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Synthesis and biological evaluation of acyclic triaryl (*Z*)-olefins possessing a 3,5-di-*tert*-butyl-4-hydroxyphenyl pharmacophore: Dual inhibitors of cyclooxygenases and lipoxygenases

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Abstract—A new class of regioisomeric acyclic triaryl (*Z*)-olefins possessing a 3,5-di-*tert*-butyl-4-hydroxyphenyl (DTBHP) 5-lipoxygenase (5-LOX) pharmacophore that is vicinal to a *para*-methanesulfonylphenyl cyclooxygenase-2 (COX-2) pharmacophore were designed for evaluation as selective COX-2 and/or 5-LOX inhibitors. The target compounds were synthesized via a highly stereoselective McMurry olefination cross-coupling reaction. This key synthetic step afforded a (*Z*):(*E*) olefinic mixture with a predominance for the (*Z*)-olefin stereoisomer. Structure–activity studies for the (*Z*)-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylalk-1-ene regioisomers showed that COX-1 inhibition decreased, COX-2 inhibition increased, and the COX-2 selectivity index (SI) increased as the chain length of the alkyl substituent attached to the olefinic double bond was increased (Et → *n*-butyl → *n*-heptyl). In this group of compounds, inhibition of both 5-LOX and 15-LOX was dependent upon the length of the alkyl substituent with the hex-1-ene compound **9c** having a *n*-butyl substituent exhibiting potent inhibition of both 5-LOX (IC_{50} = 0.3 μM) and 15-LOX (IC_{50} = 0.8 μM) relative to the inactive (IC_{50} > 10 μM) Et and *n*-heptyl analogs. Compound **9c** is of particular interest since it also exhibits a dual inhibitory activity against the COX (COX-1 IC_{50} = 3.0 μM, and COX-2 IC_{50} = 0.36 μM, COX-2 SI = 8.3) isozymes. A comparison of the relative inhibitory activities of the two groups of regioisomers investigated shows that the regioisomers in which the alkyl substituent is attached to the same olefinic carbon atom (C-2) as the *para*-methanesulfonylphenyl moiety generally exhibit a greater potency with respect to COX-2 inhibition. The 4-hydroxy substituent in the 3,5-di-*tert*-butyl-4-hydroxyphenyl moiety is essential for COX and LOX inhibition since 3,5-di-*tert*-butyl-4-acetoxyphenyl derivatives were inactive inhibitors. These structure–activity data indicate acyclic triaryl (*Z*)-olefins constitute a suitable template for the design of dual COX-2/LOX inhibitors.

1. Introduction

Arachidonic acid (AA) is the most abundant polyunsaturated fatty acid present in cell membranes. Following the phospholipase A_2 (PLA_2) catalyzed release of AA from membrane-bound phospholipids, AA is metabolized by two major enzyme families, the cyclooxygenases (COX-1, -2, and -3) and the lipoxygenases (5-, 8-, 12-, and 15-LOX). In this regard, proinflammatory prostaglandins (PGs), produced via the COX pathway, and leukotrienes (LTs), produced via the LOX pathway, are implicated in physiologic processes such as inflammation, fever, arthritis, bronchospasm,[1] and the etiology of cancer.[2] PGs that induce undesirable inflammation, fever, and pain are produced via the inducible COX-2 isozyme whereas, PGs that regulate desirable gastrointestinal cytoprotective and renal functions are produced via the constitutive COX-1 isozyme.[1,3] The initial postulate that a selective COX-2 inhibitor would reduce inflammation without causing gastric irritation was validated following the introduction of selective COX-2 inhibitors such as celecoxib and rofecoxib. However, it was subsequently observed that selective COX-2 inhibitors may alter the balance in the cyclooxygenase 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 tromboxane A_2 (TxA_2), leading to increased incidences of an adverse cardiovascular thrombotic event.[1]

Two of the three major isoforms (5-, 12-, and 15-LOX) observed in humans induce undesirable physiological effects. For example, 5-LOX is implicated in the

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production of LTs that are associated with inflammatory, bronchoconstrictor, hypersensitivity, anaphylactic, and asthmatic actions.[1,4] 15-LOX is involved in atherosclerosis since it catalyzes the oxidation of lipoproteins (LDL, HDL) to atherogenic forms.[5] It is generally agreed that a dual inhibitor of the COX/LOX enzymatic pathways offers an alternative approach for the design of more efficacious anti-inflammatory agents with an improved safety profile relative to ulcerogenic nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors. Accordingly, 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.[6–15] DTBHP derivatives have been identified that exhibit oral anti-inflammatory activity and are devoid of ulcerogenic potential. Some representative compounds (**1a**–**d**), in which the 3,5-di-*tert*-butyl-4-hydroxyphenyl moiety is linked through a vinyl bridge to a heterocyclic ring, that exhibit a dual activity against COX and 5-LOX[8,13–15] are illustrated in Figure 1. S-2474 (**1c**) was selected as an antiarthritic drug candidate that is now undergoing clinical trials.[13]

Recently, we reported that the triaryl (*Z*)-ethene regioisomers (**2**[16a] and **3**[16b], Fig. 1) having a COX-2 methanesulfonyl ($MeSO_2$) pharmacophore at the *para* position of a phenyl ring in conjunction with a R^1-alkyl substituent of appropriate chain length exhibited selective COX-2 inhibition. It was therefore of interest to design a new class of hybrid compounds in which

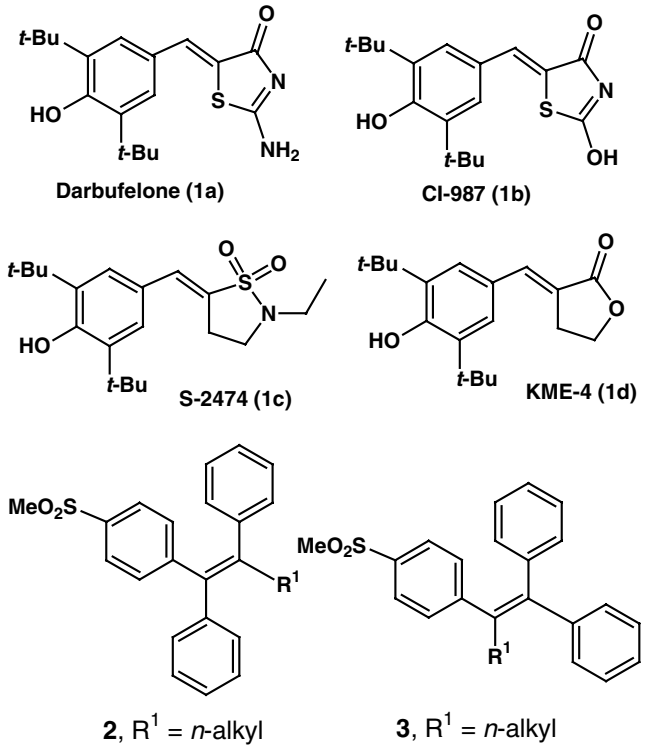


Figure 1. Some representative examples of the 3,5-di-*tert*-butyl-4-hydroxyphenyl (DTBHP) class that are dual inhibitors of cyclooxygenase and 5-lipoxygenase (**1a**–**d**), and acyclic triaryl (*Z*)-olefins that exhibit selective cyclooxygenase-2 inhibitory activity (**2**–**3**).

