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Design, synthesis, and biological evaluation of (*E*)-3-(4-methanesulfonylphenyl)-2-(aryl)acrylic acids as dual inhibitors of cyclooxygenases and lipoxygenases

Anne Moreau, Qiao-Hong Chen, P. N. Praveen Rao and Edward E. Knaus*

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

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Abstract—A group of (*E*)-3-(4-methanesulfonylphenyl)acrylic acids possessing a substituted-phenyl ring (4-H, 4-Br, 3-Br, 4-F, 4-OH, 4-OMe, 4-OAc, and 4-NHAc) attached to the acrylic acid C-2 position were prepared using a stereospecific Perkin condensation reaction. A related group of compounds having 4- and 3-(4-isopropyloxyphenyl)phenyl, 4- and 3-(2,4-difluorophenyl)phenyl and 4- and 3-(4-methanesulfonylphenyl)phenyl substituents attached to the acrylic acid C-2 position were also synthesized, using a palladium-catalyzed Suzuki cross-coupling reaction, for evaluation as dual cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) inhibitors. (*E*)-2-(3-Bromophenyl)-3-(4-methanesulfonylphenyl)acrylic acid (**9h**), and compounds having 4-(4-isopropyloxyphenyl-, 2,4-difluorophenyl-, or 4-methylsulfonylphenyl)phenyl moieties at the acrylic acid C-2 position (**11a**,**b**,**d**), were particularly potent COX-2 inhibitors with a high COX-2 selectivity index (COX-2 $IC_{50} \approx 0.32$ μM, SI > 316) similar to the reference drug rofecoxib (COX-2 $IC_{50} = 0.5$ μM, SI > 200). Acrylic acid analogs with a C-2 4-hydoxyphenyl (**9d**, $IC_{50} = 0.56$ μM), or 4-acetamidophenyl (**9g**, $IC_{50} = 0.11$ μM), substituent were particularly potent 5-LOX inhibitors that may participate in an additional specific hydrogen-bonding interaction. A number of compounds possessing a C-2 substituted-phenyl moiety (4-Br, 4-F, and 4-OH), or a 4- or 3-(2,4-difluorophenyl)phenyl moiety, showed potent 15-LOX inhibitory activity (IC_{50} values in the 0.31–0.49 μM range) relative to the reference drug luteolin ($IC_{50} = 3.2$ μM). Compounds having a C-2 4-acetylaminophenyl, or 4-(2,4-difluorophenyl)phenyl, moiety exhibited anti-inflammatory activities that were equipotent to aspirin, but less than that of celecoxib. The structure–activity data acquired indicate the acrylic acid moiety constitutes a suitable scaffold (template) to design novel acyclic dual inhibitors of the COX and LOX isozymes.

1. Introduction

Arachidonic acid (AA), following its release from membrane-bound phospholipids, undergoes biotransformation via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. In the COX pathway, the COX-1, COX-2, and COX-3 isoforms convert AA to the hydroxy-endoperoxide PGH_2 which is subsequently metabolized to prostaglandins (PGs), prostacyclin (PGI_2), and thromboxane A_2 (TxA_2). In contrast, AA is initially converted to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) that is then transformed into leukotrienes (LTs) via the 5-LOX pathway. The 8-LOX, 12-LOX, and 15-LOX isozymes similarly catalyze the transformation of AA to 8-HPETE, 12-HPTE, and 15-HPTE, respectively.[1] PGs and LTs produced in these COX and LOX pathways have been implicated as pro-inflammatory mediators in numerous inflammatory diseases, in allergic disorders,[2] in cell proliferation, and in neoangiogenesis.[3] Moreover, LTs are involved in the etiology of atherosclerosis.[4]

Rofecoxib (**1**, see Fig. 1) belongs to a class of compounds called coxibs that frequently possess two vicinal diaryl moieties attached to a central heterocyclic ring. A methanesulfonyl ($MeSO_2$), or sulfonamide (SO_2NH_2), substituent at the *para*-position of one of the phenyl rings frequently confers selective COX-2 inhibitory activity such that compounds of this type are useful for the treatment of inflammatory diseases such as rheumatoid arthritis.[5] Unlike traditional non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit both

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* Corresponding author. Tel.: +1 780 492 5993; fax: +1 780 492 1217; e-mail: eknaus@pharmacy.ualberta.ca

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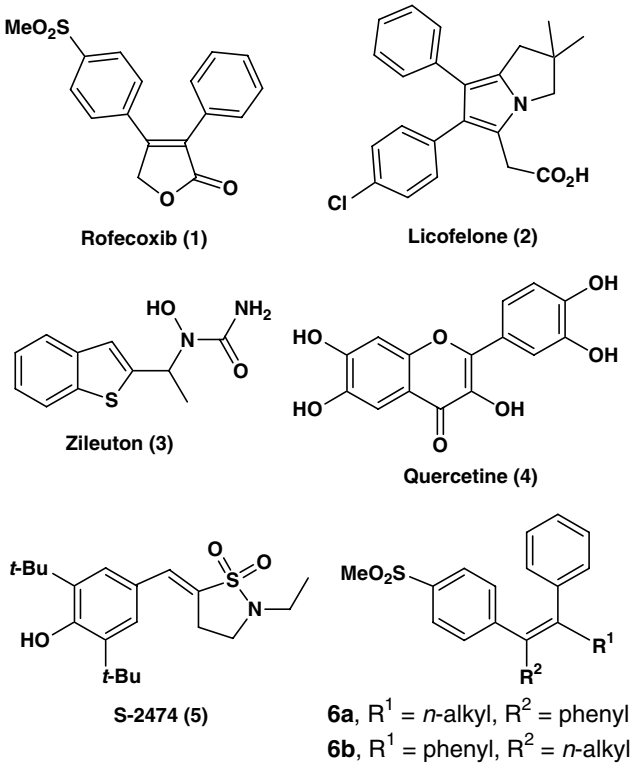


Figure 1. Some representative examples of cyclic (**1**) and acyclic (**6a**–**b**) selective COX-2 inhibitors, a dual COX-2/5-LOX inhibitor (**2**), an iron chelating 5-LOX inhibitor (**3**), and redox 5-LOX inhibitors (**4** and **5**).

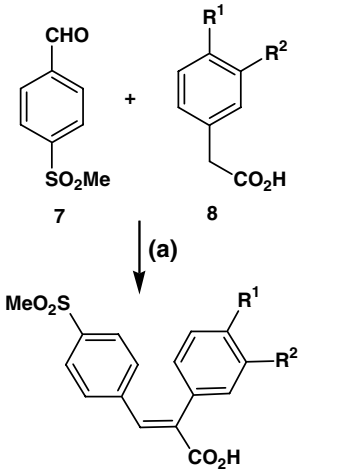
COX-1 and COX-2, selective COX-2 inhibitors do not induce adverse gastrointestinal irritation or hemorrhage. However, an increased risk of myocardial infarction and cardiovascular thrombotic events associated with the use of some selective COX-2 inhibitors has been observed. These adverse cardiovascular effects, which are attributed to a decreased level of the vasodilatory PGI_2 and an increased level of the potent platelet aggregator TxA_2, were primarily responsible for the recent withdrawal of rofecoxib (Vioxx®) and valdecoxib (Bextra®) from the market.[6]

