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Design and synthesis of acyclic triaryl (*Z*)-olefins: a novel class of cyclooxygenase-2 (COX-2) inhibitors

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Abstract—A group of acyclic 2-alkyl-1,1-diphenyl-2-(4-methylsulfonylphenyl)ethenes was designed for evaluation as selective cyclooxygenase-2 (COX-2) inhibitors. In vitro COX-1 and COX-2 isozyme inhibition structure–activity studies identified 1,1-diphenyl-2-(4-methylsulfonylphenyl)hex-1-ene as a highly potent (IC_{50} = 0.014 μM), and an extremely selective [COX-2 selectivity index (SI) >7142], COX-2 inhibitor that showed superior anti-inflammatory (AI) activity (ID_{50} = 2.5 mg/kg) relative to celecoxib (ID_{50} = 10.8 mg/kg). This initial study was extended to include the design of a structurally related group of acyclic triaryl (*Z*)-olefins possessing an acetoxy (OAc) substituent at the *para*-position of the C-1 phenyl ring that is *cis* to a C-2 4-methylsulfonylphenyl substituent. COX-1 and COX-2 inhibition studies showed that (*Z*)-1-(4-acetoxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)but-1-ene [(*Z*)-**13b**] is a potent (COX-1 IC_{50} = 2.4 μM; COX-2 IC_{50} = 0.03 μM), and selective (COX-2 SI = 81), COX-2 inhibitor which is a potent AI agent (ID_{50} = 4.1 mg/kg) with equipotent analgesic activity to celecoxib. A molecular modeling (docking) study showed that the SO_2Me substituent of (*Z*)-**13b** inserts deep inside the 2°-pocket of the COX-2 active site, where one of the *O*-atoms of SO_2 group undergoes a *H*-bonding interaction with Phe^{518}. The *p*-OAc substituent on the C-1 phenyl ring is oriented in a hydrophobic pocket comprised of Met^{522}, Gly^{526}, Trp^{387}, Tyr^{348}, and Tyr^{385}, and the C-2 ethyl substituent is oriented towards the mouth of the COX-2 channel in the vicinity of amino acid residues Arg^{120}, Leu^{531}, and Val^{349}. Structure–activity data acquired indicate that a (*Z*)-olefin having *cis* C-1 4-acetoxyphenyl (phenyl) and C-2 4-methylsulfonylphenyl substituents, and a C-1 phenyl substituent in conjunction with either a C-2 hydrogen or short alkyl substituent provides a novel template to design acyclic olefinic COX-2 inhibitors that, like aspirin, have the potential to acetylate COX-2.

1. Introduction

The constitutive COX-1 isozyme catalyzes the biosynthesis of prostaglandins that are necessary for important physiological maintenance functions that include gastrointestinal cytoprotection and vascular homeostasis. In contrast, inducible COX-2 is expressed predominately in activated cells, most often in response to mediators of inflammation. Thus, a selective COX-2 inhibitor allows the desired synthesis of cytoprotective prostaglandins, in conjunction with a simultaneous inhibition of proinflammatory prostaglandin synthesis, thereby reducing dyspepsia and ulceration.[1] Insights into the differences between the binding sites of COX-1 and COX-2 obtained from X-ray crystal structure data,[2,3] provided useful guidelines that facilitated the design of the selective COX-2 inhibitors celecoxib (**1**),[4] rofecoxib (**2**),[5] and valdecoxib (**3**)[6] (Fig. 1) having anti-inflammatory and analgesic activity with less gastrointestinal and renal toxicity.[7,8] For example, the COX-2 binding site possesses an additional 2°-pocket that is absent in COX-1, which is highly relevant to the design of selective COX-2 inhibitors. This COX-2 2°-pocket arises due to a conformational change at Tyr^{355} that is attributed to the presence of Ile^{523} in COX-1 relative to Val^{523} having a smaller side chain in COX-2.[2] It has also been reported that replacement of His^{513} in COX-1 by Arg^{513} in COX-2 plays a key role with respect to the *H*-bond network in the COX-2 binding site. Access of ligands to the 2°-pocket of COX-2 is controlled by histidine (His^{90}), glutamine (Gln^{192}), and tyrosine (Tyr^{355}).[9] Interaction of Arg^{513} with the bound drug is a requirement for time-dependent inhibition of COX-2.[10] The vast majority of selective COX-2 inhibitors investigated, or in clinical use, possess a heterocyclic or carbocyclic central ring scaffold having two vicinal aryl moieties.[11] In a different context, the nonselective COX inhibitor aspirin (**4**) is unique because of its ability to inhibit COX isozymes