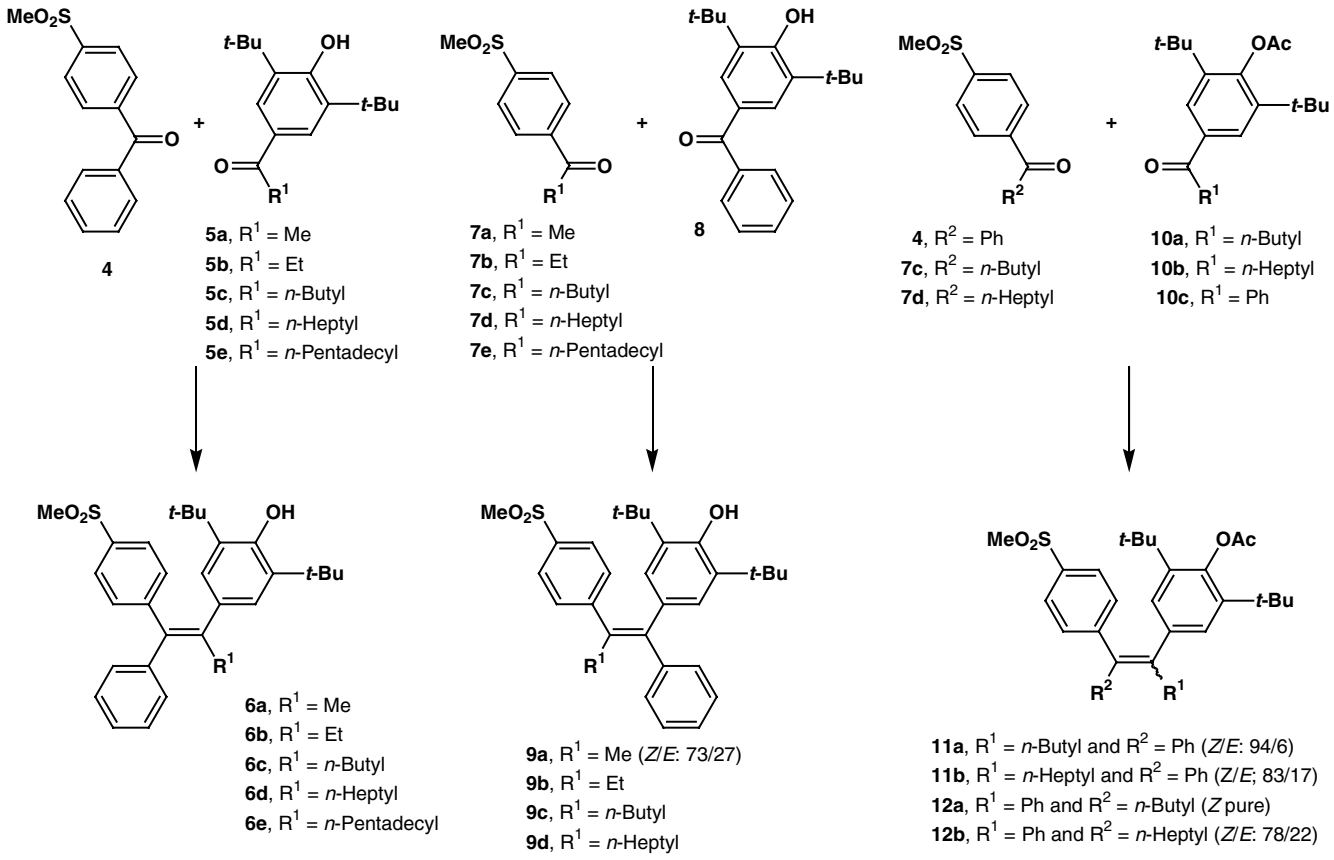
the unsubstituted-phenyl ring present in the acyclic triaryl (*Z*)-ethene regioisomers **2** and **3**, that is *cis* to the *para*-methanesulfonylphenyl substituent (COX-2 pharmacophore), is replaced by a DTBHP moiety (5-LOX pharmacophore). Accordingly, we now describe the synthesis and biological evaluation of a group of (*Z*)-2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylalk-1-enes (**6a**–**e**), (*Z*)-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylalk-1-enes (**9a**–**d**), and some 4-acetoxy-3,5-di-*tert*-butylphenyl derivatives (**11a**–**b** and **12a**–**b**).

2. Chemistry

The (*Z*)-2-(4-hydroxy-3,5-di-*tert*-butylphenyl)-1-(4-methanesulfonylphenyl)-1-phenylalk-1-enes (**6a**–**e**) (R^1 = Me, Et, *n*-butyl, *n*-heptyl, *n*-pentadecyl) were synthesized using a McMurry olefination reaction by Zn–$TiCl_4$ catalyzed reductive cross-coupling of 4-methanesulfonylbenzophenone (**4**) and a 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)alkan-1-one (**5a**–**e**) as illustrated in Scheme 1. This key synthetic step afforded a (*Z*):(*E*) olefinic mixture with a predominant selectivity for the (*Z*)-olefin stereoisomer. Subsequent consecutive recrystallizations provided the respective target (*Z*)-olefins **6a**–**e** in 30–37% isolated yield. The (*Z*)-1-(4-hydroxy-3,5-di-*tert*-butylphenyl)-2-(4-methanesulfonylphenyl)-1-phenylalk-1-ene regioisomers (**9a**–**d**) (R^1 = Me, Et, *n*-butyl, *n*-heptyl) were prepared in a similar way by reaction of a 4-(methanesulfonyl)alkanophenone (**7a**–**d**) with 3,5-di-*tert*-butyl-4-hydroxybenzophenone (**8**) in 22–41% yield. Although the product **9a** (R^1 = Me) was obtained as an inseparable (*Z*):(*E*) 73:27 olefinic mixture, the related compounds **9b**–**d** (R^1 = Et, *n*-Bu, *n*-heptyl) were obtained as a single (*Z*)-olefinic stereoisomer after several fractional recrystallizations. In contrast, the cross-coupling reaction between the ketone **8** and the 4-(methanesulfonyl)hexadecanophenone (**7e**) did not proceed and the two starting materials were recovered. The explanation for the (*Z*)-stereoselectivity observed in this McMurry olefination reaction by coupling two aryl ketones having respective methanesulfonyl and hydroxyl substituents was described in a previous study where it was shown that a sulfonyl moiety in one carbonyl compound and a hydroxyl moiety in the other carbonyl compound are a requirement for high (*Z*)-stereoselectivity.[17]

4-Methanesulfonylbenzophenone (**4**) and the 4-(methanesulfonyl)alkanophenones (**7a**–**e**) were prepared by Friedel–Crafts acylation of thioanisole followed by a subsequent oxidation of the respective intermediate methylthio compounds using Oxone® (potassium peroxymonosulfate) in 50–60% overall yield. 3,5-Di-*tert*-butyl-4-hydroxybenzophenone (**8**) and the 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)alkan-1-ones (**5a**–**e**) were also synthesized by Friedel–Crafts acylation of 2,6-di-*tert*-butylphenol using benzoyl chloride, or an appropriate alkanoyl chloride, in 45–64% yield.

The acetates **11a**–**b** and **12a**–**b** were synthesized in 45–57% yield via a McMurry coupling reaction



Scheme 1. Reagents and conditions: Zn, $TiCl_4$, THF, reflux, 4.5 h.