Dual inhibitors of COX-2 and 5-LOX represent an attractive safer alternative to selective COX-2 inhibitors: in view of a potentially greater anti-inflammatory efficacy due to their ability to synergistically block both metabolic pathways of the AA cascade.[1] In this regard, licofelone (**2**), a dihydropyrrolizidine derivative, is a dual COX-2/5-LOX inhibitor currently in clinical trials for the treatment of osteoarthritis.[7] Wouters and coworkers[8] recently proposed a model for binding of non-redox inhibitors in the human 5-LOX active site that consisted of four major anchoring points (two hydrophobic groups, one hydrogen bond acceptor, and an aromatic ring), and two secondary binding points (an acidic moiety and an additional hydrogen bond acceptor). The carboxyl group of licofelone would be expected to undergo an electrostatic interaction with Arg411 in this model for the active site of 5-LOX.[8] Licofelone, being an active site direct 5-LOX inhibitor, is more potent and selective than zileuton (**3**) which inhibits 5-LOX by iron chelation involving its hydroxyurea moiety,[9] or compounds that inhibit 5-LOX by a non-specific redox mechanism[10] such as quercetine (**4**). Compounds possessing a 3,5-di-*tert*-butyl-4-hydroxyphenyl (DTBHP) moiety that can act as a redox inhibitor, or antioxidant, to interfere with the redox cycle of the 5-LOX isozyme have been investigated extensively in the search for new dual COX/LOX inhibitors. In this regard, S-2474 (**5**), a dual COX/5-LOX inhibitor in which the DTBHP moiety is linked through a vinyl bridge to a heterocyclic ring, was selected as an anti-arthritic drug candidate that is now undergoing clinical trials.[11]

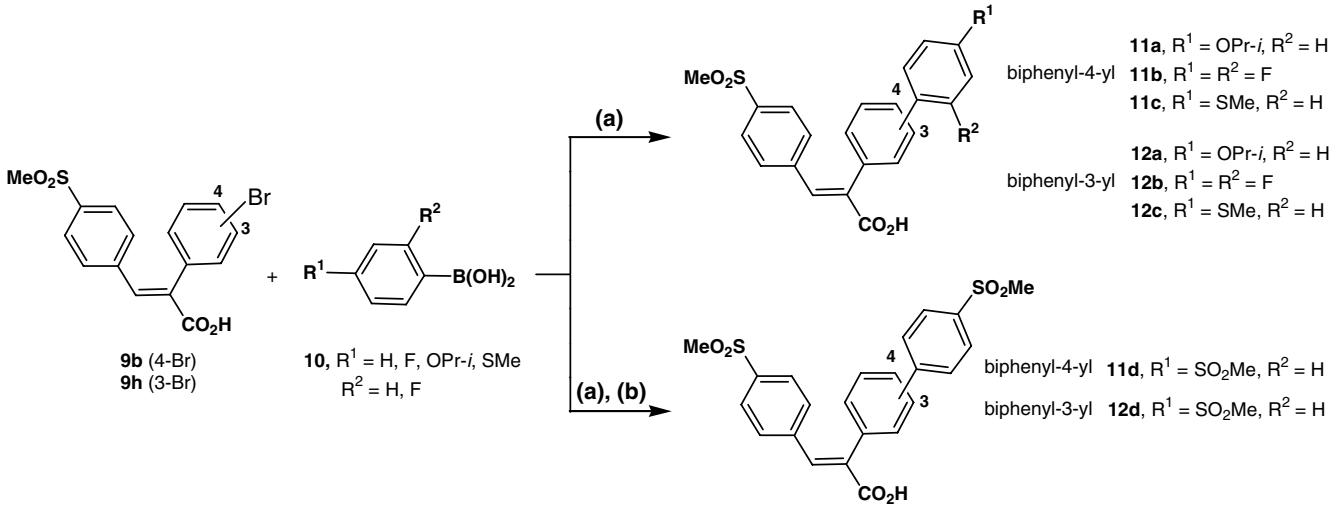
Recently, we reported that the triaryl (*Z*)-ethene regioisomers (**6a**[12a] and **6b**[12b]) having a COX-2 methanesulfonyl ($MeSO_2$) pharmacophore at the *para*-position of a phenyl ring in conjunction with a R^1 or R^2-alkyl substituent of appropriate chain length exhibited selective COX-2 inhibition. It was therefore of interest to design a new class of acyclic analogs of rofecoxib (**1**) of general structure **6** wherein R^1 is a carboxyl group that could potentially bind to Arg411 in the 5-LOX active site,[8] and R^2 is a hydrogen substituent, for evaluation as dual inhibitors of COX-2 and 5-LOX. Accordingly, we now describe the synthesis and biological evaluation of a group of (*E*)-2-(phenyl or biphenyl)-3-(4-methanesulfonylphenyl)acrylic acids (**9a**–**h**, **11a**–**d**, and **12a**–**d**).

2. Chemistry

The synthetic strategies used to prepare the target (*E*)-2-aryl-3-(4-methanesulfonylphenyl)acrylic acids are illustrated in Schemes 1 and 2. The starting reagent,



Scheme 1. Reagents and conditions: (a) Et_3N, Ac_2O, 90 °C, 12 h; (b) 10% w/v aqueous NaOH, 25 °C, 5 h.



Scheme 2. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, DME, reflux, 12 h; (b) Oxone®, MeOH, THF, 25 °C, 15 h.

4-methanesulfonylbenzaldehyde (**7**), could be prepared using either of two literature methods.[13] Thus, protection of the carbonyl function present in 4-methylsufanylbenzaldehyde as the dimethyl acetal, subsequent oxidation of the methylsulfanyl to a methylsulfonyl substituent using *meta*-chloroperbenzoic acid (MCPBA), and removal of the dimethyl acetal protecting group yielded 4-methylsufonylbenzaldehyde (**7**) in 70% overall yield. Alternatively, a nucleophilic aromatic substitution reaction involving displacement of the fluoro substituent in 4-fluorobenzaldehyde using sodium methane sulfinate in freshly distilled DMSO gave **7** in 69% yield.

The Perkin condensation[14] of 4-methanesulfonylbenzaldehyde (**7**), and a substituted-phenylacetic acid (**8**), afforded the respective (*E*)-3-(4-methanesulfonylphenyl)-2-phenylacrylic acid (**9a–c**,**e–h**) in a moderate 9–42% yield. The Perkin condensation of **7** with 4-aminophenylacetic acid (**8**, $R^1 = NH_2$, $R^2 = H$), in which the amino group is also acetylated, gave the *N*-acetylated product **9g** ($R^1 = NHCOMe$, $R^2 = H$). Alkaline hydrolysis of the acetoxy compound **9e** ($R^1 = OAc$, $R^2 = H$), obtained from the Perkin condensation of **7** with 4-acetoxyphenylacetic acid,[15] yielded the phenolic compound **9d** in 80% yield. The stereochemistry of the Perkin reaction preferentially produces the α-phenylcinnamic acid stereoisomer with *cis*-phenyl groups and an unhindered carboxyl group. This stereochemical specificity is attributed to the fact that the initial condensation reaction is not reversible, and that the reaction stereochemistry is eliminatively controlled.[14c]

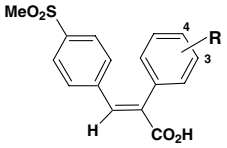
The target (*E*)-3-(4-methanesulfonylphenyl)acrylic acids **11a–c** and **12a–c**, possessing a substituted-biphenyl ring ($R^1 = H$, F, *i*-OPr, SMe; $R^2 = H$, F) attached to the acrylic acid C-2 position, were prepared using a palladium-catalyzed Suzuki cross-coupling reaction[16] according to the reaction sequence depicted in Scheme 2. Compounds **11** and **12** having the following aryl substituents were synthesized ($R^1 = i$-OPr, $R^2 = H$; $R^1 = R^2 = F$; $R^1 = SO_2Me$, $R^2 = H$) since biological data from a previous study[17] investigating *N*-acetyl-2-(or 3)-carboxymethylbenzenesulfonamides showed that compounds having a 4-isopropoxyphenyl, 2,4-difluorophenyl, or 4-methylsulfonylphenyl, substituent attached to the benzenesulfonamide ring were the most potent in vitro COX isozyme inhibitors and in vivo anti-inflammatory agents.

