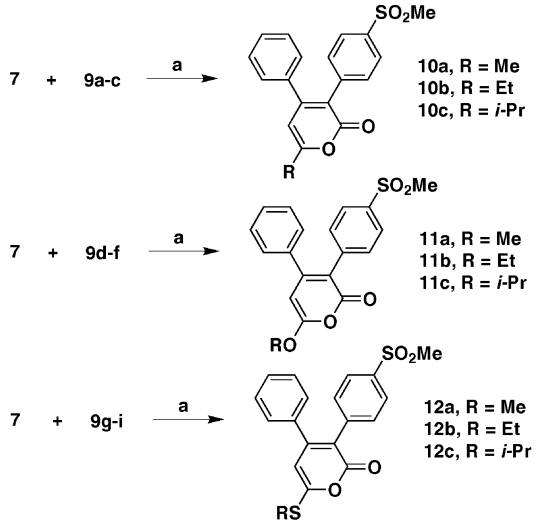
The target 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**10a–c**, **11a–c**, and **12a–c**) were prepared in low to moderate yields (9–44%) by the condensation of **7** with the respective pyridinium chlorides or bromides (**9a–c**, **9d–f**, or **9g–i**) in the presence of the base Et_3N to produce an intermediate ylide product that undergoes a ring expansion reaction to afford the title products (**10a–c**, **11a–c**, or **12a–c**) as illustrated in Scheme 3.[9] The structures of **10–12** were confirmed by microanalyses data and ^1H NMR NOE studies which showed NOE interactions between H-5 and the 6-alkyl (R), alkoxy (O-R) or alkylthio (S-R) moiety, and between H-5 and the C-4 *ortho*-phenyl hydrogens, which establishes the regiochemistry of the C-4 and C-5 phenyl rings.

The effect of the C-6 alkyl (**10a–c**), alkoxy (**11a–c**), and alkylthio (**12a–c**) substituents on the central six-membered lactone (pyran-2-one) ring on COX-2 selectivity and potency was determined by in vitro COX-1/COX-2 inhibition studies. The structure–activity relationships acquired showed that this lactone class of compounds are moderate to potent selective COX-2 inhibitors (see data in Table 1).

The 6-alkyl-3-(4-methanesulfonylphenyl)-4-phenyl pyran-2-ones (**10a–c**), show weak to moderate COX-1 inhibition (8.0–614.8 μM range) with good COX-2 inhibition in the 0.5–1.5 μM range. In the alkoxy series (**11a–c**), good COX-2 inhibitory activity and selectivity was shown by the 6-ethoxy derivative **11b** (COX-1



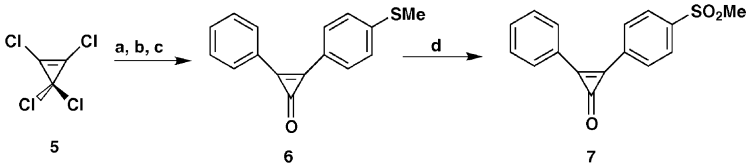
Scheme 2. Reagents and conditions: (a) pyridine, THF, 25 °C, 6 h.



Scheme 3. Reagents and conditions: (a) benzene, triethylamine, 25 °C, 16–18 h.

IC_{50} = 281.5 μM; COX-2 IC_{50} = 1.3 μM; COX-2 Selectivity Index = 216.5). Introduction of a thioethyl (EtS-) substituent at C-6 of the central pyran-2-one ring led to a dramatic increase in COX-2 selectivity and potency, with **12b** showing a weak COX-1 inhibition (COX-1 IC_{50} = 386.2 μM) and potent inhibition of COX-2 (COX-2 IC_{50} = 0.0032 μM) for a very high COX-2 S.I. > 120,000 relative to the reference drug rofecoxib (COX-2 IC_{50} = 0.4279; S.I. > 1168).

The critical difference between the binding sites for COX-1 and COX-2 is at position 523 where COX-2 has the amino acid residue Val in place of the bulkier Ile in COX-1. This difference produces a secondary pocket extending off the primary binding site in COX-2 that is absent in COX-1. Consequently, the combined volume of the primary binding site and the secondary pocket in COX-2 is about 25% larger (394 Å^3) than the volume of



Scheme 1. Reagents and conditions: (a) dry $AlCl_3$, 1,2-dichloroethane, benzene, 25 °C, 24 h; (b) thioanisole, 25 °C, 24 h; (c) H_2O, 25 °C, 10 min; (d) aqueous Oxone®, THF–MeOH (1:1), 25 °C, 4–5 h.

