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Sulfonamido, azidosulfonyl and *N*-acetylsulfonamido analogues of rofecoxib: 4-[4-(*N*-acetylsulfonamido)phenyl]-3-(4-methanesulfonylphenyl)-2(5*H*)furanone is a potent and selective cyclooxygenase-2 inhibitor

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Abstract—4-[4-(*N*-Acetylsulfonamido)phenyl]-3-(4-methanesulfonylphenyl)-2(5*H*)furanone, possessing a *N*-acetylsulfonamido pharmacophore, has been identified as a potent and selective COX-2 inhibitor that has the potential to acetylate the COX-2 isozyme.

Cyclooxygenase (COX) inhibitors such as rofecoxib (**1**)[1] and celecoxib (**4**)[2] 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 nonulcerogenic antiinflammatory drugs. However, a precautionary concern regarding the use of COX-2 inhibitors in patients at risk for an adverse cardiovascular event such as myocardial infarction has been raised. One plausible explanation for this increased incidence of a prothrombotic episode is attributed to a lower level of the vasodilator and platelet aggregation inhibitor prostacyclin (PGI_2) in conjunction with a higher level of the potent platelet activator and aggregator thromboxane A_2 (TxA_2).[3] Accordingly, there is still a need for the design of COX-2 inhibitors with a greater safety profile for the treatment of arthritis. In this regard, a novel class of isomeric acetoxy analogues of rofecoxib (**2**) were recently designed which are potent and selective COX-2 inhibitors that, like acetylsalicyclic acid (aspirin), have the potential to acetylate the COX-2 isozyme.[4] Other studies indicated that the aspirin analogue *O*-(acetoxyphenyl)hept-2-ynyl sulfide selectively acetylated and irreversibly inhibited COX-2,[5] and a diastereomeric acyl-Co-A-ketoprofen conjugate may act as a reversible inhibitor of COX-1 and an irreversible inhibitor of COX-2.[6] Extensive structural–activity studies for the diphenylheterocycle class have shown that a SO_2NH_2 or SO_2Me substituent at the *para*-position of one phenyl ring often provides optimum COX-2 selectivity and potency.[7] The SO_2Me and SO_2NH_2 *H*-bonding pharmacophores are believed to induce COX-2 selectivity by insertion into the secondary (2°) pocket of COX-2 that is absent in COX-1. The 2°-pocket in COX-2 has been attributed to the presence of isoleucine (Ile^{523}) in COX-1 relative to the smaller valine (Val^{523}) in COX-2.[8] In an earlier study we showed that the dipolar azido substituent of **3** inserts deep into the COX-2 2°-pocket binding site where it undergoes a hitherto unreported electrostatic (ion–ion) interaction with the charged guanidino moiety of Arg^{513}.[9] It is also of interest to investigate the *N*-acetylsufonamido ($SO_2NHCOMe$) moiety as an pharmacophore that is capable of acetylating the Ser^{530} hydroxyl moiety in the primary binding site of COX-2.[10] An azidosulfonyl (SO_2N_3) substituent warrants investigation as a COX-2 pharmacophore that is capable of undergoing dual *H*-bonding (SO_2) and electrostatic (N_3) interactions with amino acid residues lining the COX-2 2°-pocket. As part of our ongoing program to design COX-2 inhibitors, we now describe a novel group of regioisomeric 3,4-diphenyl-2(5*H*)furanones (**8**–**10**) that possess a SO_2Me, SO_2NH_2, $SO_2NHCOMe$ or SO_2N_3 substituent at the *para*-position of either phenyl ring.

Keywords: *N*-Acetylsulfonamido; COX-2 inhibition.
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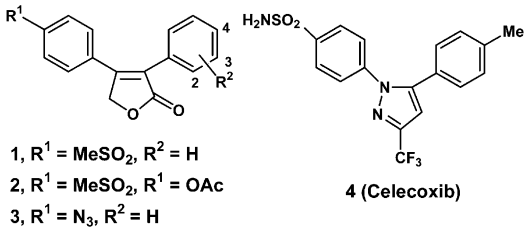
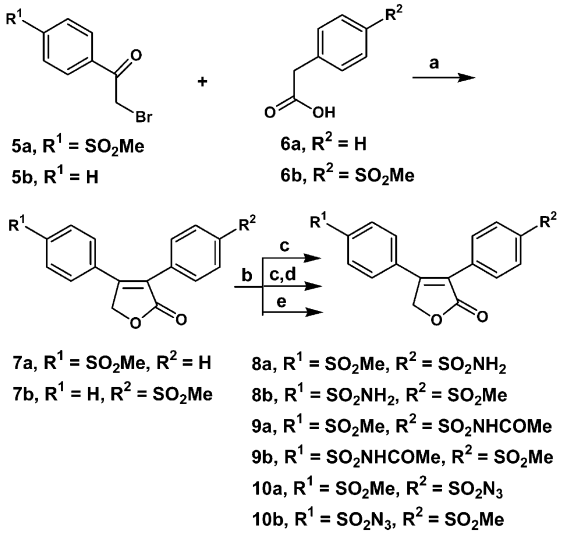