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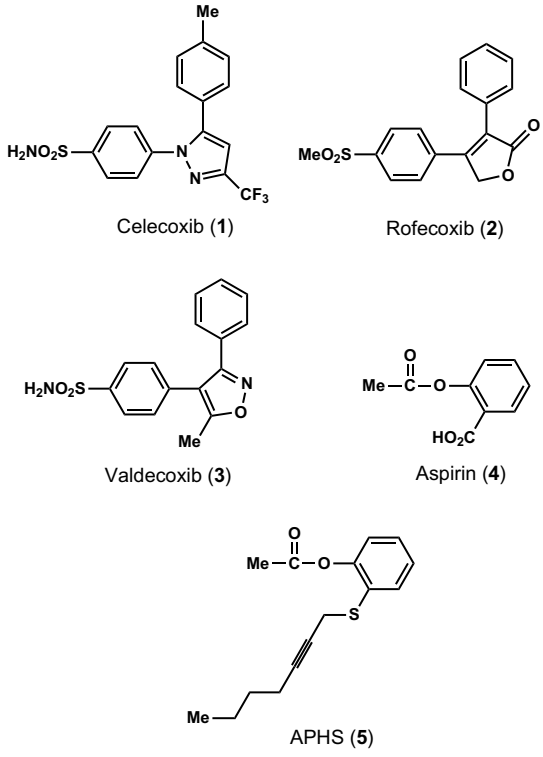
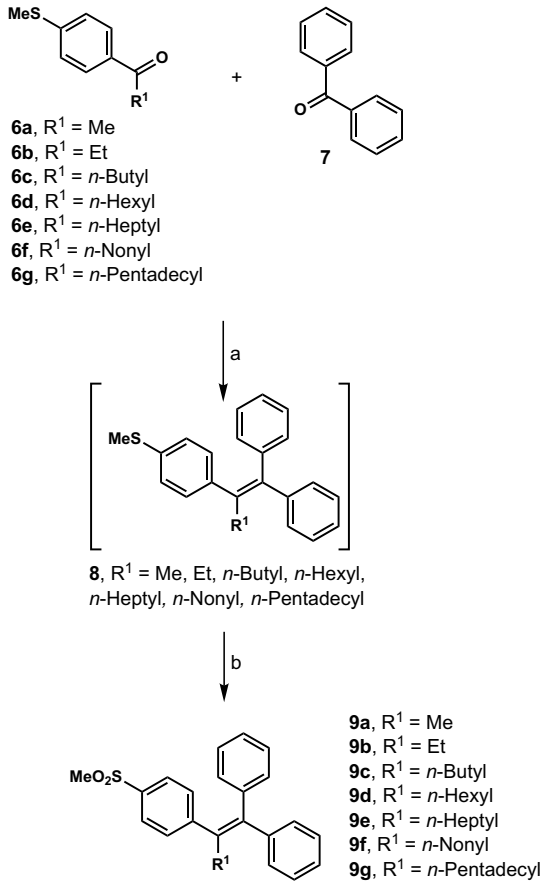


Figure 1. Representative cyclooxygenase (COX) inhibitors.