Table 1. In vitro inhibition of COX-1 and COX-2 by 6-alkyl, 6-alkoxy or 6-alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**10a–c**, **11a–c**, and **12a–c**)

Compd	COX-1 inhibition IC_{50}, μM[a]	COX-2 inhibition IC_{50}, μM[a]	COX-2 S.I.[b]	Volume (Å^3)[c]
10a	614.8	0.68	904.0	293.56
10b	8.0	1.5	5.3	310.18
10c	341.5	0.50	683.0	326.69
11a	14.7	28.3	<0.52	301.69
11b	281.5	1.3	216.5	318.75
11c	4.0	2.0	2.0	336.04
12a	>100	2.8	35.7	311.54
12b	386.2	0.0032	120,687.5	328.56
12c	>100	>100		345.65
Rofecoxib	>500	0.4279	>1,168	267.20
Celecoxib	22.9	0.0567	404	298.56

[a]Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit (Catalog No. 560101, Cayman Chemicals Inc., Ann Arbor, MI) and the 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]The volume of the molecule, after minimization using the MM3 forcefield, was calculated using the Alchemy 2000 program.

the COX-1 binding site (316 Å^3).[10] This difference in volume can be exploited to manipulate COX-2 selectivity of diarylheterocyclic classes of COX-2 inhibitors, by varying the volume of the drug and the appropriate placement of substituents with varying electronic and steric properties.[11]

It is well established for the diarylheterocyclic class of COX-2 inhibitors, that a *para*-methylsulfone or sulfonamide substituent on one of the phenyl rings is a requirement for good COX-2 potency and selectivity.[5] Accordingly, the 6-alkyl, 6-alkoxy, and 6-alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one group of compounds were designed to have a $-SO_2Me$ substituent at the *para*-position of one of the phenyl rings. Compounds **10a–c**, **11a–c**, and **12a–c** have volumes in the range of 293–345 Å^3, relative to the selective COX-2 inhibitor rofecoxib (267.2 Å^3) as shown in Table 1. In general, for this series of compounds, COX-2 selectivity and potency was dependant upon steric and electronic properties of the C-6 substituent on the central pyran-2-one ring which positions the sulfonylmethyl moiety in the vicinity of the secondary pocket of COX-2.

The orientation of the highly potent and selective COX-2 inhibitor, 6-ethylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**12b**), in the COX-2 active site was examined by a docking experiment (Fig. 1).[12] This study showed that **12b** binds in the center of the primary binding site of COX-2 with the SO_2Me moiety interacting with the secondary pocket amino acid residues Phe^{518}, Gln^{192}, Arg^{513}, Leu^{352}, Ser^{353} and Val^{523}. One of

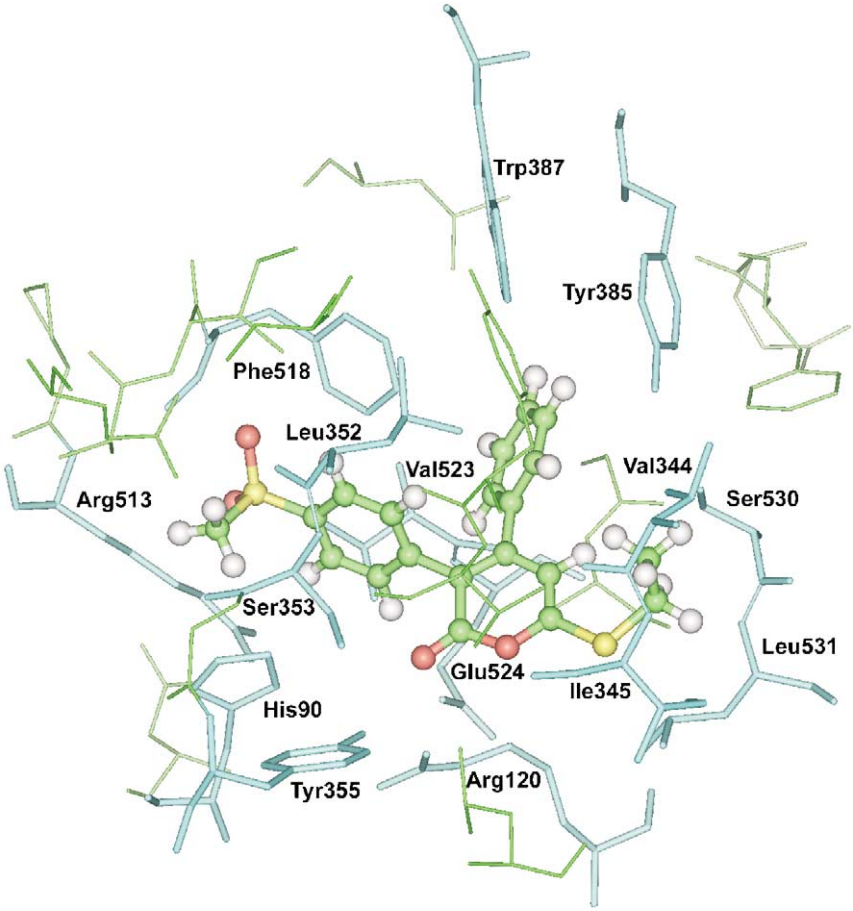


Figure 1. Docking of **12b** (ball and stick) in the active site of murine COX-2 (line and stick) ($E_{intermolecular} = -90.81$ kcal/mol). Hydrogen atoms of the amino acid residues are removed to increase clarity.