The target 3,4-diaryl-2(5*H*)furanone derivatives (**8**–**10**) were synthesized using the reaction sequence illustrated in Scheme 1. Reaction of the bromoketone (**5a**[11] or **b**) with the phenylacetic acid (**6a** or **b**[12]) proceeds via a 2-step condensation–cyclization reaction performed as a one-pot procedure.[13] Thus, treatment of a mixture of either **5a** and **6a**, or **5b** and **6b**, in acetonitrile with triethylamine at 25 °C yields an ester intermediate. Subsequent cooling to 0 °C and then addition of DBU effected the cyclization to afford the respective 4-(4-methylsulfonylphenyl)-3-phenyl-2(5*H*)furanone or 3-(4-methylsulfonyl)-4-phenyl-2(5*H*)furanone regioisomer (**7a** or **b**, 53–57% yield). Chlorosulfonation of the furanones **7a** or **b** with chlorosulfonic acid at 25 °C, and then reaction of the sulfonyl chloride intermediate with either gaseous ammonia in THF, or NaN_3 in aqueous acetone, afforded the respective sulfonamide regioisomer (**8a** or **b**, 100%), or sulfonylazide regioisomer (**10a** or **b**, 41–44% yield). Acetylation of **8a** and **b** using acetyl chloride in acetic acid afforded the respective *N*-acetylsulfonamide product (**9a** or **b**, 85–90%).

A group of rofecoxib derivatives having an additional SO_2NH_2, $SO_2NHCOMe$ or SO_2N_3 substituent at the *para*-position of the C-3 phenyl ring (**8a**–**10a**), and the corresponding rofecoxib regioisomers (**8b**–**10b**), were prepared to investigate the effect of these substituents on COX-2 selectivity and potency. In vitro COX-1/COX-2 inhibition studies showed that the rofecoxib analogues **8a** and **b** possessing an additional SO_2NH_2 substituent are inactive inhibitors of COX-1 and COX-2 (IC_{50} values >100 μM). In contrast, incorporation of a SO_2N_3 substituent at the *para*-position of the C-3 phenyl ring of rofecoxib (**10a**) conferred modest inhibitory potency against COX-1 ($IC_{50}=31.5$ μM) and COX-2 ($IC_{50}=11$ μM) with a moderate COX-2 selectivity index (S.I.) of about 3. On the other hand, the corresponding regioisomer **10b** was a selective (COX-2 S.I. >31), but not particularly potent (COX-2 $IC_{50}=3.15$ μM), inhibitor of COX-2. The *N*-acetylsulfonamide regioisomers **9a** and **9b**, in view of their potential ability to acetylate the COX-2 isozyme, could provide a lead-compound for the development of a novel type of acetylating COX-2 inhibitor. In vitro COX-1/COX-2 enzyme inhibition studies showed that incorporation of an additional *para*-*N*-acetylsulfonamido substituent on the C-3 phenyl ring of rofecoxib provided **9a** that was an approximately equipotent inhibitor of both COX-1 ($IC_{50}=3.1$ μM) and COX-2 ($IC_{50}=4.6$ μM). In contrast, the corresponding 4-[4-(*N*-acetylsulfonamido)phenyl]-3-(4-methanesulfonylphenyl)-2(5*H*)furanone (**9b**) was a highly potent (COX-2 $IC_{50}=0.05$ μM), and selective (COX-2 S.I. >2000) COX-2 inhibitor relative to the reference drug rofecoxib (see data in Table 1). These data suggest that the novel *N*-acetylsulfonamdo compound **9b** should inhibit the synthesis of inflammatory prostaglandins via the COX pathway at sites of inflammation, and be devoid of ulcerogenicity due to the fact that it does not inhibit COX-1 ($IC_{50}>100$ μM).



Scheme 1. Reagents and conditions: (a) Et_3N, MeCN, 25 °C, 30 min, and then DBU, 0 °C, 30 min; (b) $ClSO_3H$, 25 °C, 3 h; (c) NH_3 gas, THF, 5 min; (d) AcCl, AcOH, reflux, 30 min; (e) NaN_3, aqueous acetone, 0 °C, 2 h.

The orientation of the highly potent and selective COX-2 inhibitor 4-[4-(*N*-acetylsulfonamido)phenyl]-3-(4-methanesulfonylphenyl)-2(5*H*)furanone (**9b**) in the COX-2 binding site was examined by a docking experiment (Fig. 1).[14] This molecular modeling shows that **9b** binds in the primary binding site such that the C-3 *para*-SO_2Me substituent inserts into the 2°-pocket present in COX-2. One of the *O*-atoms of the SO_2Me moiety is *H*-bonding to the amide hydrogen (N*H*) of Phe^{518} (distance = 3.6 Å) whereas, the other *O*-atom is close to the NH_2 of Arg^{120} (distance = 2.8 Å). The C=*O* oxygen atom of the central furanone ring forms a hydrogen bond (distance = 3.3 Å) with the O*H* of Tyr^{355} that forms part of the entrance to the COX-2 2°-pocket. The C-4 phenyl ring with a *para*-$SO_2NHCOMe$ substituent

Table 1. In vitro inhibition of COX-1 and COX-2 by 3,4-diphenyl-2(5*H*)furanone derivatives of rofecoxib (**8**–**10**)

Compd	IC_{50} (μM)[a]		COX-2
	COX-1	COX-2	S.I.[b]
8a	>100	>100	—
8b	>100	>100	—
9a	3.2	4.6	<0.8
9b	>100	0.05	>2000
10a	31.5	11	>2.8
10b	>100	3.1	>32
Rofecoxib	>500	0.43	>1162

[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 (COX-1 IC_{50}/COX-2 IC_{50}).