The Suzuki cross-coupling reaction between the aryl bromide **9b** or **9h**, and either 4-isopropoxyphenylboronic acid, 2,4-difluorophenylboronic acid or 4-(methylthio)phenylboronic acid, in the presence of 2 M aqueous sodium carbonate in ethylene glycol dimethyl ether (DME) using tetrakis(triphenylphosphine)palladium(0) as a catalyst afforded the respective target compounds **11a–c**, or **12a–c**, in 21–67% yield. The subsequent oxidation of the thiomethyl compounds **11c** and **12c** using Oxone® afforded the corresponding methanesulfonyl compounds **11d** and **12d** in 38% and 64% yield, respectively.

3. Results and discussion

In vitro structure–activity relationships acquired for this group of (*E*)-2-(phenyl or biphenyl)-3-(4-methanesulfonylphenyl)acrylic acids (**9**, **11**, and **12**) showed that they exhibit a broad range (potent-to-inactive) of COX/LOX inhibitory activities (COX-1 $IC_{50} = 0.4$ to >100 μM range; COX-2 $IC_{50} = 0.3$ to >100 μM range; 5-LOX $IC_{50} = 0.11$ to >10 μM range; 15-LOX $IC_{50} = 0.31$ to >10 μM range; see data in Table 1). All of the compounds **9** possessing a *para*-phenyl substituent (4-H, 4-Br, 4-F, 4-OMe, and 4-NHAc), with the exception of the inactive 4-OH and 4-OAc analogs, exhibited low-to-moderate in vitro inhibition ($IC_{50} = 0.88–31.6$ μM range) of the COX-1 isozyme. In the COX-2 isozyme inhibition assay, all compounds (**9a–g**) in this group, including the 4-OH and 4-OAc analogs, exhibited weak-to-moderate activity ($IC_{50} = 1.9–36.0$ μM range). In an earlier study,[18] we reported that a hydroxy analog of rofecoxib possessing a C-3 4-hydroxyphenyl substituent (see structure of rofecoxib in Fig. 1) was an inactive

Table 1. In vitro COX-1/COX-2 and 5-LOX/15-LOX enzyme inhibition assay data for (*E*)-2-(phenyl or biphenyl)-3-(4-methanesulfonylphenyl)acrylic acids (**9**, **11**, and **12**)



Compound	R	IC_{50}[a] (μM)		Selectivity index (SI)[b]	IC_{50}[a] (μM)		Volume[c] (Å^3)
		COX-1	COX-2		5-LOX	15-LOX	
9a	4-H	1.5	3.0	0.50	>10	>10	247.4
9b	4-Br	0.88	3.6	0.24	>10	0.31	269.4
9c	4-F	0.4	36.0	—	1.3	0.32	252.4
9d	4-OH	>100	5.3	>19	0.56	0.45	256.0
9e	4-OMe	31.6	1.9	16.6	>10	5.5	273.2
9f	4-OAc	>100	2.9	>34	>10	> 10	292.6
9g	4-NHAc	3.2	2.5	1.3	0.11	>10	295.7
9h	3-Br	>100	0.31	>322	>10	>10	269.8
11a	4-(4-*i*-PrO–C_6H_4)–	>100	0.32	>312	>10	3.1	377.5
11b	4-(2,4-F_2–C_6H_3)–	>100	0.32	>312	3.1	0.49	326.1
11d	4-(4-MeO_2S–C_6H_4)–	>100	0.32	>312	>10	>10	369.7
12a	3-(4-*i*-PrO–C_6H_4)–	>100	>100	—	>10	6.2	377.6
12b	3-(2,4-F_2–C_6H_3)–	>100	>100	—	>10	0.31	327.7
12d	3-(4-MeO_2S–C_6H_4)–	>100	2.1	>47	>10	>10	369.7
Luteolin					—	3.2	231.9
Caffeic acid					3.0	—	153.5
NDGA[d]					>10	3.5	285.3
Celecoxib		33.1	0.07	472	—	—	298.5
Rofecoxib		>100	0.5	>200	—	—	267.2

[a] Values are means of two determinations acquired using an ovine COX-1/COX-2 and potato 5-LOX/soyabean 15-LOX, assay kits (Catalog Nos. 560101, 60401, and 760700, 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.

[d] NDGA, nordihydroguaiaretic acid.

inhibitor of either COX-1 or COX-2 (IC_{50} > 250 μM), whereas the C-3 4-acetoxyphenyl analog of rofecoxib was a potent COX-2 inhibitor (IC_{50} = 0.00126 μM) having an extremely high COX-2 selectivity index (SI > 79,365). In this regard, the ability of the 4-OH (**9d**) compound to inhibit COX-2 distinguishes it from the 4-hydroxy analog of rofecoxib.[18] The point of attachment of a bromo substituent was a determinant of inhibitory activity since the 3-bromo isomer (**9h**) was a more potent (IC_{50} = 0.31 μM) and selective (SI > 322) COX-2 inhibitor, relative to the less potent (IC_{50} = 3.6 μM) and selective (SI = 0.24) 4-bromo isomer (**9b**).

Although the binding sites of the COX-1 and COX-2 isozymes are highly similar such that they have virtually identical tertiary and quaternary structures, there are small structural differences between the two binding sites that can be exploited in drug design. In this context, a single amino acid residue lining the channel that differs between COX-1 and COX-2 involves the presence of valine (Val523) in COX-2 versus isoleucine (Ile523) in COX-1. Accordingly, the smaller valine side chain in COX-2 induces a conformational change at Tyr355 thereby forming an additional hydrophobic secondary internal pocket protruding off the primary binding site in COX-2 that is absent in COX-1.[19] Consequently, the total volume of the COX-2 primary binding site and its associated secondary pocket (394 Å^3) is about 25% larger than that for the COX-1 binding site (316 Å^3).[20] A comparison of the molecular volumes (see data in Table 1) of compounds **9a**–**h** possessing a smaller substituted-phenyl moiety (247–273 Å^3 range) shows that COX-1 inhibitory activity is less than that of compounds **11** and **12** having a larger substituted-biphenyl moiety (326–377 Å^3 range) that are inactive COX-1 inhibitors. These data suggest that the latter substituted-biphenyl compounds **11** and **12** are too large to bind in the smaller COX-1 binding site. In this regard, the biphenyl compounds **11a**,**b**,**d** exhibited potent (IC_{50} = 0.32 μM) and selective (SI > 312) COX-2 inhibitory activities. The point of attachment of the two phenyl rings in the biphenyl compounds **11** and **12** was a determinant of COX-2 inhibitory activity since the biphenyl-4-yl isomers **11** were more potent than the corresponding biphenyl-3-yl regioisomers (**12**).

Structure–activity relationship 5-LOX/15-LOX inhibition studies suggest that the structural requirements for 5-LOX inhibition are more stringent than for 15-LOX inhibition. Wouters and co-workers[8] recently proposed a model that serves as a first step toward characterization of human 5-LOX and its interaction with ligands. This model is comprised of four major anchoring points (two hydrophobic groups, one hydrogen bond acceptor, and an aromatic ring), and two second-

ary binding points (an acidic moiety and an additional hydrogen bond acceptor). Accordingly, it is plausible that the 4-F (**9c**), 4-OH (**9d**) or 4-NHAc (**9g**) substituent present in the 5-LOX inhibitory compounds investigated may participate in a hydrogen-bonding interaction with the 5-LOX binding site. Compounds **9c**, **9d**, and **9g** were more potent (5-LOX IC_{50} = 0.11–1.3 μM range) than the reference drug caffeic acid (IC_{50} = 3.0 μM). A much larger group of 4-substituted-phenyl compounds (**9b**, 4-Br; **9c**, 4-F; **9d**, 4-OH; **9e**, 4-OMe; IC_{50} = 0.31–5.5 μM range), and substituted-biphenyl compounds (**11a**,**b**, **12a**,**b**; IC_{50} = 0.31–6.2 μM range) showed moderate-to-highly potent 15-LOX inhibitory activity relative to the reference drugs luteolin (IC_{50} = 3.2 μM) and NDGA (IC_{50} = 3.5 μM). Compounds **11b** and **12b** having a terminal 2,4-difluorophenyl substituent exhibited optimal 15-LOX inhibition in the substituted-biphenyl group of compounds **11** and **12**.

