Design and synthesis of new rofecoxib analogs as selective cyclooxygenase-2 (COX-2) inhibitors: Replacement of the methanesulfonyl pharmacophore by an *N*-acetylsulfonamido bioisostere.

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Dedicated to the memory of Dr. Antoine Noujaim

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ABSTRACT - Purpose: A group of 3,4-diaryl-2(5*H*)furanones were synthesized to determine whether a *N*-acetylsulfonamido ($SO_2NHCOCH_3$) moiety could be used as a bioisosteric replacement for the traditional sulfonamide (SO_2NH_2) and methanesulfonyl (SO_2CH_3) COX-2 pharmacophores. **Methods:** In vitro COX-1 and COX-2 isozyme inhibition studies were carried out to acquire structure-activity relationship data with respect to the point of attachment of the *N*-acetylsulfonamide moiety at the *para*- and *meta*-positions of the C-4 phenyl ring in conjunction with a variety of substituents (H, F, Cl, Me, OMe) at the *para*-position of the C-3 phenyl ring. **Results:** COX-1 and COX-2 inhibition studies showed that all compounds were selective inhibitors of COX-2 since no inhibition of COX-1 was observed at a concentration of 100 μM. The relative COX-2 potency, and COX-2 selectivity index, profiles for the C-4 *para*-acetamidophenyl compounds, with respect to the C-3 phenyl *para*-substituent was H > F > Cl. The point of attachment of the $SO_2NHCOCH_3$ substituent on the C-4 phenyl ring was a determinant of COX-2 potency, and COX-2 selectivity, where the relative activity profile was *para*-acetylsulfonamido > *meta*-acetylsulfonamido. 4-[4-(*N*-Acetylsulfonamido)phenyl]-3-phenyl-2(5*H*)furanone was identified as a more potent (IC_{50} = 0.32 μM), and selective (S.I. > 313), COX-2 inhibitor than the parent reference compound rofecoxib (IC_{50} = 0.43 μM, S.I. > 232). **Conclusions:** The $SO_2NHCOCH_3$ moiety i) is a novel COX-2 pharmacophore that also has the potential to serve as a prodrug moiety to the traditional SO_2NH_2 COX-2 pharmacophore, and ii) it could serve as a useful COX-2 pharmacophore to study the structure-function relationship of the COX-2 isozyme in view of its potential to acetylate the NH_2 moiety of amino acid residues such as Gln192 or Arg513 that line the pocket of the secondary COX-2 binding site.

INTRODUCTION

Cyclooxygenase (COX) inhibitors such as rofecoxib **1** (1), celecoxib **2** (2), and valdecoxib **3** (3) which selectively inhibit the inducible COX-2 isozyme that causes inflammation, rather than the constitutive COX-1 isozyme that provides gastroprotection and maintains vascular homeostasis, are clinically effective anti-inflammatory agents with less gastrointestinal and renal toxicity (see structures in Figure 1). Despite the relatively safe pharmacological profile of selective COX-2 inhibitors, there is now convincing evidence that highly selective COX-2 inhibitors alter the balance in the COX pathway resulting in a decrease in the level of the vasodilatory and anti-aggregatory prostacyclin (PGI_2), relative to an increase in the level of the prothrombotic thromboxane A_2 (TxA_2), leading to increased incidences of an adverse cardiovascular thrombotic event such as myocardial infarction (4). In this regard, the clinical use of rofecoxib and valdecoxib were terminated due to adverse cardiovascular effects associated with their use (5).

Acetylsalicylic acid **4** (aspirin) is a moderately active anti-inflammatory and nonnarcotic analgesic agent that continues to be a frequent first choice drug for the treatment of some arthritic disorders. However, these beneficial actions of aspirin may also be accompanied by adverse effects that include tinnitus, hypersensitization, and contraindicated gastrointestinal irritation, bleeding and/or ulceration (6). The ability of aspirin to inhibit blood platelet aggregation is now viewed as a clinically useful prophylactic action that can reduce the incidence of thrombus formation in individuals with cardiovascular disease. Aspirin is a unique

