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Isomeric Acetoxy Analogues of Rofecoxib: A Novel Class of Highly Potent and Selective Cyclooxygenase-2 Inhibitors

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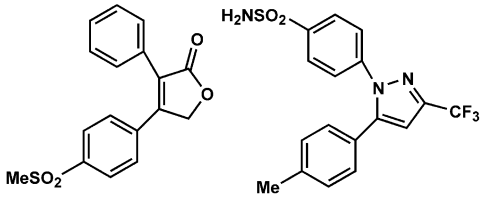
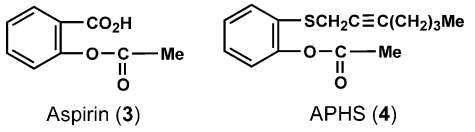
Abstract—A group of isomers possessing a 2-, 3-, or 4-acetoxy moiety on the 3-phenyl substituent of rofecoxib were synthesized that exhibit highly potent, and selective, COX-2 inhibitory activity that have the potential to acetylate the COX-2 isozyme.

The historical belief that a single cyclooxygenase (COX) enzyme catalyzed the bioconversion of arachidonic acid to prostaglandins and thromboxanes, which are responsible for both the therapeutic anti-inflammatory and associated gastrointestinal and renal toxicity exhibited by non-steroidal antiinflammatory drugs (NSAIDs), required modification following the discovery that there are two isozymes, COX-1 and COX-2.[1] The constitutive COX-1 isozyme is produced in a variety of tissues and appears to be important to the maintenance of physiological functions such as gastroprotection and vascular homeostasis.[2] Alternatively, the COX-2 isozyme is induced by mitogenic and proinflammatory stimuli[3] linking its involvement to inflammatory processes.[4] The initial concept that a selective COX-2 inhibitor would illicit effective antiinflammatory activity without the adverse ulcerogenic effect associated with the use of NSAIDs that inhibit both COX-1 and COX-2 has been validated by postmarket clinical studies which attest to the efficacy of the selective COX-2 inhibitors rofecoxib (**1**)[5] and celecoxib (**2**).[6]

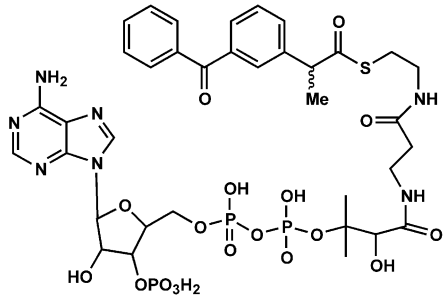
Aspirin (**3**) is a unique non-selective COX inhibitor due to its ability to acetylate the serine hydroxyl group in the COX binding site of COX-1 and COX-2. In this regard, aspirin is a 10- to 100-fold more potent inhibitor of COX-1 relative to COX-2.[7] Some of aspirin's beneficial therapeutic effects can be attributed to acetylation of COX-2, while its antithrombotic and ulcergenic effects are due to acetylation of COX-1. These observations were elegantly exploited in the design of the aspirin analogue *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS, **4**) that selectively acetylated, and irreversibly inactivated, COX-2.[8] More recently biological data was acquired that suggests the diastereomeric acyl-CoA-



Rofecoxib (**1**) Celecoxib (**2**)



Aspirin (**3**) APHS (**4**)



Acyl-CoA-ketoprofen Conjugate Diastereomers (**5**)

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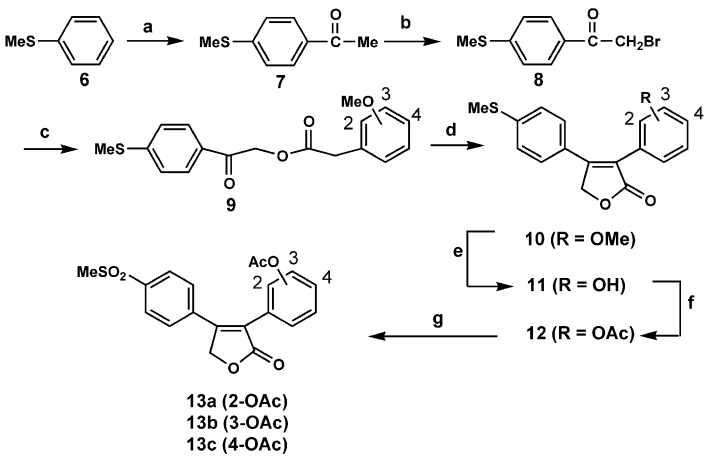


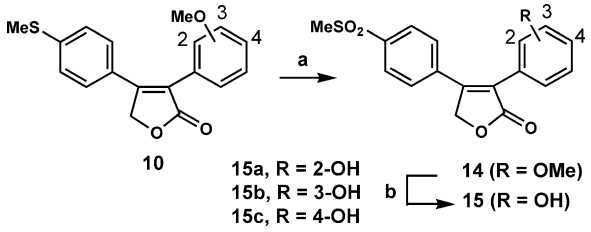
Scheme 1. Reagents and conditions: (a) $AlCl_3$, AcCl, $CHCl_3$, 0–10 °C, 1 h; (b) Br_2, $CHCl_3$, 25 °C, 30 min; (c) 2-, 3-, or 4-MeO–C_6H_4–CH_2CO_2H, Et_3N, MeCN, 25 °C, 1 h; (d) NaH, DMSO, 25 °C, 1 h; (e) pyridinium hydrochloride, 190–210 °C, 1 h; (f) AcCl, Et_3N, 0→25 °C, 1 h; (g) Oxone®, MeOH, THF, H_2O, 25 °C, 18 h.