The most stable enzyme–ligand complex of the potent and selective COX-2 inhibitor compound **11b** {(*E*)-2-[4-(2,4-difluorophenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid} docked in the COX-2 active site (Fig. 2) shows that it binds in the primary binding site such that the *para*-SO_2Me COX-2 pharmacophore on the C-3 phenyl ring is oriented in the vicinity of the secondary pocket present in COX-2 (Val523, Phe518, Ile517, Ala516, Ser353, Leu352, and His90). One of the *O*-atoms of the S*O*$_2$Me moiety is involved in a hydrogen-bonding interaction with the backbone N*H* of Ile517 (distance ≈ 2.26 Å), whereas the second *O*-atom forms a weak hydrogen bond with the N*H* of His90 (distance ≈ 3.50 Å). The 2,4-difluorophenyl moiety that is *cis* to the C-3 *p*-$MeSO_2$-phenyl group is oriented in a hydrophobic pocket at the apex of the COX-2 binding site (Met522, Phe518, Trp387, Tyr385, Leu384, and Leu352). The *p*-fluoro substituent is positioned within van der Waals contact range of the amino acid residues Met522 and Leu384 (distance < 5 Å). The distance between the centre of the C-1 4-substituted-phenyl ring and *O*H of Ser530 is about 5.04 Å. The COOH substituent attached to the central *trans* C=C olefinic bond is oriented in an area close to the mouth of the COX-2 binding site. It is interesting to note that the C-1 COOH substituent undergoes both ion–ion (electrostatic) and hydrogen-bonding interactions with polar amino acid residues. The distance between the NH_2 of the charged Arg120 and the *O*H of the COOH substituent is about 3.13 Å. In comparison, the distance between the O*H* of Tyr355 and the *O*H of the COOH group is about 4.90 Å. A hydrogen-bonding interaction was also observed between the C=*O* of the COOH substituent and the O*H* of Ser121 that is closer to the mouth of the COX-2 binding site (distance ≈ 3.19 Å).

The binding interactions of the potent 5 and 15-LOX inhibitor compound **11b** {(*E*)-2-[4-(2,4-difluorophenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid} within the 15-LOX binding site were investigated. The most stable enzyme–ligand complex for **11b** (Fig. 3) shows that **11b** is positioned in the 15-LOX binding site such that the C-3 *para*-$MeSO_2$-phenyl moiety is oriented

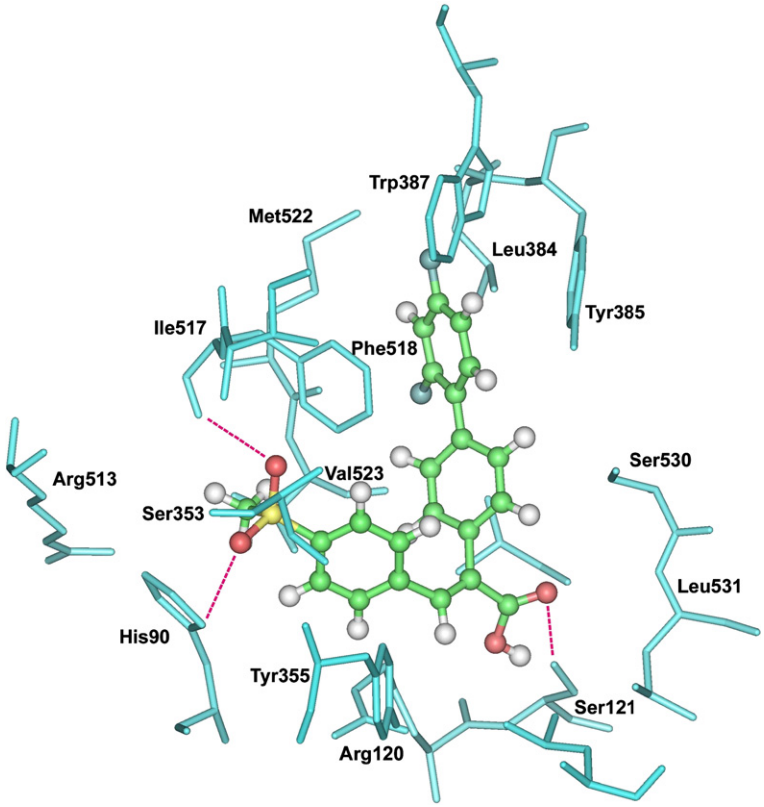


Figure 2. (*E*)-2-[4-(2,4-Difluorophenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid (**11b**) (ball and stick) docked in the active site of murine COX-2. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

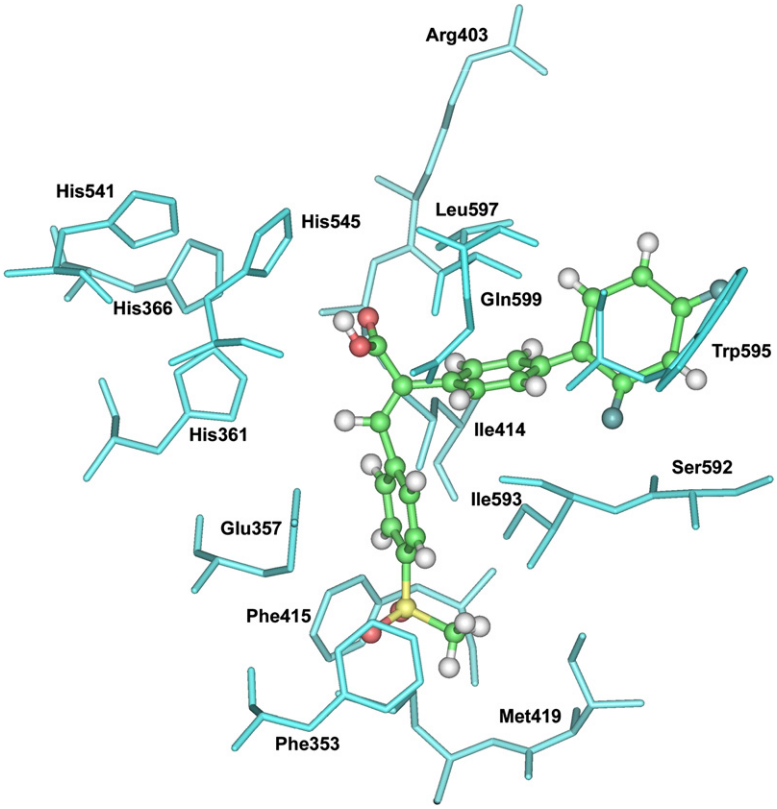


Figure 3. (*E*)-2-[4-(2,4-Difluorophenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid (**11b**) (ball and stick) docked in the active site of soyabean 15-LOX. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

toward the base of the binding site (Ile593, Met419, Ile418, and Phe415). The *p*-SO_2Me COX-2 pharmacophore is located within van der Waals contact range of the amino acid residues Ile593, Met419, and Ile418 (distance < 5 Å). The 2,4-difluorophenyl group is oriented toward a hydrophobic region comprised of the amino acid residues Leu597, Trp595, Ile414, and Leu408 closer to the base of the 15-LOX binding site (distance < 5 Å). It is interesting to note that the C-1 COOH group is oriented toward the catalytic site (His545, His366, and His361) where it undergoes an ion–ion (electrostatic) interaction. The distance between the N*H* of His361 and the *O*H of the COOH substituent is about 4.53 Å. In comparison, the distance between the N*H* of His361 and the C=*O* of the COOH substituent is about 4.06 Å. There is a relative large spatial separation between the COOH substituent and the charged Arg403 that is located at the mouth of the 15-LOX binding site (distance > 10 Å). This latter observation indicates that interaction of the C-1 CO_2H with Arg403 is not a requirement for 15-LOX inhibitory activity.