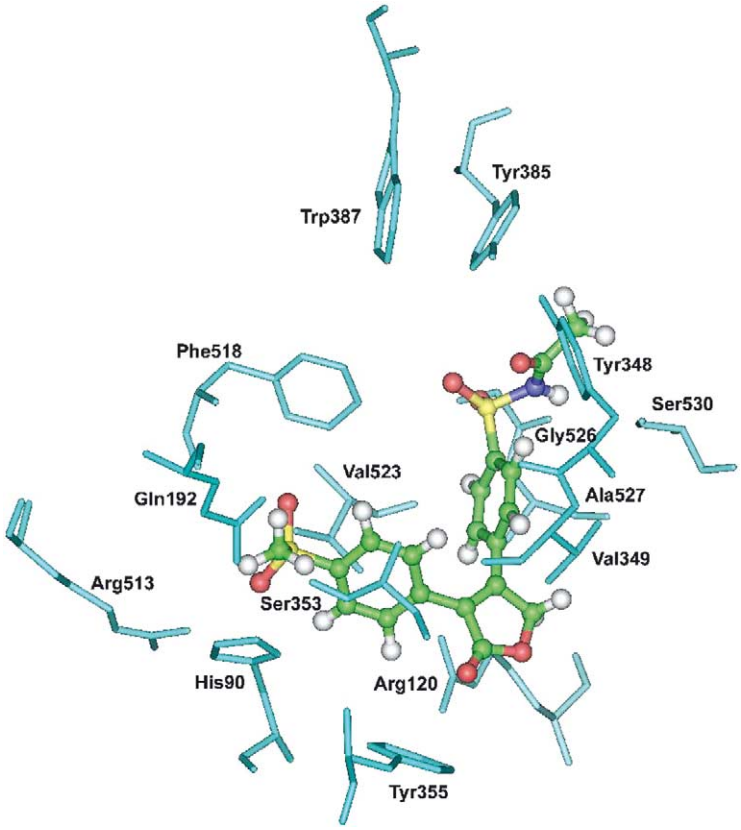


Figure 1. Docking of **9b** (ball and stick) in the active site of murine COX-2 (line and stick). Hydrogen atoms of the amino acid residues are not shown to increase clarity.

is oriented towards a hydrophobic pocket comprised of Trp^{387}, Tyr^{385} and Tyr^{348} at the top of the COX-2 primary binding site. As per our hypothesis, the SO_2NH-COMe is located in the vicinity of Ser^{530} which is the acetylation site for aspirin. In this regard, the *C*-atom of the $SO_2NHCOMe$ group is positioned about 4.0 Å away from the *O*H of Ser^{530}, and the $SO_2NHCOMe$ *N*H and Ser^{530} *O*H are separated by almost 3.3 Å. The SO_2 moiety of the $SO_2NHCOMe$ substituent undergoes a hydrophobic interaction with the backbone residues Gly^{526}, Ala^{527} and Met^{522}. It is interesting to note that, the *O*-atom of the C=*O* ($SO_2NHCOMe$) forms a hydrogen bond with the O*H* of Tyr^{385} (distance = 3.2 Å) which could potentially activate the *C*-atom of the $SO_2NHCOMe$ moiety with respect to nucleophilic attack by the *O*H of Ser^{530} that may lead to a covalent acetylation of the COX-2 isozyme Ser^{530}. This observation is consistent with the critical role of Tyr^{385} in the acetylation of Ser^{530} by aspirin in the COX binding site,[15] and the in vitro COX inhibition data provide a good explanation for the potent and selective inhibitory activity exhibited by **9b**.

The results of this investigation show (i) incorporation of a *para*-*N*-acetylsulfonamido substituent on the C-3 phenyl ring of the rofecoxib regioisomer (**9b**) provides a highly potent, and selective, COX-2 inhibitor, (ii) a molecular modeling study indicates the *para*-*N*-acetylsulfonamido substituent of **9b** is suitably positioned to acetylate the serine hydroxyl group in the COX-2 pimary binding site, and (iii) the *N*-acetylsulfonamido compound **9b** could serve as a useful probe to study the function and catalytic activity of the COX-2 isozyme.

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14. 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 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 ligand–enzyme assembly obtained after docking 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 100 step equilibration for over 1000 iterations and a time step of 1 fs using a distance dependent dielectric constant 4r. The optimal binding orientation of the ligand–enzyme assembly obtained was further minimized for 1000 iterations using the conjugate gradient method until a convergence of 0.001 kcal/mol Å was reached.
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