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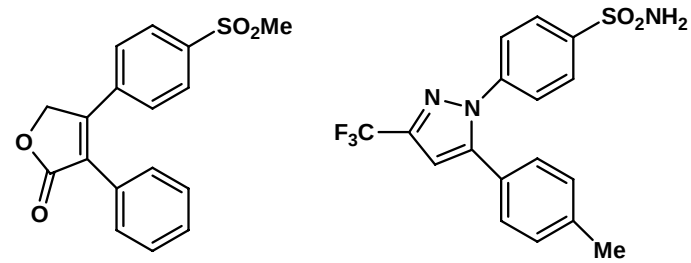
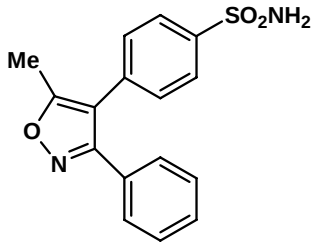


Rofecoxib **(1)**

Celecoxib **(2)**



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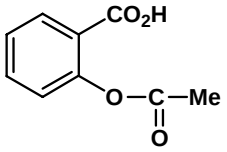
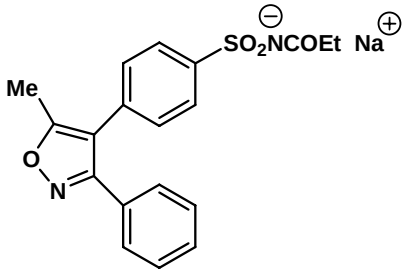


Acetylsalicyclic acid (**4**)



Parecoxib sodium (**5**)

Figure 1. Some representative examples of cyclooxygenase (COX) inhibitors.

nonselective COX inhibitor due to its ability to acetylate the Ser530 hydroxyl group in the primary COX binding site of COX-1 and COX-2. In this regard, aspirin is a 10- to 100-fold more potent inhibitor of COX-1 relative to COX-2 (7). Acetylation of the weakly nucleophilic OH of Ser530 by aspirin is thought to result from initial binding of its COOH to the Arg120 residue near the mouth of the COX binding site which positions the *ortho*-acetoxy moiety in close proximity to the Ser530 OH which it acetylates. Some of aspirin's beneficial therapeutic effects arise from acetylation of COX-2, whereas its antithrombotic and ulcerogenic effects result from acetylation of COX-1.

Extensive structure-activity relationship (SAR) studies for the diarylheterocyclic class of compounds have shown that a SO_2NH_2, or a SO_2Me, substituent at the *para*-position of one of the aryl rings is a requirement for optimum COX-2 selectivity and potency (8). It is known that the $SO_2NHCOCH_3$ moiety is a 10^5-10^6 more reactive acetylating agent of enzyme serine hydroxyls than simple amides (9). A *para*-*N*-acetylsulfonamido substituent on one of the phenyl rings of a diarylheterocyclic COX-2 inhibitor has the potential to acetylate the COX-2 isozyme. After acetylation of the COX-2 Ser530 OH by the $SO_2NHCOCH_3$ compound, the *N*-deacetylated SO_2NH_2 compound would be released that could also exhibit COX-2 inhibitory activity. This rationale is based on the observation that the water soluble nonnarcotic analgesic agent parecoxib sodium **5** (10) is a prodrug to the selective COX-2 inhibitor valdecoxib **3** (3). Accordingly, the $SO_2NHCOCH_3$ pharmacophore could serve the dual role of acetylation agent and prodrug. As part of our ongoing research program to

design selective COX-2 inhibitors, we describe herein the synthesis and biological evaluation of a novel group of 4-[4- and 3-(*N*-acetyl-sulfonamidophenyl)-3-(4-substituted-phenyl)-2-(5*H*)furanones **8a-e** that have the potential to not only inhibit, but also like aspirin to acetylate, the COX-2 isozyme.

MATERIALS AND METHODS

Materials:

The 3-(4-substituted-pheny)-4-phenyl-2(5*H*) furanones **6a-e** (R^1 = H, F, Cl, Me, OMe) were prepared using a previously reported method (11). All reagents purchased from the Aldrich Chemical Company (Milwaukee, WI) were used without further purifications.

Methods

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded as films with NaCl plates on a Nicolet 550 Series II Magna FT-IR spectrometer. Nuclear Magnetic Resonance (^{1}H NMR) spectra was recorded on a Bruker AM-300 spectrometer, in which coupling constants (*J*) were estimated in Hz. Elemental Analyses were performed for C, H and N (Micro-analytical Laboratory, Department of Chemistry, University of Alberta). Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70-230 mesh).