A similar molecular modeling study was performed where compound **9d** [(*E*)-2-(4-hydroxyphenyl)-3-(4-methanesulfonylphenyl)acrylic acid] was docked in the 15-LOX binding site (Fig. 4). This study showed that the C-3 *para*-$MeSO_2$-phenyl moiety is oriented toward the catalytic site near His545, Ile544, His366, and His361. The C-1 *p*-hydroxyphenyl group that is *cis* to the C-3 *para*-$MeSO_2$-phenyl moiety is oriented in a region comprised of Leu597, Gln548, Leu408, Glu357, and His361. The *O*-atom of the *p*-HO-phenyl group undergoes a hydrogen bond interaction with the N*H* of His361 (distance ≈ 2.0 Å). The C-1 COOH substituent is surrounded by Gln548 and Gly598. The distance between the *O*H (of COOH) and the NH_2 of Gln548 is about 4.59 Å. The C=*O* of the COOH substituent forms a hydrogen bond with the backbone N*H* of Gly598 (distance ≈ 2.73 Å). There is a large spatial separation between the COOH substituent and the charged Arg403 that is located at the mouth of the 15-LOX binding site (distance > 13 Å).

The (*E*)-3-(4-methanesulfonylphenyl)acrylic acids possessing C-2 4-hydroxyphenyl (**9d**), 4-acetylaminophenyl (**9g**), and 4-(2,4-difluorophenyl)phenyl] (**11b**) aryl moieties, based on in vitro COX and LOX isozyme inhibition data, were selected for further in vivo pharmacological evaluation to determine their anti-inflammatory (AI) and analgesic activities (see data in Table 2). In a carrageenan-induced rat paw edema assay model, the relative potency order was NHAc (**9g**, ED_{50} = 109.5 mg/kg) ≈ 2,4-F_2–C_6H_3– (**11b**, ED_{50} = 112.4 mg/kg) > OH (**9d**, ED_{50} = 363.4 mg/kg) relative to the 5-LOX inhibitor caffeic acid (8.2% inhibition for a 30 mg/kg dose), the 15-LOX inhibitor nordihydroguaiaretic acid (NDGA, ED_{50} = 205 mg/kg), the non-selective COX-1/COX-2 inhibitor aspirin (ED_{50} = 129 mg/kg), and selective COX-2 inhibitor celecoxib (ED_{50} = 10.8 mg/kg) reference drugs. One plausible explanation for the lower potency of the OH compound **9d**, relative to the NHAc (**9g**) and 2,4-F_2–C_6H_3– (**11b**) compounds, may be due to

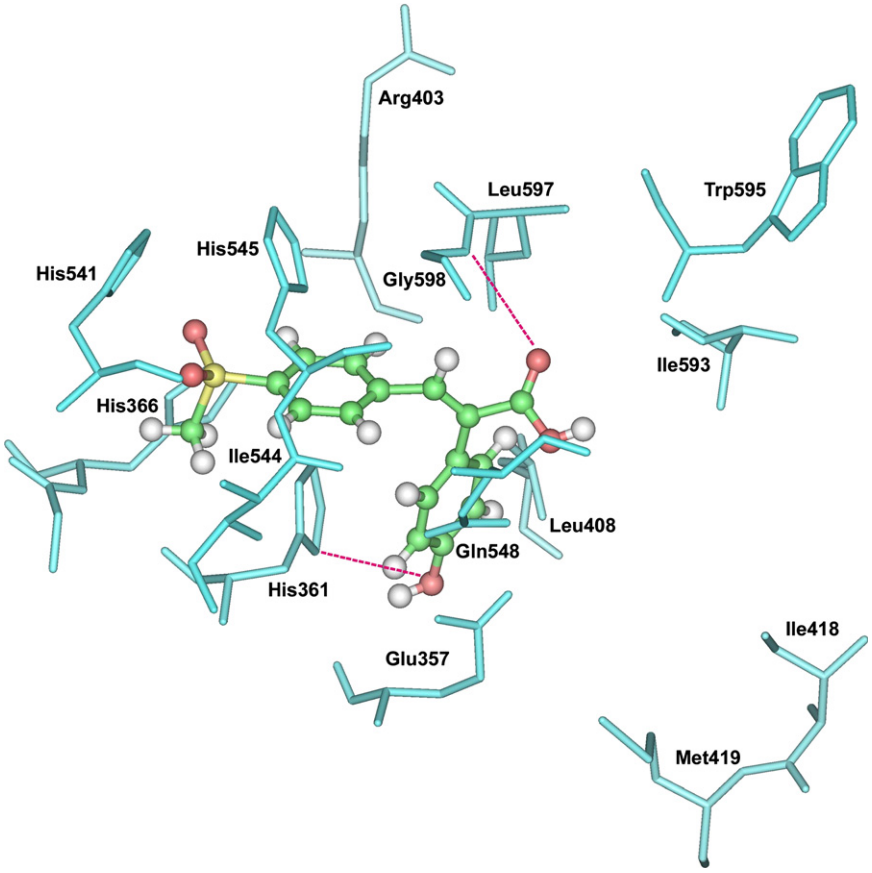
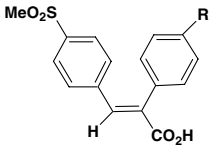


Figure 4. (*E*)-2-(4-Hydroxyphenyl)-3-(4-methanesulfonylphenyl)acrylic acid (**9d**) (ball and stick) docked in the active site of soyabean 15-LOX. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

Table 2. In vivo anti-inflammatory and analgesic activities for (*E*)-2-(4-hydroxyphenyl)-3-(4-methanesulfonylphenyl)acrylic acid (**9d**), (*E*)-2-(4-acetylaminophenyl)-3-(4-methanesulfonylphenyl)acrylic acid (**9g**), and (*E*)-2-[4-(2,4-difluorophenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid (**11b**)



Compound	R	AI activity[a]	Analgesic activity[b]	
		ED_{50} (mg/kg)	% Inhibition (30 min)	% Inhibition (60 min)
9d	–OH	363.4	54.1 ± 5.8	45.8 ± 5.8
9g	–NHAc	109.5	72.0 ± 3.3	68.0 ± 3.2
11b	(2,4-F_2-C_6H_3)–	112.4	73.3 ± 3.1	63.3 ± 7.7
Caffeic acid	—	8.2 ± 2.5[c]	47.2 ± 10.6[d]	58.3 ± 12.8[d]
NDGA	—	205.0	45.8 ± 9.2[d]	62.5 ± 4.8[d]
Aspirin	—	129.0	56.5 ± 9.8	54.3 ± 13.7
Celecoxib	—	10.8	31.7 ± 9.6	62.0 ± 7.3

[a] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as means ± SEM ($n = 4$) at 3 h following a 30 mg/kg oral dose of the test compound or as the ED_{50} value when it was determined.
[b] Inhibitory activity in the rat 4% NaCl-induced abdominal constriction assay. The results are expressed as means ± SEM ($n = 4$–5) following a 50 mg/kg oral dose of the test compound.
[c] Percent (%) inhibition of inflammation for a 30 mg/kg oral dose.
[d] Percent (%) reduction in abdominal constrictions for a 30 mg/kg oral dose.

the greater ability of **9d** to form inactive *O*-glucuronide and/or *O*-sulfate conjugates. In vitro COX isozyme inhibition studies (Table 1) showed that **9d** is a moderately selective COX-2 inhibitor (SI > 19), **9g** is a non-selective COX-2 inhibitor (SI = 1.3), and **11b** is a highly selective COX-2 inhibitor (SI > 312). Compounds **9g** and **11b** are

equipotent anti-inflammatory agents even though there is a large difference in their COX-2 potency. Accordingly, further studies are required to develop a data bank to determine the most ideal COX-2/COX-1 and 5-LOX/15-LOX values for anti-inflammatory efficacy.