ketoprofen conjugates (**5**) are reversible inhibitors of COX-1 and irreversible inhibitors of COX-2.[9]

A special communication has raised a cautionary flag regarding the use of COX-2 inhibitors in patients at risk for cardiovascular morbidity such as myocardial infarction that has been explained using the following facts.[10] The COX-1 isozyme is expressed in platelets and it mediates production of the potent platelet activator and aggregator thromboxane A_2 (TxA_2). On the other hand, COX-2 produces prostaglandins at the sites of inflammation as well as PGI_2, which is a vasodilator and inhibitor of platelet aggregation. Although selective COX-2 inhibitors have no effect on TxA_2 production, by decreasing PGI_2 production, selective COX-2 inhibitors may tip the natural balance between prothrombotic TxA_2 and antithrombotic PGI_2 that could potentially increase the possibility of a thrombotic cardiovascular event.[10] As part of our ongoing program to design COX-2 inhibitors, we now describe a novel class of highly selective and potent inhibitors of COX-2 that also have the potential to selectively acetylate COX-2 at inflammatory sites.

The target 3-(2-, 3-, or 4-acetoxyphenyl)-4-(4-methanesulfonylphenyl)-2(5*H*)furanone isomers (**13a–c**) were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, bromination of 4-methylthioacetophenone (**7**), prepared in 95% yield by Friedel–Crafts acetylation of thioanisole (**6**), afforded the bromoacetyl derivative (**8**, 90%). Condensation of **8** with either 2-, 3-, or 4-methoxyphenylacetic acid in the presence of Et_3N yielded the respective isomeric 4-methylthiophenacyl 2-, 3-, or 4-methoxyphenyl acetate (**9a–c**, 41–58%). Cyclization of the isomers (**9a–c**) using NaH in DMSO gave the respective 3-(2-, 3-, or 4-methoxyphenyl)-4-(4-methylthiophenyl)-2(5*H*)furanone isomer (**10a–c**, 58–75%) which on *O*-demethylation using neat pyridinium chloride[11] at 190–210 °C yielded the corresponding phenol derivative (**11a–c**, 57–77%). Acetylation of **11a–c** gave the respective 3-(2-, 3-, or 4-acetoxyphenyl) isomer (**12a–c**, 78–100%). Subsequent oxidation of **12a–c** using Oxone® (-SMe→-SO_2Me) afforded the corresponding isomeric product (**13a–c**, 68–78%).

The 3-(2-, 3-, or 4-hydroxyphenyl)-4-(4-methanesulfonylphenyl)-2(5*H*)furanone isomers (**15a–c**) were synthesized using the reaction sequence illustrated in Scheme 2. Thus, oxidation of the methylthio isomers (**10a–c**) to the corresponding methanesulfonyl derivative (**14a–c**, 77–82%) using Oxone®, and subsequent *O*-demethylation using pyridinium hydrochloride gave the respective 3-(2-, 3-, or 4-hydroxyphenyl) product (**15a–c**, 32–46%).



Scheme 2. Reagents and conditions: (a) Oxone®, MeOH, THF, H_2O, 25 °C, 18 h; (b) pyridinium hydrochloride, 190–210 °C, 1 h.

A group of 3-(2-, 3-, and 4-acetoxyphenyl) analogues (**13a–c**) of rofecoxib were prepared to investigate the effect of isomeric 2-, 3-, and 4-acetoxy substituents on COX-2 selectivity and potency. In vitro COX-1/COX-2 inhibition studies showed that **13a–c**, which do not inhibit COX-1 (IC_{50} values >100 μM), are potent inhibitors of COX-2 (IC_{50} values in the 0.00126–0.00350 μM range) with high COX-2 selectivity indexes (SIs in the 28,482 to >79,365 range) relative to the reference drug rofecoxib (COX-2 IC_{50} = 0.4279 μM; SI >1168) as summarized in Table 1. These data suggest that the acetoxy isomers **13a–c** should inhibit the synthesis of inflammatory prostaglandins via the cyclooxygenase pathway at sites of inflammation and be devoid of ulcerogenicty due to the absence of COX-1 inhibition.