3-Phenyl-4-(4-sulfonamidophenyl)-2(5H)furanone 7a: General procedure

Chlorosulfonic acid (10 mL, 150 mmol) was added dropwise to 3,4-diphenyl-2(5*H*)furanone **6a** (1.0 g, 4.2 mmol) at 0 °C with vigorous stirring. The cooling bath was removed and the reaction was allowed to proceed for 6 hours at 25 °C with stirring. The cooled reaction mixture was poured slowly onto crushed ice and the benzenesulfonyl chloride intermediate was isolated as a pale yellow solid by rapid filtration to reduce the exposure time to water and from moisture. This crude benzenesulfonyl chloride intermediate product was dissolved in THF (30 mL) and then gaseous ammonia was bubbled through the solution for 10 min at 25 °C. Removal of the solvent in vacuo gave a residue that was extracted with ethyl acetate (2 × 30 mL). The combined organic extracts were washed with water, the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo to afford a yellow syrup. Purification of this syrup by silica gel column chromatography using ethyl acetate:hexane (4:1, v/v) as eluent gave **7a** (380 mg, 28%) as white needles; mp 248-250 °C [lit. (11) mp 248-250 °C]; IR (film) 3430, 3230 (NH_2), 1740 (furanone CO), 1320, 1140 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.43 (s, 2H, C*H*$_2$), 7.36-7.42 (m, 5H, phenyl hydrogens), 7.44 (s, 2H, N*H*$_2$), 7.52 (d, *J* = 8.2 Hz, 2H, 4-sulfonamidophenyl H-2, H-6), 7.85 (d, *J* = 8.2 Hz, 2H, 4-sulfonamidophenyl H-3, H-5).

Compounds **7b-e** were prepared using a similar sequence of chlorosulfonation and then amidation reactions as described above where **6b-e** were used in place of **6a**. The physical and spectral data for 7**b-e** are listed below.

3-(4-Fluorophenyl)-4-(4-sulfonamidophenyl)-2(5H)furanone 7b

Compound **7b** was obtained as a white solid in 32% yield; mp 192-193 °C [lit. (12) mp 187-188 °C]; IR (film) 3470, 3280 (NH_2), 1755 (furanone CO), 1320, 1155 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.39 (s, 2H, C*H*$_2$), 7.26 (d, J_{HCCF} = 8.5 of d, J_{HCCH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.40 (d, J_{HCCH} = 8.5 Hz of d, J_{HCCCF} = 5.4 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.45 (s, 2H, N*H*$_2$), 7.54 (d, *J* = 8.2 Hz, 2H, 4-sulfonamidophenyl H-2, H-6), 7.83 (d, *J* = 8.2 Hz, 2H, 4-sulfonamidophenyl H-3, H-5).

3-(4-Chlorophenyl)-4-(4-sulfonamidophenyl)-2(5H)furanone 7c

Compound **7c** was obtained as a white solid in 41% yield; mp 197-198 °C; IR (film) 3430, 3290 (NH_2), 1745 (furanone CO), 1315, 1160 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 5.40 (s, 2H, C*H*$_2$), 7.38 (d, *J* = 6.6 Hz, 2H, 4-chlorophenyl H-3, H-5), 7.47 (s, 2H, N*H*$_2$), 7.51 (d, *J* = 6.6 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.57 (d, *J* = 8.2 Hz, 2H, 4-sulfonamidophenyl H-2, H-6), 7.83 (d, *J* = 8.2 Hz, 2H, 4-sulfonamidophenyl H-3, H-5).

3-(4-Methylphenyl)-4-(3-sulfonamidophenyl)-2(5H)furanone 7d

Compound **7d** was obtained as a white solid in 34% yield; mp 248-249 °C; IR (film) 3450, 3295 (NH_2), 1745 (furanone CO), 1325, 1160 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.61 (s, 3H, C*H*$_3$) 5.40 (s, 2H, C*H*$_2$), 7.35-7.50 (m, 9H, 4-methylphenyl hydrogens,

3-sulfonamidophenyl H-4, H-5, H-6, N*H*$_2$), 7.94 (s, 1H, 3-sulfonamidophenyl H-2).

3-(4-Methoxyphenyl)-4-(3-sulfonamidophenyl)-2(5H)furanone 7e

Compound **7e** was obtained as a white solid in 37% yield; mp 220-221 ^{o}C; IR (film) 3420, 3290 (NH_2), 1745 (furanone CO), 1330, 1135 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d$_6$): δ 3.93 (s, 3H, OC*H*$_3$), 5.37 (s, 2H, C*H*$_2$), 7.12 (s, 2H, N*H*$_2$), 7.16 (d, *J* = 8.8 Hz, 1H, 3-sulfonamidophenyl H-6), 7.38-7.52 (m, 6H, 4-methoxyphenyl hydrogens, 3-sulfonamidophenyl H-4, H-5), 7.79 (d, *J* = 2.1 Hz, 1H, 3-sulfonamidophenyl H-2).