(Scheme 1) between 4-methanesulfonylbenzophenone (**4**) or a 4-(methanesulfonyl)alkanophenone (**7c**, **d**) and the respective acetoxy compounds (**10a**, **10b** or **10c**) that were previously obtained by acetylation of the respective parent-phenol compounds **5c**, **5d** or **8**. Initial attempts to synthesize the acetates **11a**–**b** and **12a**–**b** by acetylation of the respective (*Z*)-olefinic compounds **6c**, **6d**, **9c** or **9d** using a variety of acylation conditions such as (i) TEA (1.5 equiv), AcCl (1.5 equiv) in diethyl ether, (ii) TEA (3 equiv), AcCl (3 equiv) in freshly distillated THF, or (iii) *n*-butyllithium (1.2 equiv), AcCl (1.5 equiv) in freshly distillated THF, as well as varying the reaction temperature and time, afforded only traces of the target acetylated products. To circumvent this low reactivity, it was decided to acetylate the phenolic substituent prior to performing the McMurry olefination reaction. Accordingly, the acetoxy compounds **10a**–**c** were prepared by reaction of the respective 1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)alkan-1-one (**5c** or **5d**), or 3,5-di-*tert*-butyl-4-hydroxybenzophenone (**8**), with acetyl chloride (3 equiv) in the presence of TEA (3 equiv) in 66–78% yield. However, the subsequent McMurry olefination reaction using the acetoxy compounds (**10a**–**c**) and a methanesulfonyl ketone (**4**, **7c** or **7d**) was less (*Z*)-stereoselective. In this regard, the product was isolated as a mixture of (*Z*)- and (*E*)-olefins for **11a** (*Z*:*E* ratio = 94:6), **11b** (*Z*:*E* ratio = 83:17), and **12b** (*Z*:*E* ratio = 78:22; oil). On the other hand, **12a** was obtained as a single (*Z*)-stereoisomer after several recrystallizations.

The structure of the methanesulfonyl compounds **7a**–**e**, the acetates **10a**–**c**, and the target olefins **6a**–**e**, **9a**–**d**, **11a**–**b**, and **12a**–**b** was consistent with their spectral and/or microanalytical data. The stereochemistry of the target olefins possessing a phenolic moiety (**6a**–**e**, **9a**–**d**) was not determined by ^{1}H NMR difference nuclear Overhauser effect (NOE) studies since these compounds undergo slow isomerization in solution ($CDCl_3$ or DMSO-d_6) where new resonances appear that are attributed to formation of the more stable (*E*)-stereoisomer. This observation in conjunction with X-ray crystal structure data previously used to establish the high (*Z*)-stereoselectivity observed in the McMurry olefination reaction[17] provides credence for the (Z)-stereoselectivity observed in this study.

3. Results and discussion

In vitro structure–activity relationships acquired for these (*Z*)-2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1-4-methanesulfonylphenyl)-1-phenylalk-1-ene (**6a**–**e**) and (*Z*)-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylalk-1-ene (**9b**–**d**) regioisomers showed that they exhibit a broad range (potent-to-inactive) of COX/LOX inhibitory activities (COX-1 IC_{50} = 1 to >100 μM range; COX-2 IC_{50} = 0.1 to >100 μM range; 5-LOX IC_{50} = 0.3 to >10 μM range; 15-LOX IC_{50} = 0.6 to >10 μM range; see data in Table 1). Alk-1-enes **6a**–**d** having a shorter R^1 alkyl

Table 1. In vitro COX-1/COX-2 and 5-LOX/15-LOX enzyme inhibition assay data for (*Z*)-2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylalk-1-enes (**6a–e**), (*Z*)-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylalk-1-enes (**9b–d**), and some 4-acetoxy-3,5-di-*tert*-butylphenyl derivatives (**11a** and **12a**)

Compound	R^1	R^2	IC_{50}[a] (μM)		Selectivity index (SI)[b]	IC_{50}[a] (μM)	
			COX-1	COX-2		15-LOX	5-LOX
6a	Me	—	>100	3.6	>27.7	>10	0.5
6b	Et	—	1.0	32.0	—	0.6	>10
6c	*n*-Butyl	—	>100	3.3	>30.3	3.8	>10
6d	*n*-Heptyl	—	4.1	1.0	4.1	>10	>10
6e	*n*-Pentadecyl	—	>100	>100	—	>10	>10
9b	Et	—	1.0	1.77	—	>10	>10
9c	*n*-Butyl	—	3.0	0.36	8.3	0.8	0.3
9d	*n*-Heptyl	—	31.0	0.1	310	>10	>10
11a[c]	*n*-Butyl	—	>100	>100	—	>10	>10
12a[d]	—	*n*-Butyl	>100	>100	—	>10	>10
Luteolin						3.2	—
Caffeic acid						—	3.0
NDGA						3.5	>10
Celecoxib			33.1	0.07	472	—	—
Rofecoxib			>100	0.5	>200	—	—

[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] Mixture of isomers, (*Z*):(*E*) ratio = 94:6.
[d] Pure (*Z*)-isomer.

substituent (Me, Et, *n*-butyl, *n*-heptyl) exhibited the highest COX-2 inhibitory potency (IC_{50} = 1.0–32 μM range) relative to the inactive R^1 pentadecyl [–$(CH_2)_{14}CH_3$] analog **6e** (IC_{50} > 100 μM). The requirement for a shorter R^1 alkyl chain was more stringent for inhibition of 5-LOX and 15-LOX since active inhibitors (**6a–c**) possess a R^1-alkyl chain length of 1–4 carbon atoms. Among the group of compounds **6a–e**, the prop-1-ene (**6a**) having a R^1 Me substituent exhibited modest COX-2 inhibition (IC_{50} = 3.6 μM) and selectivity [COX-2 selectivity index (SI) > 27] in conjunction with a potent inhibition of 5-LOX (IC_{50} = 0.5 μM). By comparison, the related hex-1-ene (**6c**) having a R^1 *n*-butyl substituent showed a similar COX-2 potency (IC_{50} = 3.3 μM) and selectivity (SI > 30), but with a simultaneous potent inhibition of 15-LOX (IC_{50} = 3.8 μM). These LOX inhibitory data for **6a–e** show that the small R^1 Me substituent provides 5-LOX selectivity whereas, compounds having a slightly longer R^1 Et or *n*-Bu substituent show 15-LOX selectivity. In contrast, compound **6e** having a long R^1 pentadecyl chain is an inactive inhibitor of both COX and LOX isozymes.

Similar structure–activity in vitro enzyme inhibition data for the alk-1-ene regioisomers (**9b–d**) showed that COX-1 inhibition decreased, COX-2 inhibition increased, and the COX-2 SI increased as the chain length of the R^1 alkyl substituent was increased (Et → *n*-butyl → *n*-heptyl). In the group of compounds **9b–d**, inhibition of both 5-LOX and 15-LOX was highly dependent upon the length of the R^1 substituent since **9c** (R^1 = *n*-Bu) was a potent inhibitor of both 5-LOX (IC_{50} = 0.3 μM) and 15-LOX (IC_{50} = 0.8 μM) relative to the inactive (IC_{50} > 10 μM) R^1 Et (**9b**) and *n*-heptyl (**9d**) analogs. The hex-1-ene (**9c**, R^1 = *n*-butyl) is of particular interest since it exhibits a dual inhibitory activity against the COX (COX-1 IC_{50} = 3.0 μM, and COX-2 IC_{50} = 0.36 μM, COX-2 SI = 8.3), in conjunction with a well-balanced inhibition of the 15-LOX (IC_{50} = 0.8 μM) and 5-LOX (IC_{50} = 0.3 μM), isozymes. A comparison of the relative inhibitory activities of the two groups of regioisomers **6** and **9** shows that regioisomers **9** in which the R^1 alkyl substituent is attached to the same olefinic carbon atom (C-2) as the *para*-methanesulfonylphenyl moiety generally exhibit a greater potency with respect to COX-2 inhibition (R^1 = Et, **9b > 6b**; R^1 = *n*-Bu, **9c > 6c**; R^1 = *n*-heptyl, **9d > 6d**), 15-LOX inhibition (R^1 = *n*-butyl, **9c > 6c** but R^1 = Et is an exception where **6b ≫ 9b**), and 5-LOX inhibition (R^1 = *n*-butyl, **9c ≫ 6c**). The 4-hydroxy substituent in the 3,5-di-*tert*-butyl-4-hydroxyphenyl moiety (such as in **6c** and **9c**) is essential for COX and LOX inhibition since the 3,5-di-*tert*-butyl-4-acetoxyphenyl derivatives **11a** and **12a** were inactive inhibitors.