In a rat 4% NaCl-induced abdominal constriction (analgesic) assay, a 50 mg oral dose of the (*E*)-olefins **9d**, **9g**, and **11b** exhibited good analgesic activities (45–73% range) comparable to the reference drugs caffeic acid, NDGA, aspirin, and celecoxib at 30 and 60 min post-drug administration (see data in Table 2).

4. Conclusions

A new class of (*E*)-2-(aryl)-3-(4-methanesulfonylphenyl)acrylic acids were designed that possess *para*-methanesulfonylphenyl (COX-2), and substituted-phenyl or -biphenyl (5-LOX), pharmacophores for evaluation as dual acting COX/LOX inhibitors. The structure–activity data acquired indicate that two compounds (**9d** and **11b**) exhibit an interesting combination of COX-2/5-LOX/15-LOX inhibitory activities. (*E*)-2-(4-Hydroxyphenyl)-3-(4-methanesulfonylphenyl)acrylic acid (**9d**) is a weak, but moderately selective (SI > 19), COX-2 inhibitor that is simultaneously a potent balanced inhibitor of both 5-LOX (IC_{50} = 0.56 μM) and 15-LOX (IC_{50} = 0.45 μM). In comparison, (*E*)-2-[4-(2,4-difluorophenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid (**11b**) is a potent (IC_{50} = 0.32 μM) and selective (SI > 312) COX-2 inhibitor that is also an effective inhibitor of 5-LOX (IC_{50} = 3.1 μM) and 15-LOX (IC_{50} = 0.49 μM). The modeling studies show that substituent steric bulk (volume), regiochemistry, and electronic parameters modulate COX-2 and 15-LOX ligand–enzyme binding interactions which can facilitate the interpretation of in vitro enzyme inhibition structure–activity data.

5. Experimental

Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded as films on NaCl plates using a Nicolet 550 Series II Magna FT-IR spectrometer. ^{1}H NMR spectra were measured on a Bruker AM-300 spectrometer in $CDCl_3$ or $CDCl_3$ + DMSO-d_6 as solvent with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz. Spin multiplets are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Microanalyses were performed for C and H (Microanalytical Service Laboratory, Department of Chemistry, University of Alberta), and were within ±0.4% of theoretical values. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70–230 mesh). Luteolin, caffeic acid, and nordihydroguaiaretic acid (NDGA) were purchased from Cayman Chemicals (Ann Arbor, MI). All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification.

5.1. General procedure for the synthesis of (*E*)-acrylic acids (9a–c and 9e–h)

A solution of 4-methanesulfonylbenzaldehyde (**7**, 1.38 g, 7.5 mmol), the appropriate phenylacetic acid (**8**, 7.5 mmol), and triethylamine (1.02 mL, 7.3 mmol) in acetic anhydride (6.95 mL) was stirred at 90 °C for 16 h. After cooling to 25 °C, diethyl ether (30 mL) and water (20 mL) were added, and the organic phase was extracted with 10% w/v aqueous NaOH (3× 30 mL). The combined aqueous layers were acidified to pH 2 using concentrated HCl, the precipitate was filtered off, the precipitate was dissolved in EtOAc, and the EtOAc solution was dried (Na_2SO_4). Removal of the solvent under vacuum afforded a residue which was purified by silica gel column chromatography using EtOAc–petroleum ether (7:3, v/v) as eluant. Physical, spectral, and microanalytical data for **9a**–**c** and **9e**–**h** are listed below.

5.2. (*E*)-3-(4-Methanesulfonylphenyl)-2-phenylacrylic acid (9a)

Yield, 42%; white powder; mp 176–178 °C; IR (film): 3463–2530 (COOH), 1686 (C=O), 1304 (SO_2), 1219, 1149 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.02 (s, 3H, CH_3SO_2), 7.19–7.26 (m, 4H, 4-methanesulfonylphenyl H-2, H-6, phenyl H-3, H-5), 7.36–7.43 (m, 3H, phenyl H-2, H-6, H-4), 7.75 (d, *J* = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.96 (s, 1H, H-3). Anal. Calcd for $C_{16}H_{14}O_4S$: C, 63.56; H, 4.67. Found: C, 63.76; H, 4.93.

5.3. (*E*)-2-(4-Bromophenyl)-3-(4-methanesulfonylphenyl)acrylic acid (9b)

Yield, 9%; white powder; mp 250–252 °C; IR (film) 3430–2712 (COOH), 1688 (C=O), 1307 (SO_2), 1223, 1150 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.14 (s, 3H, CH_3SO_2), 7.11 (d, *J* = 8.2 Hz, 2H, 4-bromophenyl H-3, H-5), 7.29 (d, *J* = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.55 (d, *J* = 8.2 Hz, 2H, 4-bromophenyl H-2, H-6), 7.73 (d, *J* = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.82 (s, 1H, H-3). Anal. Calcd for $C_{16}H_{13}BrO_4S$: C, 50.41; H, 3.44. Found: C, 50.29; H, 3.49.

5.4. (*E*)-2-(4-Fluorophenyl)-3-(4-methanesulfonylphenyl)acrylic acid (9c)

Yield, 11%; white crystals; mp 243–245 °C; IR (film) 3473–2713 (COOH), 1685 (C=O), 1307 (SO_2), 1222, 1151 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.17 (s, 3H, CH_3SO_2), 7.16–7.22 (m, *J* = 7.3 Hz, 4H, 4-fluorophenyl hydrogens), 7.29 (d, *J* = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, *J* = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.83 (s, 1H, H-3). Anal. Calcd for $C_{16}H_{13}FO_4S$: C, 59.99; H, 4.09. Found: C, 59.73; H, 4.13.

5.5. (*E*)-2-(4-Acetoxyphenyl)-3-(4-methanesulfonylphenyl)acrylic acid (9e)

Yield, 32% (from 4-acetoxyphenylacetic acid); bright white crystals; mp 242–243 °C; IR (film): 3513–2487

(COOH), 1764 (C=O of OAc), 1686 (C=O), 1298 (SO_2), 1219, 1149 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.23 (s, 3H, $COCH_3$), 2.94 (s, 3H, CH_3SO_2), 7.02 (d, J = 8.5 Hz, 2H, 4-acetoxyphenyl H-3, H-5), 7.14 (d, J = 8.5 Hz, 2H, 4-acetoxyphenyl H-2, H-6), 7.15 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.64 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.78 (s, 1H, H-3). Anal. Calcd for $C_{18}H_{16}O_6S$: C, 59.99; H, 4.47. Found: C, 59.68; H, 4.39.

5.6. (*E*)-3-(4-Methanesulfonylphenyl)-2-(4-methoxyphenyl)acrylic acid (9f)

Yield, 41%; yellow powder; mp 210–211 °C; IR (film): 2610–3482 (COOH), 1684 (C=O), 1293 (SO_2), 1219, 1149 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.03 (s, 3H, CH_3SO_2), 3.85 (s, 3H, OCH_3), 6.92 (d, J = 8.5 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.14 (d, J = 8.5 Hz, 2H, 4-methoxyphenyl H-2, H-6), 7.28 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.76 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.91 (s, 1H, H-3). Anal. Calcd for $C_{17}H_{16}O_5S$: C, 61.43; H, 4.85. Found: C, 61.17; H, 4.93.

5.7. (*E*)-2-(4-Acetylaminophenyl)-3-(4-methanesulfonylphenyl)acrylic acid (9g)

Yield, 32% (from 4-aminophenylacetic acid); pale yellow powder; mp 275–277 °C; IR (film): 1733 (C=O), 1683 (C=O), 1218 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.06 (s, 3H, $COCH_3$), 3.18 (s, 3H, CH_3SO_2), 7.09 (d, J = 8.5 Hz, 2H, 4-acetylaminophenyl H-2, H-6), 7.32 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.58 (d, J = 8.5 Hz, 2H, 4-acetylaminophenyl H-3, H-5), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.78 (s, 1H, H-3), 10.03 (br s, 1H, N*H*), 12.93 (br s, 1H, CO_2H). Anal. Calcd for $C_{18}H_{17}NO_5S$: C, 60.15; H, 4.77. Found: C, 59.93; H, 4.80.