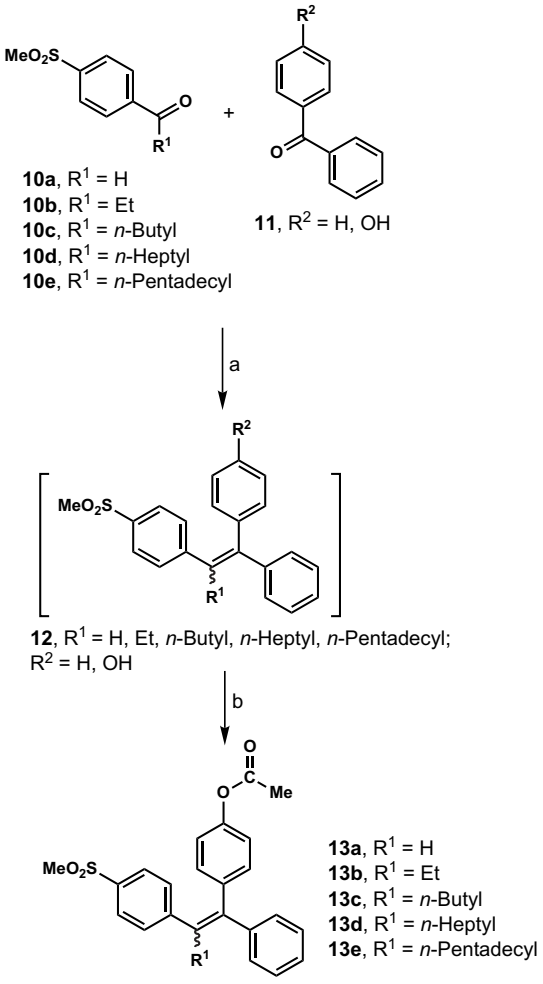
irreversibly by transferring its acetyl group to Ser^{530} as a covalent modifier.[12] The beneficial therapeutic effects of aspirin can be attributed to the acetylation of COX-2, while its anti-thrombotic and ulcerogenic effects are due to acetylation of COX-1. These properties were exploited in the design of the aspirin analog *o*-(acetoxyphenyl)hept-2-ynyl sulfide (**5**, APHS) that selectively acetylated, and irreversibly inactivated, COX-2.[13,14] Recently, we designed a new class of highly potent and selective COX-2 inhibitors having an acyclic triaryl olefinic structure (**9**).[15] As part of our ongoing program to develop new drug design concepts, we now provide complete details of this initial study,[15] and also report a group of related acyclic triaryl (*Z*)-olefins, possessing a 4-acetoxyphenyl moiety, from which a number of compounds (**13**) have been identified that exhibit highly potent and selective COX-2 inhibition.

2. Chemistry

Using a McMurry olefination reaction, intermediate olefins **8** (R^1 = alkyl) were generated in situ by reductive cross-coupling of the respective ketone **6**, prepared by Friedel–Crafts acylation of thioanisole,[16–19] and benzophenone **7**.[20] Subsequent oxidation of the 4-methylthiophenyl olefins **8** with Oxone® (potassium peroxymonosulfate)[21] afforded the target 4-methylsulfonylphenyl olefins **9** (R^1 = Me, Et, *n*-butyl, *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-nonyl, *n*-pentadecyl) in 62–76% yield (Scheme 1). On the other hand, carbonyl compounds **10** (R^1 = H, Et, *n*-butyl, *n*-heptyl, *n*-pentadecyl) were prepared by oxidation of the respective methylthio compounds using either Oxone®[21] or *m*-CPBA.[22] The intermediate (*Z*)-olefins **12** (R^1 = H, Et, *n*-butyl, *n*-heptyl, *n*-pentadecyl, R^2 = H, OH) were generated in situ using a (*Z*)-selective McMurry olefination reaction[23] by Zn–$TiCl_4$ catalyzed reductive cross-coupling of the respective 4-(methylsulfonyl)alkanophenone (**10**) with 4-hydroxybenzophenone (**11**, R^2 = OH). Subsequent acetylation[24] of the 4-hydroxyphenyl intermediate **12** afforded the target 4-acetoxyphenyl (*Z*)-olefin product **13** (R^1 = H, Et, *n*-butyl, *n*-heptyl, *n*-pentadecyl) in 67–72% overall yield (Scheme 2). Alternatively, when 4-(methylthio)propiophenone (**6**, R^1 = Et) was used, in place of 4-(methylsulfonyl)propiophenone (10, R^1 = Et) which gave (*Z*)-**13b** as the sole product, a mixture of the isomeric (*E*)- and (*Z*)-**13b** olefins (ratio of 1:4) was obtained. The structure of the starting 4-methylthiophenyl ketones **6**, the cross-coupled products 1,1,2-triaryl-2-alkyl-1-ethenes (**9**), 4-methylsulfonylphenyl carbonyl compounds **10**, the isolated olefins **12a** and **12b**, and final target (*Z*)-1-(4-acetoxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)-2-alkyl-1-ethenes (**13a–e**) were consistent with their spectral and microanalytical data. The stereochemistry of (*Z*)-**13e** (R^1 = *n*-pentadecyl) was confirmed by ^{1}H NMR difference nuclear Overhauser enhancement (NOE) studies (Fig. 2). The percent NOE enhancements ($CDCl_3$, 22 °C) of 6.4% and 3.2% for the respective H-2 (δ 6.84) and H-3 (δ 6.76) protons of the 4-acetoxyphenyl substituent upon irradiation of the H-3 (δ 7.74) proton of the 4-methyl-