A molecular modeling study of the most potent and selective COX-2 inhibitor, (*Z*)-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylnon-1-ene (**9d**) (COX-2 IC_{50} = 0.10 μM; SI = 310) docked in the COX-2 active site (Fig. 2), shows that it binds in the primary binding-site such that the *p*-SO_2Me COX-2 pharmacophore on the C-2 phenyl ring inserts into the 2°-pocket present in COX-2 (distance = 1.50 Å) where it undergoes interactions with Val523, Phe518, Ile517, Thr94, and His90. One of the O-atoms of SO_2Me moiety forms a H-bond with the O*H* of Thr94 (distance = 1.69 Å), whereas the distance between O-atom and the NH_2 (guanidine) of Arg513 was about 7.59 Å. The other O-atom (of SO_2Me) forms a weak hydrogen bond with the amide bond (N*H*) of Ile517 (distance = 3.62 Å). The Me of the SO_2Me group is within van der Waals contact range of Phe518 (distance < 5 Å), and the C-2 phenyl ring (of the *p*-$MeSO_2$-phenyl) is involved in a π–π stacking interaction with the phenyl ring

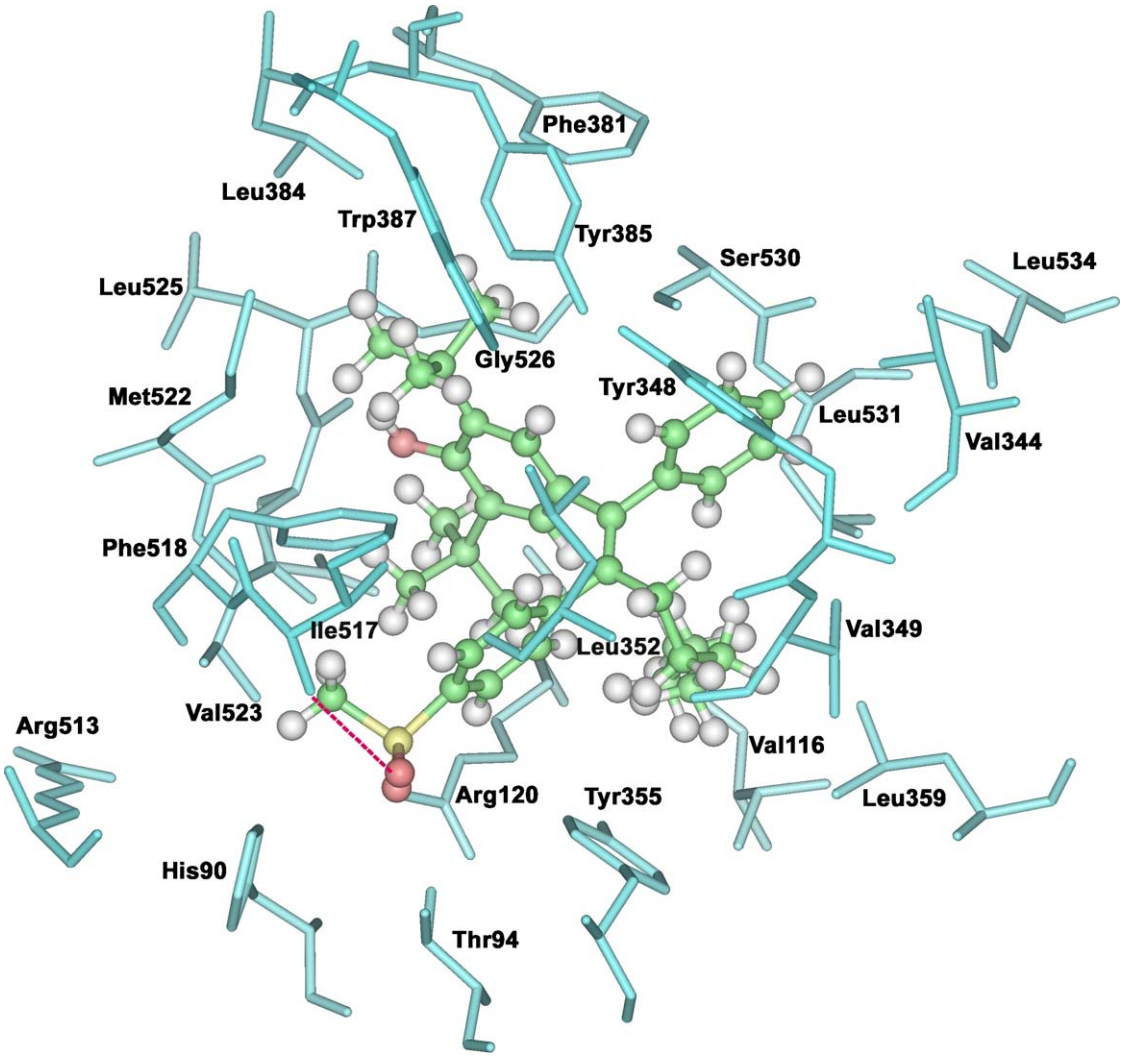


Figure 2. Docking (*Z*)-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylnon-1-ene (**9d**) (ball and stick) in the active site of murine COX-2. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

of Phe518. The C-1 di-*tert*-butylphenol moiety is oriented toward a hydrophobic pocket at the apex of the COX-2 binding site where it is surrounded by Leu525, Met522, Trp387, Leu384, and Phe381. The O*H* of the di-*tert*-butylphenol group is positioned in the vicinity of the backbone C=*O* of Glu524‘ (distance = 5.20 Å), whereas the C-1 unsubstituted phenyl ring is oriented toward a region comprised of Leu534, Leu531, Ser530, Tyr385, Tyr348, and Val344. The distance between the center of the C-1 phenyl ring and the *O*H of Ser530 is about 4.97 Å. The C-2 *n*-heptyl substituent is oriented closer to the mouth of the COX-2 binding site which allows it to interact with amino acid residues such as Leu359, Tyr355, Val349, Ser121, and Val116. The terminal Me of the *n*-heptyl substituent is within van der Waals contact range of the Me side chain of Leu359 (distance < 5 Å). This computational study shows that the stereochemical disposition of alkyl, *p*-$MeSO_2$-phenyl, or di-*tert*-butylphenol substituents about the C=C bond controls the optimal protein–ligand binding interactions in the active site of COX-2.

A molecular modeling (docking) simulation was performed to investigate the binding interaction of the most potent 15-LOX inhibitor, (*Z*)-2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylbut-1-ene (**6b**), (15-LOX IC_{50} = 0.6 μM) within the 15-LOX binding site (Fig. 3, some amino acid residues such as His356, Gln548, and Phe415 listed in the text below are not shown in Fig. 3 to improve clarity). The C-1 *p*-$MeSO_2$-phenyl COX-2 pharmacophore is oriented toward the catalytic center of 15-LOX where it is able to interact with amino acid residues such as Phe552, His545, Ile544, Leu362, His361, Glu357, and His356. The Me of the SO_2Me group is located within van der Waals contact range of Phe552, Gln548, and Glu357 (distance < 5 Å). The phenyl ring (of *p*-$MeSO_2$-phenyl) is involved in a π–π stacking interaction with the imidazole ring of His361. It is interesting to note that the *O*H of the C-2 di-*tert*-butylphenol moiety participates in a hydrogen bonding interaction with the O*H* of Glu357 (distance = 1.74 Å). The di-*tert*-butylphenol moiety is oriented toward the base of the 15-LOX binding site where it is positioned in a hydrophobic pocket