Aspirin treatment of human prostaglandin endoperoxide H synthase (hPGHS-1, hCOX-1) expressed in *cos*-1 cells causes a time dependent inactivation of oxygenase

Table 1. In vitro inhibition of COX-1 and COX-2 by 3-(2-, 3-, and 4-acetoxyphenyl) (**13a–c**), and 3-(2-, 3-, and 4-hydroxyphenyl) (**15a–c**) analogues of rofecoxib

Compd	R	COX-1 inhibition IC_{50}, μM[a]	COX-2 inhibition IC_{50}, μM[a]	COX-2 S.I.[b]
13a	2-OAc	>100	0.00350	>28,482
13b	3-OAc	>100	0.00168	>59,220
13c	4-OAc	>100	0.00126	>79,365
15a	2-OH	>250	1.832	>136
15b	3-OH	>250	3.96	>63
15c	4-OH	>250	>250	—
Rofecoxib		>500	0.4279	>1,168
Celecoxib		22.9	0.0567	404

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

activity. In contrast, treatment of PGHS-2 (COX-2) produced an enzyme that retained oxygenase activity, but which formed the unnatural (15*R*)-hydroxy-5,8,11,13-eicosatetraenoic acid [(15*R*)-HETE] exclusively that is a precursor to leukotrienes via the lipoxygenase (5-LO) pathway rather than prostaglandin H_2 (PGH_2) produced via the cyclooxygenase pathway. The K_m values for arachidonate of native and aspirin-treated hPGHS-2 were similar suggesting that arachidonate binds to both aspirin-treated and native hPGHS-2 in a similar manner.[12] A recent study has shown that (15*R*)-HETE inhibits the release of the potent inflammatory mediator LTB_4 from blood polymorphonuclear cells via the 5-LO pathway.[13] Based on these reports, it is possible that the acetoxy compounds **13a–c**, in addition to inhibiting COX-2, could also acetylate COX-2 to produce (15*R*)-HETE that would prevent the formation of inflammatory leukotrienes such as LTB_4 via the 5-LO pathway.

In view of the fact that the acetoxy compounds **13a–c** may undergo in vivo bioconversion by esterases to the phenolic compounds **15a–c**, the ability of **15a–c** to inhibit COX-1/COX-2 was also investigated. All three phenolic isomers **15a–c** were inactive inhibitors of COX-1 (IC_{50} values >250 μM). The relative COX-2 inhibition potency order for **15a–c**, which were much less potent inhibitors of COX-2 than the corresponding acetoxy analogues, was 2-OH (**15a**) > 3-OH (**15b**) > inactive 4-OH (**15c**). Compounds **15a** and **15b** exhibited COX-2 selectivity indexes of >136 and >63, respectively (Table 1).

A molecular modeling study was performed where 3-(3-acetoxyphenyl)-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**13b**) was docked in the active site of human COX-2 (1CX2 PDB file) using a procedure previously reported.[14] The objective of this docking experiment was to determine the orientation of **13b** within the COX-2 binding site and the spatial orientation of the acetoxy group relative to the serine hydroxyl group which it could potentially acetylate to produce acetylated COX-2. This docking study showed (see Fig. 1) **13b** binds in the center of human COX-2 primary active site such that the phenolic *O*H of Ser^{530} is about 6.05 Å from the *O*-atom of the C=O (3-OAc phenyl), and that the *S*-atom of the $MeSO_2$ moiety is inserted deep inside (4.53 Å) the entrance to the secondary COX-2 pocket (Val^{523}). In addition, the carbonyl *O*-atom of the central lactone ring is about 4.31 Å from one hydrogen atom of Arg^{120} (guanidino moiety).

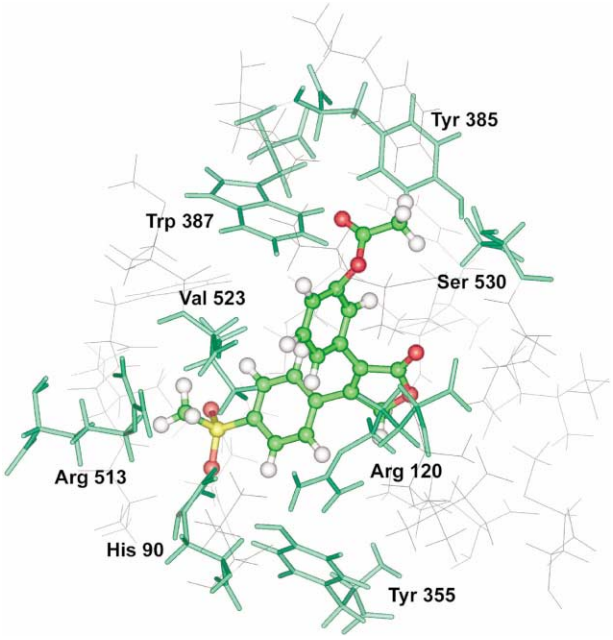


Figure 1. Docking the 3-OAc analogue of rofecoxib (**13b**) (ball-and-stick) in the active site of human COX-2 (line and stick) ($E_{intermolecular}$ = −60.74 kcal/mol).

The results of this investigation show (i) incorporation of a 2-, 3-, or 4-OAc substituent on the 3-phenyl ring of rofecoxib provides highly potent, and selective, COX-2 inhibitors, (ii) molecular modeling studies indicate the 3-OAc substituent of **13b** is suitably positioned to acetylate the serine hydroxyl group in the COX-2 primary binding site, and (iii) the acetoxy compounds **13a–c** could serve as useful probes to study the function and catalytic activity of the COX-2 isozyme.

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