Scheme 1. Reagents and conditions: (a) Zn, $TiCl_4$, THF, reflux 4.5 h; (b) Oxone® (potassium peroxymonosulfate), MeOH, THF, H_2O, 25 °C, 15 h.



Scheme 2. Reagents and conditions: (a) Zn, $TiCl_4$, THF, reflux 4.5 h; (b) AcCl, TEA, ether, 25 °C, 1.5 h.

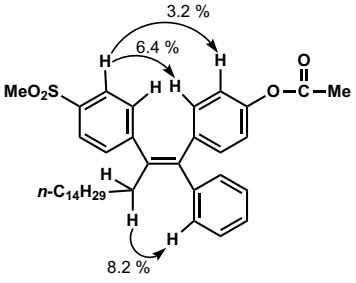


Figure 2. Some NOE determinations to determine the stereochemistry of the olefinic substituents for (*Z*)-**13e** in $CDCl_3$ at 22 °C.

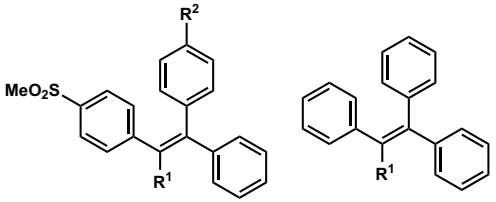
sulfonyl moiety indicate that these two aryl moieties are *cis* to each other [(*Z*)-stereoisomer]. This (*Z*)-stereochemical assignment for **13e** is also consistent with the observation that irradiation of the C=C–CH_2 (δ 2.45) protons resulted in a 8.2% enhancement of the C-1 *ortho*-phenyl (δ 7.36) protons of the unsubstituted *cis* phenyl ring. In regard to the stereochemical aspects of this (*Z*)-selective olefination reaction, it has been proposed that the (*Z*)-isomer arises from a consecutive induction by the active Ti° surface to the polydentate pinacolic intermediate formed by homolytic coupling of a radical anion species generated from reduction of the two carbonyl compounds[25] prior to subsequent demetallation and deoxygenation reactions.[26] In this regard, the 'phenoxy-Ti-sulfone' induction plays the key role for (*Z*)-stereoselection by forcing the phenoxy and sulfone moieties to be positioned on the same side *cis* to each other.[23]

3. Results and discussion

In a preliminary study regarding the design of acyclic triaryl olefins,[15] we reported that simple acyclic olefins exhibit selective COX-2 inhibition when (i) two geminal unsubstituted phenyl substituents are present at the C-1 position, (ii) a 4-methylsulfonylphenyl substituent is located at the C-2 position, and (iii) a *n*-alkyl substituent of appropriate chain length is attached to the C-2 position. Initial structure–activity studies, where the length of the C-2 alkyl substituent was varied, showed that maximal COX-2 potency (IC_{50} = 0.014 μM) and COX-2 selectivity [selectivity index (SI) > 7142] was exhibited by compound **9c** having a C-2 *n*-butyl substituent. In contrast, compound **9g** having a longer hydrophobic C-2 *n*-pentadecyl substituent did not inhibit COX-1 or COX-2 (IC_{50} > 100 μM) (see Table 1).