the *O*-atoms of the SO_2Me substituent forms a hydrogen bond with the amide hydrogen of Phe^{518} (1.92 Å). The ring *O*-atom of the central lactone (pyran-2-one) is oriented in the direction of the polar amino acid Arg^{120} at the mouth of the channel, where this *O*-atom is about 4.24 Å away from the NH_2 (guanidino) group. The C=*O* of the central pyran-2-one is hydrogen bonding with the O*H* of Tyr^{355} (1.70 Å). These interactions may disrupt the salt bridge between His^{90}, Arg^{120}, Tyr^{355} and Glu^{524} at the mouth of the COX-2 active site. The unsubstituted phenyl ring lies in a hydrophobic cavity lined by Tyr^{385}, Trp^{387}, Tyr^{348} and Ser^{530}. Interestingly, the C-6 EtS-substituent is located in a hydrophobic region formed by Val^{344}, Ile^{345}, Val^{349}, Ser^{530} and Leu^{531}, with the *S*-atom forming a weak hydrogen bond with the O*H* of Ser^{530} (4.41 Å). This shows the importance of the C-6 substituent in orienting the molecule such that the methylsulfone moiety inserts into the secondary pocket of COX-2. A similar docking study for the less potent, and less selective, COX-2 inhibitory C-6 OEt analogue (**11b**) showed that the SO_2Me moiety is inserted less deeply into the secondary pocket than the C-6 SEt of **12b**, the lactone ring oxygen atom in **11b** is closer to the NH_2 of Arg^{120} (3.26 Å) relative to 4.24 Å in **12b**, that the C-6 *O*Et oxygen atom is not within hydrogen bonding distance of the O*H* of Ser^{530} (6.63 Å), and the intermolecular energy for the ligand-enzyme complex for **11b** is higher (−87.60 kcal/mol). These observations together with the larger volume (328.5 $Å^3$), provides a good explanation for the potent and selective inhibitory activity of **12b**.

The results of this investigation show (i) a C-6 SEt substituent (**12b**)[13] in this 3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one class of diarylheterocycles provides potent and selective inhibition of the COX-2 isozyme, (ii) molecular modeling studies indicate the SO_2Me moiety inserts deep into the COX-2 secondary pocket and the C-6 *S*Et sulfur atom forms a weak hydrogen bond with the O*H* atom of Ser^{530} and (iii) these C-6 alkyl, alkoxy and alkylthio compounds **10**–**12** could serve as useful probes to study the function and catalytic activity of the COX-2 isozyme.

Acknowledgements

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12. Docking studies were performed using Insight II software Version 2000.1 (Accelrys Inc.) running on a Silicon Graphics Octane 2 R14000A workstation. The coordinates of the X-ray crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme was obtained from the RCSB Protein Data Bank (1cx2) and hydrogens were added. The ligand molecules were constructed using the Builder module and were energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The energy minimized ligands were superimposed on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The resulting structure (ligand–enzyme assembly) was minimized using the Discover module for 5000 iterations or until an RMSD of 0.05 Å was reached using consistent valence force field (CVFF). Further optimization of the ligand–enzyme complex was obtained using the Affinity command in the Docking module of Insight II by defining subsets of the enzyme such that residues within 10 Å of the ligand were allowed to relax, while the rest of the enzyme residues were fixed. The optimal binding orientation was achieved by utilizing 300 steps of steepest descent followed

by the conjugate gradient method. The CVFF was employed for all docking purposes. These docked structures were very similar to the minimized structures obtained initially. The quality of the docked structures were evaluated by measuring the intermolecular energy of the ligand–enzyme assembly.

13. Analytical data for **12b**. Mp 175–176 °C; IR (KBr): 1718 (C=O), 1314, 1153 (SO_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.46 (3H, t, $J=7.3$ Hz, SCH_2CH_3), 3.03 (3H, s, SO_2CH_3), 3.15 (2H, q, $J=7.3$ Hz, SCH_2CH_3), 6.35 (1H, s, pyranone H-5), 7.04–7.07 (2H, m, phenyl H-2, H-6), 7.21–7.31 (3H, m, phenyl H-3, H-4, H-5), 7.35 (2H, d, $J=8.5$ Hz, 4-$MeSO_2$–C_6H_4– H-2, H-6), 7.78 (2H, d, $J=8.5$ Hz, $MeSO_2$–C_6H_4– H-3, H-5). Anal. calcd for $C_{20}H_{18}O_4S_2$: C 62.16, H 4.69. Found: C 62.04, H 4.85.