5.8. (*E*)-2-(3-Bromophenyl)-3-(4-methanesulfonylphenyl)acrylic acid (9h)

Yield, 18%; white crystals; mp 200–201 °C; IR (film) 3460–2698 (COOH), 1679 (C=O), 1306 (SO_2), 1228, 1146 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.19 (s, 3H, CH_3SO_2), 7.17 (d, J = 7.6 Hz, 1H, bromophenyl H-4), 7.30–7.37 (m, 3H, 4-methanesulfonylphenyl H-2, H-6, bromophenyl H-5), 7.44 (t, J = 1.3 Hz, 1H, bromophenyl H-2), 7.58 (dd, J = 8.2, 1.3 Hz, 1H, bromophenyl H-6), 7.78 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.87 (s, 1H, H-3), 13.09 (br s, 1H, CO_2H). Anal. Calcd for $C_{16}H_{13}BrO_4S$: C, 50.41; H, 3.44. Found: C, 50.31; H, 3.53.

5.9. Synthesis of (*E*)-2-(4-hydroxyphenyl)-3-(4-methanesulfonylphenyl)acrylic acid (9d)

A solution of 10% w/v aqueous NaOH (25 mmol, 1.0 g) was added to a solution of (*E*)-2-(4-acetoxyphenyl)-3-(4-methanesulfonylphenyl)acrylic acid (**9e**, 1.80 g, 5.0 mmol) in methanol (20 mL). The reaction mixture was stirred at 25 °C for 5 h and then concentrated under vaccuo. The residue obtained was extracted with ethyl acetate (3× 20 mL), the combined organic layers were washed with brine, and the EtOAc solution was dried ($MgSO_4$). Removal of the organic solvent under vacuum furnished a residue which was purified by silica gel column chromatography using EtOAc–petroleum ether (8:2, v/v) as eluant to yield **9d** (80%) as a pale yellow powder; mp 219–221 °C; IR (film) 3488–2844 (COOH), 1685 (C=O), 1297 (SO_2), 1217, 1146 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.18 (s, 3H, CH_3SO_2), 6.75 (d, J = 8.5 Hz, 2H, 4-hydroxyphenyl H-3, H-5), 6.96 (d, J = 8.5 Hz, 2H, 4-hydroxyphenyl H-2, H-6), 7.32 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.72 (s, 1H, H-3), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 9.59 (br s, 1H, OH), 12.82 (br s, 1H, CO_2H). Anal. Calcd for $C_{16}H_{14}O_5S$: C, 60.37; H, 4.43. Found: C, 59.97; H, 4.57.

5.10. General procedure for the synthesis of (*E*)-acrylic acids (11a–c and 12a–c)

An aqueous Na_2CO_3 solution (5.8 mL of 2 M), and tetrakis(triphenylphosphine)palladium (67 mg, 0.058 mmol) were added to a solution of either (*E*)-2-(4-bromophenyl)-3-(4-methanesulfonylphenyl)acrylic acid **(9b)**, or (*E*)-2-(3-bromophenyl)-3-(4-methanesulfonylphenyl)acrylic acid **(9h**, 0.74 g, 1.94 mmol), and a substituted-phenylboronic acid (**10**, 2.91 mmol), in DME (20 mL). The reaction was allowed to proceed at reflux for 16 h, the reaction mixture was cooled to 25 °C, and the solvent was removed in vaccuo. Ethyl acetate (30 mL) and water (10 mL) were added, and the mixture was acidified to pH 3 using an aqueous solution of HCl (10% w/v) prior to extraction with ethyl acetate (2× 20 mL). The organic layers were combined, washed with brine, and the organic fraction was dried (Na_2SO_4). Removal of the solvent under vacuum gave a residue which was purified by silica gel column chromatography using chloroform–methanol (9.5:0.5, v/v) as eluant. Physical, spectral, and microanalytical data for **11**–**c** and **12a**–**c** are listed below.

5.11. (*E*)-2-[4-(Isopropoxyphenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid (11a)

Yield, 28%; pale yellow cotton form; mp 228–230 °C; IR (film) 3453–2728 (COOH), 1683 (C=O), 1306 (SO_2), 1244, 1150 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.29 (d, J = 6.1 Hz, 6H, $CH(CH_3)_2$), 3.18 (s, 3H, CH_3SO_2), 4.67 (septet, J = 6.1 Hz, 1H, $CH(CH_3)_2$), 7.00 (d, J = 8.8 Hz, 2H, 4-isopropoxyphenyl H-3, H-5), 7.23 (d, J = 8.2 Hz, 2H, 1,4-disubstituted-phenyl H-2, H-6), 7.36 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.62–7.66 (m, 4H, 4-isopropoxyphenyl H-2, H-6, 1,4-disubstituted-phenyl H-3, H-5), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.88 (s, 1H, H-3). Anal. Calcd for $C_{25}H_{24}O_5S$: C, 68.79; H, 5.54. Found: C, 68.55; H, 5.45.

5.12. (*E*)-2-[4-(2,4-Difluorophenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid (11b)

Yield, 60%; white powder; mp 226–227 °C; IR (film) 3347–2773 (COOH), 1682 (C=O), 1304 (SO_2), 1221,

1149 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.99 (s, 3H, CH_3SO_2), 6.90–7.00 (m, 2H, 2,4-difluorophenyl H-3, H-5), 7.21 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.22 (d, J = 8.2 Hz, 2H, 1,4-disubstituted-phenyl H-2, H-6), 7.40–7.49 (m, 1H, 2,4-difluorophenyl H-6), 7.44 (d, J = 8.2 Hz, 2H, 1,4-disubstituted-phenyl H-3, H-5), 7.66 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.80 (s, 1H, H-3). Anal. Calcd for $C_{22}H_{16}F_2O_4S$: C, 63.76; H, 3.89. Found: C, 63.61; H, 3.93.

5.13. (*E*)-3-(4-Methanesulfonylphenyl)-2-[4-(methylsulfanylphenyl)phenyl]acrylic acid (11c)

Yield, 44%; yellow powder; mp 244–245 °C; IR (film) 3388–2667 (COOH), 1685 (C=O), 1306 (SO_2), 1221, 1153 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.52 (s, 3H, SCH_3), 3.18 (s, 3H, CH_3SO_2), 7.26 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.36 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.37 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-2, H-6), 7.68 and 7.70 (two d, J = 8.2 Hz, 2H each, 1,4-disubstituted-phenyl H-2, H-6 and H-3, H-5), 7.76 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.85 (s, 1H, H-3), 13.01 (br s, 1H, CO_2H). Anal. Calcd for $C_{23}H_{20}O_4S_2$: C, 65.07; H, 4.75. Found: C, 64.78; H, 4.82.

5.14. (*E*)-2-[3-(4-Isopropoxyphenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid (12a)

Yield, 50%; white crystals; mp 177–179 °C; IR (film) 3503–2703 (COOH), 1686 (C=O), 1306 (SO_2), 1247, 1151 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.27 (d, J = 5.8 Hz, 6H, $CH(CH_3)_2$), 3.17 (s, 3H, CH_3SO_2), 4.64 (septet, J = 5.8 Hz, 1H, $CH(CH_3)_2$), 6.96 (d, J = 8.8 Hz, 2H, 4-isopropoxyphenyl H-3, H-5), 7.10 (d, J = 8.2 Hz, 1H, 1,3-disubstituted-phenyl H-4), 7.36 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.40–7.46 (m, 2H, 1,3-disubstituted-phenyl H-2, H-5), 7.48 (d, J = 8.5 Hz, 2H, 4-isopropoxyphenyl H-2, H-6), 7.62 (d, J = 8.2 Hz, 1H, 1,3-disubstituted-phenyl H-6), 7.75 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.87 (s, 1H, H-3) 13.00 (br s, 1H, CO_2H). Anal. Calcd for $C_{25}H_{24}O_5S$: C, 68.79; H, 5.54. Found: C, 68.78; H, 5.43.