The nonselective COX-2 inhibitor aspirin (**4**, COX-1 IC_{50} 0.35 μM, COX-2 IC_{50} 2.4 μM; COX-2 SI = 0.14) is a 10-fold more potent inhibitor of COX-1 than COX-2 (see Table 1). Marnett and co-workers reported the aspirin derivative, *o*-(acetoxyphenyl)hept-2-ynyl sulfide (**5**, APHS), is a selective inhibitor of COX-2 that acetylates the hydroxyl group of Ser^{530} in the COX-2 binding site.[13,14] These structure–activity data prompted us to investigate the effect of an acetoxy substituent on COX-2 selectivity and potency for a group of 4-acetoxyphenyl (*Z*)-olefins **13** (R^1 = H, Me, Et, *n*-butyl, *n*-heptyl, *n*-pentadecyl), that are analogs of the acyclic triaryl olefins **9** reported previously as a letter.[15] Accordingly, an initial study showed that the *p*-acetoxyphenyl olefin (*E*,*Z*)-**13a** (R^1 = H, isomeric ratio = 1:6.4) is a potent inhibitor of COX-2 (COX-1 IC_{50} = 11.1 μM; COX-2 IC_{50} = 0.07 μM; COX-2 SI = 158), that is 26-fold more potent and 3-fold more selective than the parent triaryl olefin **12a** (R^1 = R^2 = H; COX-1 IC_{50} > 100 μM; COX-2 IC_{50} = 1.8 μM; COX-2 SI > 54). Replacement of the vinyl hydrogen at the C-2 position of (*E*,*Z*)-**13a** by an ethyl substituent [(*Z*)-**13b** (R^1 = Et)], increased COX-2 potency and decreased COX-1 selectivity slightly (COX-1 IC_{50} = 2.4 μM, COX-2 IC_{50} = 0.03 μM; COX-2 SI = 81). In comparison, (*Z*)-**13b** was a 40-fold more potent, and 3-fold more selective, inhibitor of COX-2 than the parent triaryl olefin **9b** (R^1 = Et; COX-1 IC_{50} = 31.6 μM, COX-2 IC_{50} = 1.2 μM; COX-2 SI = 25). It is noteworthy that the 4-acetoxyphenyl olefin (*Z*)-**13b** is about 2.3-fold more potent than celecoxib (**1**) and 16-fold more potent than rofecoxib (**2**) (Table 1). A comparative study showed that (*E*,*Z*-**13b**) (R^1 = Et, isomeric ratio = 1:4) is a selective inhibitor of COX-2 (COX-1 IC_{50} > 100 μM; COX-2 IC_{50} = 3.2 μM; COX-2 SI > 31), but 100-fold less potent than (*Z*)-**13b**. Further

Table 1. In vitro COX-1/COX-2 enzyme inhibition assay data for **9a–g**, **12a–b**, **13a–e**, **14**, and in vivo anti-inflammatory and analgesic activity assay data for **9c–d**, **13b**, and **14**



9a-g or **12a-b** or **13a-e** **14**

Compound	R^1	R^2	Vol (Å^3)[a]	COX-1 IC_{50} (μM)[b]	COX-2 IC_{50} (μM)[b]	COX-2 SI[c]	AI activity[d] ID_{50} (mg/kg)	Analgesic activity[e]	
								%Inhibition (30 min)	%Inhibition (1 h)
9a	Me	H	319	0.47	0.63	0.74	—	—	—
9b	Et	H	337	31.6	1.2	25	—	—	—
9c	*n*-C_4H_9	H	369	>100	0.014	>7142	2.5	49.3 ± 9.0	54.3 ± 7.5
9d	*n*-C_6H_{13}	H	403	1.7	0.03	54	1.6	52.4 ± 6.0	61.9 ± 12.5
9e	*n*-C_7H_{15}	H	420	>100	0.15	>666	—	—	—
9f	*n*-C_9H_{19}	H	453	>100	1.1	91	—	—	—
9g	*n*-$C_{15}H_{31}$	H	555	>100	>100	1	—	—	—
12a	H	H	303	>100	1.8	>54	—	—	—
(*Z*)-**12b**	Et	OH	343	31.6	1.9	16	—	—	—
(*E*,*Z*)-**13a**[f]	H	OAc	347	11.1	0.07	158	—	—	—
(*Z*)-**13b**	Et	OAc	380	2.4	0.03	81	4.1	43.0 ± 7.0	62.7 ± 9.7
(*E*,*Z*)-**13b**[g]	Et	OAc	380	>100	3.2	>31	—	—	—
(*Z*)-**13c**	*n*-C_4H_9	OAc	414	4.6	31.6	0.14	—	—	—
(*Z*)-**13d**	*n*-C_7H_{15}	OAc	464	3.1	7.9	0.39	—	—	—
(*Z*)-**13e**	*n*-$C_{15}H_{31}$	OAc	599	>50	>50	1	—	—	—
14	*n*-C_4H_9	—	318	>100	7.9	>12	22.3	55.5 ± 6.8	44.4 ± 6.8
Celecoxib	—	—	298	33.1	0.07	472	10.8	69.3 ± 12.1[h]	79.5 ± 2.0[h]
Rofecoxib	—	—	266	>100	0.50	>200	—	—	—
Aspirin	—	—	153	0.35	2.4	0.14	—	—	—