4-[4-(N-Acetylsulfonamido)phenyl]-3-phenyl-2(5H)furanone 8a. General procedure

Acetyl chloride (1 mL, 14.0 mmol) was added to a solution of **7a** (0.3 g, 0.95 mmol) in glacial acetic acid (2 mL), and the mixture was refluxed for 30 min. After cooling to 25 °C, the excess acetyl chloride was removed in vacuo, and this solution was poured onto water prior to extraction with ethyl acetate (2 × 30 mL). The combined organic extracts were washed with water, the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo to yield a pale yellow syrup. This syrup was purified by silica gel column chromatography using ethyl acetate:hexane (2:1, v/v) as eluent to give **8a** as white needles in 91% yield; mp 185-186 ^{o}C; IR (film): 3235 (NH), 1750 (furanone CO), 1715 ($COCH_3$) 1345, 1155 (SO_2) cm^{-1}; ^{1}H NMR (deuteriochloroform): δ 2.07 (s, 3H, COC*H*$_3$), 5.24 (s, 2H, C*H*$_2$), 7.29 (d, *J* = 7.8 Hz, 2H, phenyl H-2, H-6), 7.37-7.45 (m, 3H, phenyl H-3, H-4, H-5)), 7.63 (d, *J* = 8.4 Hz, 2H, acetylsulfonamidophenyl H-2, H-6), 7.85 (d, *J* = 8.4 Hz, 2H, acetylsulfonamidophenyl H-3, H-5), 8.75 (s, 1H, N*H*). *Anal*. Calcd. for $C_{18}H_{15}NO_5S$. 1/10 H_2O: C, 60.19; H, 4.27; N, 3.90. Found: C, 59.99; H, 4.18; N, 3.69.

Compounds **8b-e** were prepared using a similar acetylation reaction to that described above, where **7b-e** were used in place of **7a**. The physical and spectral data for **8b-e** are listed below.

4-[4-(N-Acetylsulfonamido)phenyl]-3-(4-fluorophenyl)-2(5H)furanone 8b

Compound **8b** was obtained as white needles in 84% yield; mp 120-122 ^{o}C; IR (film): 3245 (NH), 1755 (furanone CO), 1715 ($COCH_3$), 1340, 1160 (SO_2) cm^{-1}; ^{1}H NMR (deuteriochloroform): δ 2.06 (s, 3H, COC*H*$_3$), 5.20 (s, 2H, C*H*$_2$), 7.11 (d, J_{HCCF} = 8.5, of d, J_{HCCH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.40 (d, J_{HCCH} = 8.5, of d, J_{HCCCF} = 5.4 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.53 (d, *J* = 8.3 Hz, 2H, acetylsulfonamidophenyl H-2, H-6), 8.02 (d, *J* = 8.3 Hz, 2H, acetylsulfonamidophenyl H-3, H-5), 9.21 (br s, 1H, N*H*). *Anal*. Calcd. for $C_{18}H_{14}FNO_5S$.1/8 H_2O: C, 57.25; H, 3.80; N, 3.71. Found: C, 56.95; H, 3.97; N, 3.51.

4-[4-(N-Acetylsulfonamido)phenyl]-3-(4-chlorophenyl)-2(5H)furanone 8c

Compound **8c** was obtained as white needles in 89% yield; mp 137-138 ^{o}C; IR (film): 3370 (NH) 1750 (furanone CO), 1715 ($COCH_3$) 1340, 1150 (SO_2) cm^{-1}; ^{1}H NMR (deuteriochloroform): δ 2.08 (s, 3H, COC*H*$_3$), 5.20 (s, 2H, C*H*$_2$), 7.30 (d, *J* = 6.6 Hz, 2H, 4-chlorophenyl H-3, H-5), 7.40 (d, *J* = 6.6 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.50 (d, *J* = 8.4 Hz, 2H, acetylsulfonamidophenyl H-2, H-6), 8.04 (d, *J* = 8.4 Hz, 2H, acetylsulfonamidophenyl H-3, H-5), 8.85 (br s, 1H, N*H*). *Anal*. Calcd. for $C_{18}H_{14}ClNO_5S$: C, 55.18; H, 3.60; N, 3.57. Found: C, 54.85; H, 3.57; N, 3.40.