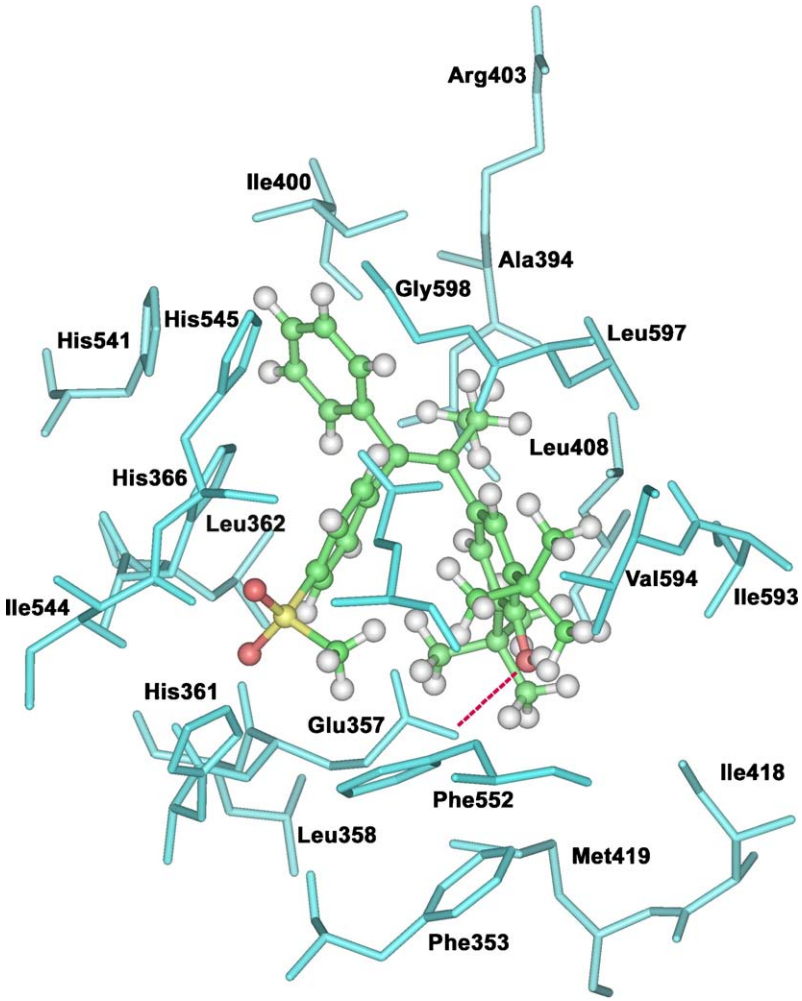


Figure 3. Docking of (*Z*)-2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylbut-1-ene (**6b**) (ball and stick) in the active site of soyabean 15-LOX. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

comprised of Leu597, Val594, Ile593, Phe552, Met419, Ile418, Phe415, Leu358, and Phe353. The C-1 unsubstituted phenyl ring is oriented in the vicinity of amino acid residues such as His545, Gly598, Ile400, His366, and His361, and it undergoes a π–π stacking interaction with the imidazole ring of His366. The C-2 ethyl substituent that is *cis* to the unsubstituted phenyl ring is oriented toward a hydrophobic pocket closer to the entrance of the 15-LOX binding site (Arg403) where it is within van der Waals contact range of Leu597, Leu408, and Ala394.

The (*Z*)-olefins **6a**, **6b**, **9c**, **9d** and the acetate **12a** (acetoxy derivative of **9c**), based on in vitro enzyme 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, qualitative assays using a 30 mg/kg oral dose showed that compounds **6a**, **6b**, **9c**, and **9d** inhibited inflammation at 3 h post-drug administration in the 13–33% range relative to the 5-LOX inhibitor caffeic acid (8.2% inhibition) and the 15-LOX inhibitor nordihydroguaiaretic acid (NDGA, 15.1% inhibition) reference drugs. A comparison of the anti-inflammatory activity of the 3,5-di-*tert*-butyl-4-acetoxyphenyl compound (**12a**, 8.1% inhibition) with that of the parent 3,5-di-*tert*-butyl-4-hydroxyphenyl compound (**9c**, 17.9% inhibition) indicates that in vivo cleavage of the acetoxy substituent to a hydroxyl group following oral administration must not be rapid or complete. Further studies showed that the most active compounds **6b** (ED_{50} = 131.9 mg/kg) and **9d** (ED_{50} = 70.2 mg/kg) were more potent anti-inflammatory agents than the 15-LOX inhibitor NDGA (ED_{50} = 205 mg/kg), but less potent than the selective COX-2 inhibitor celecoxib (ED_{50} = 10.8 mg/kg).

In a rat 4% NaCl-induced abdominal constriction (analgesic) assay, a 30 mg oral dose of the (*Z*)-olefins **6a**, **6b**, **9c**, and **9d** exhibited good analgesic activities (40–70% range) compared to the reference drugs caffeic acid, NDGA, and celecoxib at 30 and 60 min post-drug administration (see data in Table 2).

4. Conclusions

A new class of acyclic triaryl (*Z*)-olefin regioisomers were designed that possess *para*-methanesulfonylphenyl (COX-2) and 3,5-di-*tert*-butyl-4-hydroxyphenyl (5-

Table 2. In vivo anti-inflammatory and analgesic activities for (*Z*)-2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylalk-1-enes (**6a–b**), (*Z*)-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylalk-1-enes (**9c–d**), and the 4-acetoxy-3,5-di-*tert*-butylphenyl derivative (**12a**)

Compound	R^1	R^2	AI activity[a]		Analgesic activity[b]	
			% inhibition (30 mg/kg)	ED_{50} (mg/kg)	% inhibition (30 min)	% inhibition (60 min)
6a	Me	—	13.0 ± 4.6	–	40.0 ± 18.9	43.3 ± 14.6
6b	Et	—	33.3 ± 4.0	131.9	70.7 ± 6.5	68.3 ± 12.2
9c	*n*-Butyl	—	17.9 ± 1.4	—	64.7 ± 3.6	61.9 ± 6.8
9d	*n*-Heptyl	—	25.0 ± 3.0	70.2	41.3 ± 8.3	63.3 ± 1.6
12a	—	*n*-Butyl	8.1 ± 3.1	—	27.3 ± 4.8	39.4 ± 13.8
Caffeic acid			8.2 ± 2.5	—	47.2 ± 10.6	58.3 ± 12.8
NDGA			15.1 ± 1.7	205.0	45.8 ± 9.2	62.5 ± 4.8
Celecoxib	—	—	79.9 ± 1.9[c]	10.8	69.3 ± 12.1[c]	79.5 ± 2.0[c]

[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 ($n = 3$–4) following a 30 mg/kg oral dose of the test compound.

[c] 5 mg/kg oral dose.

LOX) pharmacophores for evaluation as dual acting COX/LOX inhibitors. Structure–activity data acquired identified (*Z*)-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylnon-1-ene (**9d**) as a highly potent ($IC_{50} = 0.1$ μM) and a particularly selective (SI = 310) COX-2 inhibitor that showed good anti-inflammatory activity ($ED_{50} = 70.2$ mg/kg) even though it did not inhibit either 5-LOX or 15-LOX. In contrast, (*Z*)-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylhex-1-ene (**9c**) was found to be a potent COX-2 inhibitor ($IC_{50} = 0.36$ μM) with a modest COX-2 selectivity (SI = 8.3) that also effectively inhibited both 15-LOX ($IC_{50} = 0.8$ μM) and 5-LOX ($IC_{50} = 0.3$ μM). The structure–activity data acquired in this investigation show that optimum dual COX/LOX inhibitory activity is observed when the acyclic triaryl (*Z*)-olefin possesses a hex-1-ene (R^1 *n*-butyl substituent) moiety in combination with a *para*-methanesulfonylphenyl COX-2 pharmacophore and a 3,5-di-*tert*-butyl-4-hydroxyphenyl LOX pharmacophoric group.