[a] The volume of the molecule, after minimization using PM3 geometry optimization, was calculated using the ALCHEMY 2000 program.
[b] The in vitro test compound concentration required to produce 50% inhibition of COX-1 or COX-2. The result (IC_{50}, μM) is the mean of two determinations.
[c] Selectivity Index (SI) = COX-1 IC_{50}/COX-2 IC_{50}.
[d] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as the ID_{50} value (mg/kg) at 3 h after oral administration of the test compound.
[e] Inhibitory activity in the rat 4% NaCl-induced abdominal constriction assay. The results are expressed as the mean % inhibition value ± SEM (n = 4) following a 5 mg/kg oral dose of the test compound.
[f] Ratio (*E*):(*Z*) = 1:6.4.
[g] Ratio (*E*):(*Z*) = 1:4.
[h] Oral dose (50 mg/kg).

elongation of the alkyl substituent chain length at C-2 to *n*-butyl [(*Z*)-**13c**] and *n*-heptyl [(*Z*)-**13d**] decreased COX-2 potency and selectivity. The C-2 *n*-pentadecyl compound (*Z*)-**13e** was an inactive inhibitor of both COX-1 and COX-2 (IC_{50} > 50 μM). It is noteworthy that replacement of the C-1 phenyl substituent in compound **9c** (R^1 = *n*-Bu) and **9e** (R^1 = *n*-heptyl) by a 4-acetoxyphenyl substituent in the respective compounds (*Z*)-**13c** and (*Z*)-**13d** reduced COX-2 potency and selectivity dramatically. On the other hand, the C-1 4-acetoxyphenyl compound (*Z*)-**13b** was a more potent and selective COX-2 inhibitor than the corresponding C-1 4-hydroxyphenyl compound (**12b**). These structure-activity data suggest that the acyclic olefin (*Z*)-**13b**, which possesses the best combination of COX-2 potency and selectivity among the group of compounds **13**, should inhibit the synthesis of inflammatory prostaglandins via the COX pathway at sites of induced inflammation.