4-[3-(N-Acetylsulfonamido)phenyl]-3-(4-methylphenyl)-2(5H)furanone 8d

Compound **8d** was obtained as white needles in 88% yield; mp 120-122 ^{o}C; IR (film): 3230 (NH), 1750 (furanone CO), 1710 ($COCH_3$) 1330, 1150 (SO_2) cm^{-1}; ^{1}H NMR (deuteriochloroform): δ 2.07 (s, 3H, COC*H*$_3$), 2.70 (s, 3H, -C$_6$H$_4$-C*H*$_3$), 5.22 (s, 2H, C*H*$_2$), 7.30-7.50 (m, 6H, 4-methylphenyl hydrogens, acetylsulfonamidophenyl H-5, H-6), 7.66 (d, *J* = 7.9 of d, *J* = 1.8 Hz, 1H, acetylsulfonamidophenyl H-4), 8.18 (s, 1H, acetylsulfonamidophenyl H-2), 8.28 (s, 1H, N*H*). *Anal*. Calcd. for $C_{19}H_{17}NO_5S$: C, 61.44; H, 4.61; N, 3.77. Found: C, 61.24; H, 4.73; N, 3.74.

4-[3-(N-Acetylsulfonamido)phenyl]-3-(4-methoxylphenyl)-2(5H)furanone 8e

Compound **8e** was obtained as white needles in 89% yield; mp 207-208 ^{o}C; IR (film): 3210 (NH), 1750 (furanone CO), 1715 ($COCH_3$) 1315, 1150 (SO_2) cm^{-1}; ^{1}H NMR (deuteriochloroform): δ 2.09 (s, 3H, COC*H*$_3$), 4.03 (s, 3H, OC*H*$_3$), 5.20 (s, 2H, C*H*$_2$), 7.12 (d, *J* = 8.6 Hz, 1H, acetylsulfonamidophenyl H-6), 7.30-7.50 (m, 5H, 4-methoxyphenyl hydrogens, acetylsulfonamidophenyl H-5), 7.81 (d, *J* = 8.1, 1H, acetylsulfonamidophenyl H-4), 8.08 (s, 1H, acetylsulfonamidophenyl H-2), 8.38 (s, 1H, N*H*).

Anal. Calcd. for $C_{19}H_{17}NO_6S$. H_2O: C, 56.29; H, 4.72; N, 3.45. Found: C, 56.57; H, 4.54; N, 3.51.

In vitro cyclooxygenase (COX-1 and COX-2) inhibition assays

The ability of the test compounds **8a-e** listed in Table 1 to inhibit ovine COX-1 and COX-2 (IC_{50} value, μM) was determined using an enzyme immuno assay (EIA) kit (catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method (13).

Molecular modeling (docking) study

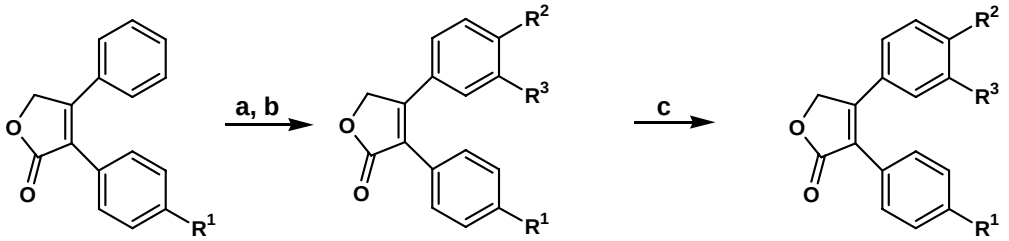
The docking experiment was performed using Insight II software Version 2000.1 (Accelrys Inc.) running on a Silicon Graphics Octane 2 R14000A workstation according to a previously reported method (14).