5. Experimental section

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 $CDCl_3$ with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hz. Spin multiples are given as s (singlet), d (double), t (triplet), q (quartet), m (multiplet), and br (broad). Microanalyses were performed for C, 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 Inc., Ann Arbor, MI, USA. All other reagents were purchased from the Aldrich Chemical Company (Milwaukee, WI) and used without further purification. Male Sprague–Dawley rats, used in the anti-inflammatory–analgesic screens, were purchased from Animal Health Services at the University of Alberta, and experiments were carried out using protocols approved by the Animal Welfare Committee, University of Alberta.

5.1. General procedure for the synthesis of 4-(methanesulfonyl)benzophenone (4) and 4-(methanesulfonyl)alkanophenones (7a–e)

A solution of Oxone® (potassium peroxymonosulfate) (4.06 g, 6.6 mmol) in water (20 mL) was added dropwise at 0 °C to a solution of 4-(methylthio)benzophenone or the corresponding 4-(methylthio)alkanophenone (3.3 mmol) in THF/MeOH (1:1, v/v, 10 mL). The reaction was allowed to proceed for 15 h at 25 °C with stirring and the solvent was removed in vacuo. Water (20 mL) was added to the residue, this mixture was extracted with EtOAc (3 × 30 mL), the combined organic extracts were washed with water, and the organic layer was dried (Na_2SO_4). Removal of the solvent in vacuo afforded a solid residue which was recrystallized from petroleum ether/dichloromethane. Some physical and spectral data for compounds **4** and **7a–e** are listed below.

5.2. 4-(Methanesulfonyl)benzophenone (4)

Yield, 82%; white crystals; mp 140–141 °C (lit.[18] 141 °C); IR (film) 1661 (C=O), 1599 (C=C), 1319 (SO_2), 1156 cm^{-1}, ^{1}H NMR ($CDCl_3$) δ 3.13 (s, 3H, SO_2CH_3), 7.53 (t, $J = 7.6$ Hz, 1H, phenyl H-4), 7.66 (t, $J = 7.6$ Hz, 2H, phenyl H-3, H-5), 7.81 (d, $J = 7.6$ Hz, 2H, phenyl H-2, H-6), 7.96 (d, $J = 8.2$ Hz, 2H, methanesulfonylphenyl H-3, H-5), 8.09 (d, $J = 8.2$ Hz, 2H, methanesulfonylphenyl H-2, H-6).

5.3. 1-(4-Methanesulfonylphenyl)ethan-1-one (7a)

Yield, 79%; white crystals; the spectral data were identical to those previously reported.[19]

5.4. 1-(4-Methanesulfonylphenyl)propan-1-one (7b)

Yield, 84%; white crystals; mp 109–110 °C (lit.[20] 108 °C); IR (film) 1686 (C=O), 1317 (SO_2),

1151 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 3.05 (t, $J = 7.3$ Hz, 2H, $COCH_2$), 3.08 (s, 3H, SO_2CH_3), 8.04 (d, $J = 8.5$ Hz, 2H, phenyl H-3, H-5), 8.13 (d, $J = 8.5$ Hz, 2H, phenyl H-2, H-6).

5.5. 1-(4-Methanesulfonylphenyl)pentan-1-one (7c)

Yield, 82%; white foam; mp 84–85 °C; IR (film) 1686 (C=O), 1315 (SO_2), 1156 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 1.43 (sextet, $J = 7.3$ Hz, 2H, $CH_2CH_2CH_3$), 1.74 (quintet, $J = 7.3$ Hz, 2H, $CH_2CH_2CH_2$), 3.02 (t, $J = 7.3$ Hz, 2H, $COCH_2$), 3.09 (s, 3H, SO_2CH_3), 8.05 (d, $J = 8.2$ Hz, 2H, phenyl H-3, H-5), 8.14 (d, $J = 8.2$ Hz, 2H, phenyl H-2, H-6).

5.6. 1-(4-Methanesulfonylphenyl)octan-1-one (7d)

Yield, 81%; white crystals; mp 104–106 °C; IR (film) 1689 (C=O), 1293 (SO_2), 1149 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 1.29–1.38 [m, 8H, $(CH_2)_4CH_3$], 3.00 (t, $J = 7.3$ Hz, 2H, $COCH_2$), 3.09 (s, 3H, SO_2CH_3), 8.05 (d, $J = 8.5$ Hz, 2H, phenyl H-3, H-5), 8.13 (d, $J = 8.5$ Hz, 2H, phenyl H-2, H-6).

5.7. 1-(4-Methanesulfonylphenyl)hexadecane-1-one (7e)

Yield, 76%; bright white crystals; mp 85–87 °C; IR (film) 1681 (C=O), 1473, 1397 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (t, $J = 6.6$ Hz, 3H, CH_2CH_3), 1.29-1.38 [m, 26H, $(CH_2)_{13}CH_3$], 2.53 (s, 3H, SO_2CH_3), 2.92 (t, $J = 7.5$ Hz, 2H, $COCH_2$), 7.27 (d, $J = 8.5$ Hz, 2H, phenyl H-3, H-5), 7.88 (d, $J = 8.5$ Hz, 2H, phenyl H-2, H-6).

5.8. General procedure for the synthesis of acetates (10a–c)

Acetyl chloride (1.47 mL, 20.7 mmol) was added dropwise at 0 °C with stirring to a solution of the appropriate phenol **5c**, **5d** or **8** (6.9 mmol) and TEA (2.89 mL, 20.7 mmol) in freshly distilled THF (40 mL). The reaction was allowed to proceed for 24 h at 25 °C with stirring, the mixture was diluted with water (30 mL), extracted with EtOAc (3 × 30 mL), and the combined organic layers were dried (Na_2SO_4). Removal of the solvent in vacuo furnished a residue which was purified by silica gel column chromatography using petroleum ether/ethyl acetate (9:1, v/v) as eluant.

5.9. 1-(3,5-Di-*tert*-butyl-4-acetoxyphenyl)pentan-1-one (10a)

Yield, 69%; viscous yellow oil; IR (film) 1766 (C=O of OAc), 1684 (C=O), 1367, 1186, 1109 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 1.33–1.47 [m, 20H, CH_2CH_3 + two $(CH_3)_3C$], 1.73 (quintet, $J = 7.3$ Hz, 2H, $CH_2CH_2CH_2$), 2.38 (s, 3H, $OCOCH_3$), 2.95 (t, $J = 7.3$ Hz, 2H, $COCH_2$), 7.96 (s, 2H, phenyl H-2, H-6).

5.10. 1-(3,5-Di-*tert*-butyl-4-acetoxyphenyl)octan-1-one (10b)

Yield, 78%; viscous pale yellow oil (lit.[21] bp 234–236 °C/10 Torr); IR (film) 1762 (C=O of OAc), 1686 (C=O), 1367, 1200, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, $J = 7.3$ Hz, 3H, CH_2CH_3), 1.30–1.82 [m, 28H, $(CH_2)_5CH_3$ + two $(CH_3)_3C$], 2.38 (s, 3H, $OCOCH_3$), 2.94 (t, $J = 7.3$ Hz, 2H, $COCH_2$), 7.96 (s, 2H, phenyl H-2, H-6).

5.11. 3,5-Di-*tert*-butyl-4-acetoxybenzophenone (10c)

Yield, 78%; viscous pale yellow oil; IR (film) 1765 (C=O of OAc), 1659 (C=O), 1367, 1186, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37 [s, 18H, two $(CH_3)_3C$], 2.40 (s, 3H, $OCOCH_3$), 2.97 (s, 3H, $OCOCH_3$), 7.47–7.52 (m, 2H, phenyl H-3, H-5), 7.57–7.60 (m, 1H, phenyl H-4), 7.81–7.84 (m, 4H, acetoxyphenyl H-2, H-6, phenyl H-2, H-6).