5.15. (*E*)-2-[3-(2,4-Difluorophenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid (12b)

Yield, 21%; white solid; mp 214–216 °C; IR (film) 3438–2733 (COOH), 1684 (C=O), 1307 (SO_2), 1222, 1150 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.18 (s, 3H, CH_3SO_2), 7.13–7.24 (m, 2H, 2,4-difluorophenyl H-3, H-5), 7.30–7.38 (m, 2H, 1,3-disubstituted-phenyl H-4, H-5), 7.35 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.43–7.50 (m, 2H, 1,3-disubstituted-phenyl H-6, 2,4-difluorophenyl H-6), 7.52 (s, 1H, 1,3-disubstituted-phenyl H-2), 7.76 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.88 (s, 1H, H-3), 13.05 (br s, 1H, CO_2H). Anal. Calcd for $C_{22}H_{16}F_2O_4S$: C, 63.76; H, 3.89. Found: C, 63.53; H, 3.97.

5.16. (*E*)-3-(4-Methanesulfonylphenyl)-2-[3-(4-methylsulfanylphenyl)phenyl]acrylic acid (12c)

Yield, 67%; beige powder; mp 183–185 °C; IR (film) 3438–2708 (COOH), 1683 (C=O), 1303 (SO_2), 1218, 1148 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.49 (s, 3H, SCH_3), 3.17 (s, 3H, CH_3SO_2), 7.15 (d, J = 7.6 Hz, 1H, 1,3-disubstituted-phenyl H-4), 7.31 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.36 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.45 (s, 1H, 1,3-disubstituted-phenyl H-2), 7.46 (t, J = 7.6 Hz, 1H, 1,3-disubstituted-phenyl H-5), 7.53 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-2, H-6), 7.66 (d, J = 7.6 Hz, 1H, 1,3-disubstituted-phenyl H-6), 7.75 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.88 (s, 1H, H-3), 13.03 (br s, 1H, CO_2H). Anal. Calcd for $C_{23}H_{20}O_4S_2$: C, 65.07; H, 4.75. Found: C, 64.79; H, 4.73.

5.17. General procedure for the synthesis of (*E*)-acrylic acids (11d and 12d)

A solution of Oxone® (potassium peroxymonosulfate) (1.45 g, 2.36 mmol) in water (8 mL) was added dropwise at 0 °C to a solution of **11c** or **12c** (0.50 g, 1.18 mmol) in THF–MeOH (1:1, v/v, 4 mL). The reaction was allowed to proceed for 15 h at 25 °C with stirring, and then the solvent was removed in vacuo. Water (20 mL) was added to the residue and this mixture was extracted with EtOAc (3× 30 mL), the combined organic layers were washed with water and then dried (Na_2SO_4). Removal of the solvent in vacuo afforded a solid residue which was recrystallized from 95% EtOH. The physical, spectral and microanalytical data for **11d** and **12d** are listed below.

5.18. (*E*)-2-[4-(Methanesulfonylphenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid (11d)

Yield, 38%; white solid; mp 255–256 °C; IR (film) 3453–2688 (COOH), 1683 (C=O), 1304 (SO_2), 1219, 1153 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.18 (s, 3H, CH_3SO_2), 3.27 (s, 3H, biphenyl CH_3SO_2), 7.34 and 7.36 (two d, J = 8.2 Hz, 2H each, 4-methanesulfonylphenyl H-2, H-6), 7.76 and 7.80 (two d, J = 8.2 Hz, 2H each, 1,4-disubstituted-phenyl H-2, H-6 and H-3, H-5), 7.88 (s, 1H, H-3), 8.00–8.04 (m, 4H total, 4-methanesulfonylphenyl H-3, H-5), 13.05 (br s, 1H, CO_2H). Anal. Calcd for $C_{23}H_{20}O_6S_2$: C, 60.51; H, 4.42. Found: C, 60.37; H, 4.45.

5.19. (*E*)-2-[3-(4-Methanesulfonylphenyl)phenyl]-3-(4-methanesulfonylphenyl)acrylic acid (12d)

Yield, 64%; white cotton form; mp 204–205 °C; IR (film) 3433–2683 (COOH), 1683 (C=O), 1304 (SO_2), 1219, 1149 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.17 (s, 3H, CH_3SO_2), 3.24 (s, 3H, biphenyl CH_3SO_2), 7.25 (d, J = 7.6 Hz, 1H, 1,3-disubstituted-phenyl H-4), 7.36 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.53 (t, J = 7.8 Hz, 1H, 1,3-disubstituted-phenyl H-5), 7.58 (s, 1H, 1,3-disubstituted-phenyl H-2), 7.75 (d, J = 8.2 Hz, 2H, biphenyl 4-methanesulfonylphenyl H-2, H-6), 7.77 (d, J = 7.6 Hz, 1H, 1,3-disubstituted-phen-

yl H-6), 7.85 (s, 1H, H-3), 7.89 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.98 (d, $J = 8.5$ Hz, 2H, biphenyl 4-methanesulfonylphenyl H-3, H-5), 13.06 (br s, 1H, CO_2H). Anal. Calcd for $C_{23}H_{20}O_6S_2$: C, 60.51; H, 4.42. Found: C, 60.59; H, 4.69.

6. 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 structure of the enzyme COX-2 and 15-LOX were obtained from the RCSB Protein Data Bank and hydrogens were added. The ligand molecules were constructed using the Builder module and 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. The coordinates for 15-LOX were obtained from PDB file1lox and the energy-minimized ligand was superimposed on the inhibitor RS75091 after which RS75091 was deleted. In all these experiments 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 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 after docking was further minimized for 1000 iterations using the conjugate gradient method until a convergence of 0.001 kcal/mol Å was reached after which $E_{\text{intermolecular}}$ (kcal/mol) of the ligand–enzyme assembly was evaluated.

7. In vitro cyclooxygenase (COX) inhibition assays

The ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and COX-2 (IC_{50} value, μM) was determined using an enzyme immunoassay (EIA) kit (Catalog No. 560101, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method.[12a]

8. In vitro lipoxygenase (LOX) inhibition assays

The ability of the test compounds to inhibit potato 5-LOX (Catalog No. 60401, Cayman Chemical, Ann Arbor, MI, USA) and soybean 15-LOX (Catalog No. 760700, Cayman Chemical, Ann Arbor, MI, USA) (IC_{50} values, μM) was determined using an enzyme immunoassay (EIA) kit according to the manufacturer's instructions. The Cayman Chemical lipoxygenase inhibitor screening assay detects and measures the hydroperoxides produced in the lipoxygenation reaction using a purified lipoxygenase. Stock solutions of test compounds, prepared immediately before use, were dissolved in a minimum volume of DMSO and were diluted using the supplied buffer solution (0.1 M Tris–HCl, pH 7.4). To a 90 μl solution of 5- or 15-LOX enzyme in 0.1 M Tris–HCl, pH 7.4 buffer, 10 μl of various concentrations of test drug solutions (0.001, 0.01, 0.1, 1, and 10 μM in a final volume of 210 μl) was added and the lipoxygenase reaction was initiated by the addition of 10 μl (100 μM) of linoleic acid (LA). After maintaining the 96-well plate on a shaker for 5 min, 100 μl of chromogen was added and the plate was retained on a shaker for 5 min. The lipoxygenase activity was determined after measuring absorbance at a wavelength of 490 nm. Percent inhibition was calculated by the 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).

9. Anti-inflammatory assay

Anti-inflammatory activity was performed using a method described by Winter et al.[21]

10. Analgesic assay

Analgesic activity was determined using a 4% sodium chloride-induced writhing (abdominal constriction) assay previously reported.[22]

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