RESULTS

A group of 3-(4-substituted-phenyl)-4-(4-sulfonamidophenyl)-2(5*H*)furanones **7a-c** (R^1 = H, F, Cl), and 3-(4-substituted-phenyl)-4-(3-sulfonamidophenyl)-2(5*H*)furanones **7d-e** (R^1 = Me, OMe), were synthesized using a one-pot sequence of reactions in 28-41% isolated yield (see Scheme 1). Accordingly, chlorosulfonation of the 3-(4-substituted-phenyl-4-phenyl-2(5*H*) furanones **6a-e** using chlorosulfonic acid, and then amidation of the sulfonyl chloride product with ammonia gas, afforded the respective sulfonamide product **7a-e**. The observation that chloro-sulfonation of **6a-c** (R^1 = H, F, Cl) occurs at the *para*-position of the C-4 phenyl ring, and that chlorosufonation of **6d-e** (R^1 = Me, OMe) occurs at the *meta*-position of the C-4 phenyl ring, is consistent with previously related chlorosulfonation reactions (11). Subsequent *N*-acetylation of the sulfonamides **7a-e** using acetyl chloride in acetic acid furnished the respective target *N*-acetylsufonamido product **8a-e** (84-89% yield).

The ability of the *N*-acetylsulfonamides **8a**-e to inhibit the COX-1 and COX-2 isozymes was determined using enzyme immuno assays (see enzyme inhibition data in Table 1). None of the compounds **8a-e** inhibited COX-1 at a concentration of 100 μM (IC_{50} values > 100 μM). In contrast, all compounds **8a**-e were selective inhibitors of the COX-2 isozyme with IC_{50} values in the highly potent to weakly active 0.32 to 9.97 μM range, and COX-2 selectivity indexes (S.I.) in the > 10 to > 313 range. 4-[4-(*N*-Acetylsulfonamido)phenyl]-3-phenyl-2(5*H*) furanone **8a** was a more potent (IC_{50} = 0.32 μM), and selective (S.I. > 313), COX-2 inhibitor than the parent reference compound rofecoxib (IC_{50} = 0.43 μM, S.I. > 232).

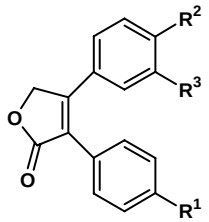
A molecular modeling study showed that the *N*-acetylsulfonamido compound **8a** binds in the center of the primary COX-2 binding site such that the C-4 *para*-$SO_2NHCOMe$ substituent is oriented, like the C-4 *para*-SO_2Me moiety in rofecoxib, in the vicinity of amino acid residues lining the COX-2 secondary (2˚) pocket (Ala516, Ile517, Phe518, Arg513 and Gln192) as illustrated in Figure 2.



6a, R^1 = H	**7a**, R^1 = H, R^2 = SO_2NH_2, R^3 = H	**8a**, R^1 = H, R^2 = $SO_2NHCOMe$, R^3 = H
6b, R^1 = F	**7b**, R^1 = F, R^2 = SO_2NH_2, R^3 = H	**8b**, R^1 = F, R^2 = $SO_2NHCOMe$, R^3 = H
6c, R^1 = Cl	**7c**, R^1 = Cl, R^2 = SO_2NH_2, R^3 = H	**8c**, R^1 = Cl, R^2 = $SO_2NHCOMe$, R^3 = H
6d, R^1 = Me	**7d**, R^1 = Me, R^2 = H, R^3 = SO_2NH_2	**8d**, R^1 = Me, R^2 = H, R^3 = $SO_2NHCOMe$
6e, R^1 = OMe	**7e**, R^1 = OMe, R^2 = H, R^3 = SO_2NH_2	**8e**, R^1 = OMe, R^2 = H, R^3 = $SO_2NHCOMe$

Scheme 1. Reagents and conditions: (**a**) $ClSO_3H$, 25 ^oC, 6 h; (**b**) NH_3 (gas), THF, 10 min; (**c**) AcCl, AcOH, reflux, 30 min.

Table 1. In vitro COX-1 and COX-2 inhibition data for 4-[4-(*N*-acetylsulfonamido)phenyl]-3-(4-substituted-phenyl)-2(5*H*)furanones **8a-c**, and 4-[3-(*N*-acetylsulfonamido)phenyl]-3-(4-substituted-phenyl)-2(5*H*)furanones **8d-e**.



8a-e

Compound	R^1	R^2	R^3	IC_{50} (μM)[a]		COX-2 S.I.[b]	Volume (Å^3)[c]
				COX-1	COX-2		
8a	H	$SO_2NHCOMe$	H	>100	0.32	>313	296.3
8b	F	$SO_2NHCOMe$	H	>100	1.03	>97	300.6
8c	Cl	$SO_2NHCOMe$	H	>100	4.48	>22	310.0
8d	Me	H	$SO_2NHCOMe$	>100	4.85	>20	314.4
8e	OMe	H	$SO_2NHCOMe$	>100	9.97	>10	323.2
1 (Rofecoxib)				>100	0.43	>232	266.1
4 (Aspirin)				0.35	2.4	0.14	154.8

[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, USA), and the deviation from the mean is <10% of the mean value.
[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/ COX-2 IC_{50}).
[c] The volume of the molecule after minimization using the PM3 force field was calculated using the Alchemy 2000 program (Alchemy 32 Version 2.0, 1997, Tripos Inc., St Louis, MO, USA).