5.12. General procedure for the synthesis of (*Z*)-2-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylalk-1-enes (6a–e), (*Z*)-1-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylalk-1-enes (9a–d), and the 4-acetoxy-3,5-di-*tert*-butylphenyl derivatives (11a–b, 12a–b)

Titanium tetrachloride (1.30 mL, 11.7 mmol) was added dropwise to a stirred suspension of Zn powder (1.57 g, 24.0 mmol) in dry THF (30 mL), under Ar, at −10 °C, and the reaction was allowed to proceed at reflux for 2 h. A solution of the first ketone **4**, **7a**–**d**, **4** or **7c**–**d** (3.0 mmol), respectively, and the second ketone **5a**–**e**, **8**, **10a**–**b** or **10c** (3.0 mmol), respectively, in THF (65 mL) was added dropwise to the cooled suspension of the titanium reagent at 0 °C, and the reaction was allowed to proceed at reflux for 2.5 h. The reaction mixture was cooled to 25 °C, poured into 10% aqueous K_2CO_3 solution (100 mL), and after vigorous stirring for 5 min, the dispersed insoluble material was removed by vacuum filtration using a Celite 545® pad. The organic layer was separated, the aqueous layer was extracted with EtOAc (3 × 50 mL), the combined organic fractions were washed with water (100 mL), and the organic fraction was dried (Na_2SO_4). Evaporation of solvent in vacuo left a residue which was purified by silica gel column chromatography using petroleum ether/ethyl acetate (3:1, v/v) as eluant to afford a white solid which was recrystallized several times from 95% EtOH.

5.13. (*Z*)-2-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylprop-1-ene (6a)

Yield, 30%; white solid; mp 185–186 °C; IR (film) 3628 (OH), 1590 (C=C), 1436, 1315 (SO_2), 1231, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 [s, 18H, two $(CH_3)_3C$], 2.19 (s, 3H, CH_3C=C), 2.97 (s, 3H, CH_3SO_2), 5.15 (s, 1H, O*H*), 6.90 (s, 2H, phenolic H-2, H-6), 7.05 (d, $J = 8.5$ Hz, 2H, phenyl H-2, H-6), 7.22–7.41 (m, 5H, phenyl H-3, H-4, H-5, 4-methanesulfonylphenyl H-2, H-6), 7.61 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonyl-

phenyl H-3, H-5). Anal. Calcd for $C_{30}H_{36}O_3S$: C, 75.59; H, 7.61. Found: C, 75.41; H, 8.00.

5.14. (*Z*)-2-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylbut-1-ene (6b)

Yield, 35%; white foam; mp 210–211 °C; IR (film) 3629 (OH), 1590 (C=C), 1435, 1314 (SO_2), 1228, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.02 (t, J = 7.3 Hz, 3H, CH_3CH_2), 1.28 [s, 18H, two $(CH_3)_3C$], 2.52 (q, J = 7.3 Hz, 2H, CH_3CH_2), 2.95 (s, 3H, CH_3SO_2), 5.12 (s, 1H, O*H*), 6.84 (s, 2H, phenolic H-2, H-6), 6.95 (d, J = 8.5 Hz, 2H, phenyl H-2, H-6), 7.22–7.41 (m, 5H, phenyl H-3, H-4, H-5, 4-methanesulfonylphenyl H-2, H-6), 7.58 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{31}H_{38}O_3S$: C, 75.88; H, 7.81. Found: C, 75.49; H, 8.03.

5.15. (*Z*)-2-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylhex-1-ene (6c)

Yield, 32%; white foam; mp 192–194 °C; IR (film) 3629 (OH), 1591 (C=C), 1435, 1315 (SO_2), 1234, 1151 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (t, J = 7.0 Hz, 3H, CH_3CH_2), 1.24–1.40 [m, 22H, two $(CH_3)_3C$ + $CH_3(CH_2)_2$], 2.47 (t, J = 7.0 Hz, 2H, $C{=}CCH_2CH_2$), 2.95 (s, 3H, CH_3SO_2), 5.12 (s, 1H, O*H*), 6.84 (s, 2H, phenolic H-2, H-6), 7.02 (d, J = 8.2 Hz, 2H, phenyl H-2, H-6), 7.21–7.40 (m, 5H, phenyl H-3, H-4, H-5, 4-methanesulfonylphenyl H-2, H-6), 7.58 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{33}H_{42}O_3S$: C, 76.41; H, 8.16. Found: C, 76.26; H, 8.34.

5.16. (*Z*)-2-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylnon-1-ene (6d)

Yield, 34%; white foam; mp 163–165 °C; IR (film) 3635 (OH), 1590 (C=C), 1434, 1315 (SO_2), 1231, 1150 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (t, J = 7.3 Hz, 3H, CH_3CH_2), 1.19–1.39 [m, 28H, two $(CH_3)_3C$ + $CH_3(CH_2)_5$], 2.46 (t, J = 7.3 Hz, 2H, $C{=}CCH_2CH_2$), 2.95 (s, 3H, CH_3SO_2), 5.11 (s, 1H, O*H*), 6.83 (s, 2H, phenolic H-2, H-6), 7.02 (d, J = 8.2 Hz, 2H, phenyl H-2, H-6), 7.21–7.40 (m, 5H, phenyl H-3, H-4, H-5, 4-methanesulfonylphenyl H-2, H-6), 7.58 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{36}H_{48}O_3S$: C, 77.10; H, 8.63. Found: C, 76.78; H, 9.01.

5.17. (*Z*)-2-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylheptadec-1-ene (6e)

Yield, 37%; white foam; mp 149–150 °C; IR (film) 3629 (OH), 1591 (C=C), 1434, 1312 (SO_2), 1233, 1151 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (t, J = 7.0 Hz, 3H, CH_3CH_2), 1.16–1.43 [m, 44H, two $(CH_3)_3C$ + $CH_3(CH_2)_{13}$], 2.46 (t, J = 7.0 Hz, 2H, $C{=}CCH_2CH_2$), 2.95 (s, 3H, CH_3SO_2), 5.10 (s, 1H, O*H*), 6.83 (s, 2H, phenolic H-2, H-6), 7.02 (d, J = 8.2 Hz, 2H, phenyl H-2, H-6), 7.21–7.40 (m, 5H, phenyl H-3, H-4, H-5, 4-methanesulfonylphenyl H-2, H-6), 7.57 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{44}H_{64}O_3S$: C, 78.52; H, 9.58. Found: C, 78.31; H, 9.92.

5.18. (*Z*, *E*)-1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylprop-1-ene (9a)

Yield, 38%; (*Z*):(*E*) ratio = 73:27 (after several recrystallizations); white solid; mp 113–115 °C; IR (film) 3629 (OH), 1590 (C=C), 1436, 1314 (SO_2), 1236, 1152 cm^{-1}; major (*Z*)-isomer; ^1H NMR ($CDCl_3$) δ 1.17 [s, 18H, two $(CH_3)_3C$], 2.13 (s, 3H, $CH_3C{=}C$), 3.01 (s, 3H, CH_3SO_2), 5.05 (s, 1H, O*H*), 6.60 (s, 2H, phenolic H-2, H-6), 6.86-7.05 (m, 2H, phenyl H-2, H-6), 7.28–7.41 (m, 5H, phenyl H-3, H-4, H-5, 4-methanesulfonylphenyl H-2, H-6), 7.67-7.76 (m, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{30}H_{36}O_3S$: C, 75.59; H, 7.61. Found: C, 75.24; H, 7.63.