DISCUSSION

A group of 3-(4-substituted-phenyl)-4-(4-sulfonamidophenyl)-2(5*H*)furanones **8a-c** (R^1 = H, F, Cl), and 3-(4-substituted-phenyl)-4-(3-sulfonamidophenyl)-2(5*H*)furanones **8d-e** (R^1 = Me, OMe), were synthesized for evaluation as cyclooxygenase inhibitors. The objectives of these studies were i) to determine whether a *N*-acetylsulfonamido ($SO_2NHCOCH_3$) moiety could be used as a bioisosteric replacement for the traditional sulfonamide (SO_2NH_2) and methylsulfonyl (SO_2CH_3) COX-2 pharmacophores, and ii) to acquire some exploratory structure-activity relationship data with respect to the point of attachment of the *N*-acetylsulfonamide moiety at the *para*- and *meta*-positions of the C-4 phenyl ring in conjunction with a variety substituents (H, F, Cl, Me, or OMe) at the *para*-position of the C-3 phenyl ring. In vitro COX-1 and COX-2 isozyme inhibition studies showed that all compounds **8a-e** were selective inhibitors of COX-2 since no inhibition of COX-1 occurred at a concentration of 100 μM. The relative COX-2 potency, and COX-2 selectivity index, profiles, for the C4 *para*-acetamidophenyl compounds **8a-c**, with respect to the C-3 phenyl R^1

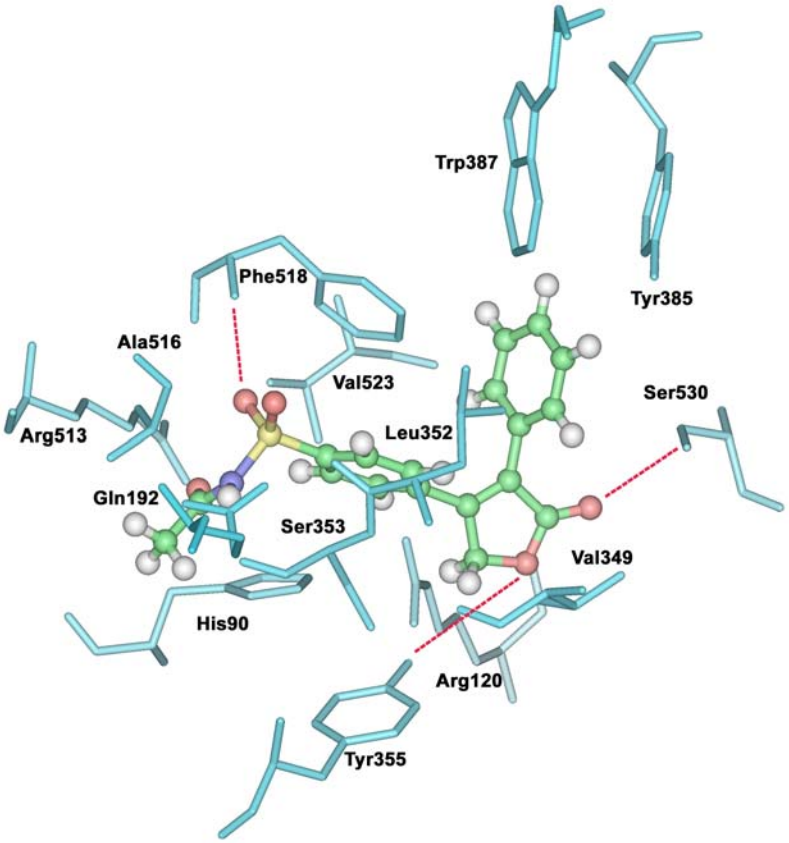


Figure 2. Docking of 4-[4-(*N*-acetylsulfonamido)phenyl]-3-phenyl-2(5*H*)furanone **8a** (ball-and-stick) in the active site of murine COX-2. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