5.19. (*Z*)-1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylbut-1-ene (9b)

Yield, 22%; bright white crystals; mp 194–195 °C; IR (film) 3635 (OH), 1590 (C=C), 1436, 1315 (SO_2), 1236, 1152 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (t, J = 7.0 Hz, 3H, CH_3CH_2), 1.17 [s, 18H, two $(CH_3)_3C$], 2.50 (q, J = 7.0 Hz, 2H, CH_3CH_2), 3.03 (s, 3H, CH_3SO_2), 5.04 (s, 1H, O*H*), 6.59 (s, 2H, phenolic H-2, H-6), 7.26-7.41 (m, 7H, phenyl hydrogens, 4-methanesulfonylphenyl H-2, H-6), 7.76 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{31}H_{38}O_3S$: C, 75.88; H, 7.81. Found: C, 75.52; H, 8.11.

5.20. (*Z*)-1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylhex-1-ene (9c)

Yield, 25%; white foam; mp 195–196 °C; IR (film) 3634 (OH), 1590 (C=C), 1436, 1315 (SO_2), 1236, 1152 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78 (t, J = 7.0 Hz, 3H, CH_3CH_2), 1.16–1.26 [m, 22H, two $(CH_3)_3C$ + $CH_3(CH_2)_2$], 2.45 (t, J = 7.0 Hz, 2H, $C{=}CCH_2CH_2$), 3.03 (s, 3H, CH_3SO_2), 5.03 (s, 1H, O*H*), 6.55 (s, 2H, phenolic H-2, H-6), 7.26-7.41 (m, 7H, phenyl hydrogens, 4-methanesulfonylphenyl H-2, H-6), 7.76 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{33}H_{42}O_3S$: C, 76.41; H, 8.16. Found: C, 76.09; H, 8.16.

5.21. (*Z*)-1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylnon-1-ene (9d)

Yield, 27%; white foam; mp 183–184 °C; IR (film) 3635 (OH), 1590 (C=C), 1436, 1313 (SO_2), 1236, 1152 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (t, J = 7.0 Hz, 3H, CH_3CH_2), 1.16–1.34 [m, 28H, two $(CH_3)_3C$ + $CH_3(CH_2)_5$], 2.44 (t, J = 7.0 Hz, 2H, $C{=}CCH_2CH_2$), 3.03 (s, 3H, CH_3SO_2), 5.03 (s, 1H, O*H*), 6.58 (s, 2H, phenolic H-2, H-6), 7.25–7.40 (m, 7H, phenyl hydrogens, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{36}H_{48}O_3S$: C, 77.10; H, 8.63. Found: C, 76.80; H, 8.61.

5.22. (*Z*, *E*)-2-(3,5-Di-*tert*-butyl-4-acetoxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylhex-1-ene (11a)

Yield, 57%; ratio (*Z*):(*E*) = 94:6 (after several recrystallizations); bright white crystals; mp 157–158 °C; IR (film) 1759 (C=O), 1591 (C=C), 1428, 1367, 1316 (SO_2), 1152,

1109 cm^{-1}; major (*Z*)-isomer; ^1H NMR ($CDCl_3$) δ 0.83 (t, J = 7.3 Hz, 3H, CH_3CH_2), 1.17–1.47 [m, 22H, two $(CH_3)_3C$ + $CH_3(CH_2)_2$], 2.30 (s, 3H, CH_3CO), 2.48 (t, J = 7.3 Hz, 2H, C=CCH_2CH_2), 2.93 (s, 3H, CH_3SO_2), 6.97-7.02 (m, 4H, acetoxyphenyl H-2, H-6, phenyl H-2, H-6), 7.22–7.41 (m, 5H, phenyl H-3, H-4, H-5, 4-methanesulfonylphenyl H-2, H-6), 7.59 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{35}H_{44}O_4S$: C, 74.96; H, 7.91. Found: C, 74.58; H, 8.24.

5.23. (*Z*, *E*)-2-(3,5-Di-*tert*-butyl-4-acetoxyphenyl)-1-(4-methanesulfonylphenyl)-1-phenylnon-1-ene (11b)

Yield, 57%; ratio (*Z*):(*E*) = 83:17 (after several recrystallizations); white solid; mp 114–115 °C; IR (film) 1761 (C=O), 1592 (C=C), 1427, 1367, 1316 (SO_2), 1151, 1109 cm^{-1}; major (*Z*)-isomer; ^1H NMR ($CDCl_3$) δ 0.86 (t, J = 7.0 Hz, 3H, CH_3CH_2), 1.12–1.52 [m, 28H, two $(CH_3)_3C$ + $CH_3(CH_2)_5$], 2.30 (s, 3H, CH_3CO), 2.48 (t, J = 7.0 Hz, 2H, C=CCH_2CH_2), 2.93 (s, 3H, CH_3SO_2), 6.97-7.02 (m, 4H, acetoxyphenyl H-2, H-6, phenyl H-2, H-6), 7.22–7.41 (m, 5H, phenyl H-3, H-4, H-5, 4-methanesulfonylphenyl H-2, H-6), 7.59 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{38}H_{50}O_4S$: C, 75.71; H, 8.36. Found: C, 75.47; H, 8.61.

5.24. (*Z*)-1-(3,5-Di-*tert*-butyl-4-acetoxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylhex-1-ene (12a)

Yield, 55%; white solid; mp 116–117 °C; IR (film) 1760 (C=O), 1592 (C=C), 1427, 1367, 1315 (SO_2), 1151, 1109 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.78 (t, J = 7.0 Hz, 3H, CH_3CH_2), 1.07–1.32 [m, 22H, two $(CH_3)_3C$ + $CH_3(CH_2)_2$], 2.25 (s, 3H, CH_3CO), 2.45 (t, J = 7.0 Hz, 2H, C=CCH_2CH_2), 3.00 (s, 3H, CH_3SO_2), 6.76 (s, 2H, acetoxyphenyl H-2, H-6), 7.28–7.41 (m, 7H, phenyl hydrogens, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{35}H_{44}O_4S$: C, 74.96; H, 7.91. Found: C, 74.69; H, 8.10.

5.25. (*Z*, *E*)-1-(3,5-Di-*tert*-butyl-4-acetoxyphenyl)-2-(4-methanesulfonylphenyl)-1-phenylnon-1-ene (12b)

Yield, 45%; ratio (*Z*):(*E*) = 78:22; viscous pale yellow oil; IR (film) 1759 (C=O), 1592 (C=C), 1427, 1367, 1316 (SO_2), 1152, 1110 cm^{-1}; major (*Z*)-isomer; ^1H NMR ($CDCl_3$) δ 0.83 (t, J = 7.3 Hz, 3H, CH_3CH_2), 1.08–1.33 [m, 28H, two $(CH_3)_3C$ + $CH_3(CH_2)_5$], 2.25 (s, 3H, CH_3CO), 2.46 (t, J = 7.3 Hz, 2H, C=CCH_2CH_2), 2.99 (s, 3H, CH_3SO_2), 6.77 (s, 2H, acetoxyphenyl H-2, H-6), 7.28–7.39 (m, 7H, phenyl hydrogens, 4-methanesulfonylphenyl H-2, H-6), 7.76 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. Calcd for $C_{38}H_{50}O_4S$: C, 75.71; H, 8.36. Found: C, 75.34; H, 8.42.

5.26. 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 enzymes 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.

5.27. 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 immuno assay (EIA) kit (catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method.[16a] The solution of the test compound was prepared immediately before starting the assay to minimize the potential isomerization reaction.

5.28. In vitro lipoxygenase (LOX) inhibition assays

The ability of the test compounds to inhibit potato 5-LOX (catalog number 60401, Cayman Chemical, Ann Arbor, MI, USA) and soybean 15-LOX (catalog number 760700, Cayman Chemical, Ann Arbor, MI, USA) (IC_{50} values, μM) was determined using an enzyme immuno assay (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).

5.29. Anti-inflammatory assay

Anti-inflammatory activity was performed using a method described by Winter et al.[22]

5.30. Analgesic assay

Analgesic activity was determined using a 4% sodium chloride-induced writhing (abdominal constriction) assay previously reported.[23]

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