substituent, was H > F > Cl. Compounds having a C-4 *para*-acetamidophenyl substituent **8a-c** (R^1 = H, F, Cl) were more potent and selective COX-2 inhibitors than the regioisomeric C-4 *meta*-acetamidophenyl compounds **8d-e** (R^1 = Me, OMe). It is interesting to note that the COX-2 inhibitory potency, and COX-2 selectivity index, decreased as the molecular volume (see data in Table 2) increased (**8a**, 296.3 Å^3; **8b**, 300.6 Å^3; **8c**, 310.0 Å^3; **8d**, 314.4 Å^3; **8e**, 323.23 Å^3). In this regard, 3-phenyl)-4-(4-sulfonamidophenyl)-2(5*H*)furanone **8a**, having the smallest molecular volume (296.3 Å^3), was a more potent (IC_{50} = 0.32 μM), and selective (S.I. > 313), COX-2 inhibitor than the parent reference compound rofecoxib **1** having a traditional SO_2CH_3 COX-2 pharmacophore in place of the $SO_2NHCOCH_3$ moiety present in **8a**.

A molecular modeling study was performed where the most selective COX-2 inhibitor 4-[4-(*N*-acetylsulfonamido)phenyl]-3-phenyl-2(5*H*)furanone **8a** was docked in the binding site of the COX-2 isozyme (Figure 2). This molecular modeling study showed that **8a** binds in the center of the primary binding site such that the C-4 *para*-$SO_2NHCOMe$ substituent is oriented in the vicinity of amino acid residues lining the COX-2 secondary (2˚) pocket (Ala516, Ile517, Phe518, Arg513 and Gln192). One of the *O*-atoms of the *para*-$SO_2NHCOMe$ moiety forms a hydrogen bond with the amide hydrogen (N*H*) of Phe518 (distance ≈ 2.3 Å). The distance between the other *O*-atom (of the *para*-sulfone moiety) and the NH_2 of Gln192 is about 5.2 Å. The *N*-atom of the *para*-$SO_2NHCOMe$ group is positioned about 5.4 Å from the NH_2 (guanidino group) of Arg513, with the *N*-acetyl carbonyl oxygen of the *para*-$SO_2NHCOMe$ undergoing an ion-ion (electrostatic) interaction with the NH_2 of Arg513 (distance ≈ 3.2 Å). In addition, the N*H* of the *para*-$SO_2NHCOCH_3$ has a spatial separation of about 4.1 Å from the amide nitrogen of Ala516 that lines the COX-2 2˚-pocket. This molecular

modeling study indicates that the $SO_2NHCOMe$ group, which inserts into the COX-2 2°-pocket, is too far removed to acetylate the Ser530 hydroxyl group. On the other hand, the $SO_2NHCOMe$ moiety is suitably positioned to potentially acetylate the NH_2 group of Gln192 or Arg513 that line the 2°-pocket into which the $SO_2NHCOMe$ group is inserted. The unsubstituted C-3 phenyl ring is oriented towards a hydrophobic pocket comprised of Trp387, Tyr385 and Tyr348 at the apex of the COX-2 binding site. It is noteworthy that the C=*O* of the central furanone ring is hydrogen bonded to the O*H* of Ser530 (distance ≈ 2.6 Å). A recent study has shown the importance of Ser530 in the COX-2 inhibitory activity of rofecoxib (15). In addition, the *O*-atom of the central furanone ring system is also hydrogen bonded to the O*H* of Tyr355 (distance ≈ 3.6 Å) and it is also interacting with the NH_2 of Arg120 at the mouth of the COX-2 binding site (distance ≈ 5.2 Å).

In summary, the $SO_2NHCOCH_3$ moiety i) is a novel COX-2 pharmacophore that also has the potential to serve as a prodrug moiety to the respective SO_2NH_2 COX-2 pharmacophore, ii) unlike the acetoxy ($OCOCH_3$) moiety present in aspirin which acetylates the hydroxyl group of Ser530, the $SO_2NHCOMe$ moiety in **8a** has the potential to acetyalte a NH_2 group present in Gln192 or Arg513 that line the COX-2 2°-pocket, and iii) could serve as a useful agent to study the structure-function relationship of the COX-2 isozyme. In addition, a compound such as 4-[4-(*N*-acetylsulfonamido)phenyl]-3-(4-fluorophenyl)-2(5*H*)furanone **8b**, when radiolabeled with a fluorine-18 positron emission tomography (PET) radionuclide, has biological properties that may be suitable for use as a potential candidate COX-2 imaging radiopharmaceutical for the in vivo imaging of COX-2 expression (16-17).

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