Aspirin treatment of human prostaglandin endoperoxide H synthase (hPGHS-1, hCOX-1) expressed in *cos*-1 cells causes a time-dependent inactivation of oxygenase activity. In contrast, treatment of PGHS-2 (COX-2) produced an enzyme that retained oxygenase activity, but which formed the unnatural (15*R*)-hydroxy-5,8,11,13-eicosatetraenoic acid [(15*R*)-HETE] exclusively that is a precursor of leukotrienes via the 5-lipoxygenase (5-LO) pathway rather than prostaglandin H_2 (PGH_2) produced via the cyclooxygenase pathway. The K_m values for arachidonate of native and aspirin-treated hPGHS-2 were similar suggesting that arachidonate binds to both aspirin-treated and native hPGHS-2 in a similar manner.[27] A recent study has shown that (15*R*)-HETE inhibits the release of the potent inflammatory mediator LTB_4 from blood polymorphonuclear cells via the 5-LO pathway.[28] Based on these reports, it is possible that the 4-acetoxyphenyl (*Z*)-olefins **13a**–**b**, in addition to inhibiting COX-2, could also acetylate COX-2 to produce (15*R*)-HETE that would prevent the formation of inflammatory leukotrienes such as LTB_4 via the 5-LO pathway. Since the 4-acetoxyphenyl olefin (*Z*)-**13b** may undergo in vivo bioconversion by esterases to the 4-hydroxylphenyl olefin (*Z*)-**12b** (R^1 = Et, R^2 = OH), the COX-1 and COX-2 enzyme inhibition activities of (*Z*)-**12b** were determined where the assay showed a reduction in potency (COX-1 IC_{50} = 31.6 μM, COX-2 IC_{50} = 1.9 μM; COX-2 SI = 16) (Table 1). The combined volume of the COX-2 primary and secondary binding site is 394 Å^3, which is 25% larger than the COX-1 binding site (316 Å^3).[29] In this regard, it is possible that (*Z*)-**13b** (R^1 = Et) with a molecular volume of 380 Å^3 may easily enter the COX-2 active site (394 Å^3) with a favorable orientation to induce COX-2 inhibitory activity. In contrast, the 4-acetoxyphenyl olefin (*Z*)-**13e** (R^1 = *n*-pentadecyl) having a molecular volume of 599 Å^3 is likely too large to insert into the COX-2 active site resulting in a loss of COX-2 inhibitory activity. A molecular modeling study of the 4-acetoxyphenyl olefin (*Z*)-**13b** docked in the COX-2 active site (Fig. 3) shows that (*Z*)-**13b** binds in the primary binding-site such that the *p*-SO_2Me substituent on the C-2 phenyl ring inserts into the 2°-pocket present in COX-2 (distance = 1.76 Å) undergoing interactions with Phe^{518}, Arg^{513}, Gln^{192}, and

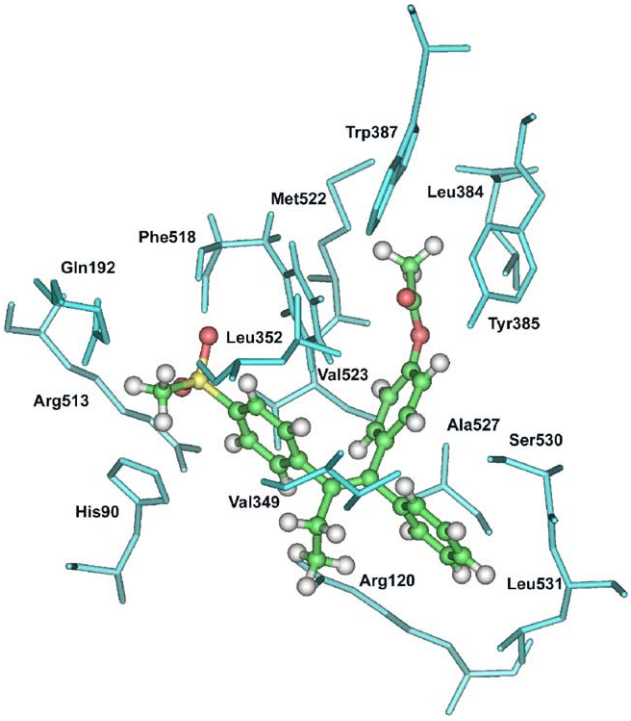


Figure 3. Docking of (*Z*)-**13b** in the binding site of murine COX-2. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

His^{90}. One of the *O*-atoms of SO_2Me moiety forms a *H*-bond with an amide hydrogen (N*H*) of Phe^{518} (distance = 2.06 Å), whereas the other *O*-atom is closer to the NH_2 of Arg^{513} (distance of 5.54 Å). The C-1 4-acetoxyphenyl substituent, that is *cis* to the C-2 4-methylsulfonylphenyl substituent is oriented toward a hydrophobic pocket comprised of Met^{522}, Gly^{526}, Trp^{387}, Tyr^{348}, and Tyr^{385} at the apex of the binding site. The distance between the center of C-1 4-acetoxyphenyl ring at the olefinic C-1 position and the *O*H of Ser^{530} is about 6.19 Å, and this moiety is within van der Waal's contact range (distance less than 5 Å) of Leu^{384} and Tyr^{385}. The *O*H of Ser^{530} is about 6.55 Å from the *C*-atom of the *C*=O, and the C=*O* oxygen atom forms a *H*-bond with the O*H* of Tyr^{385} (distance = 3.08 Å). This *H*-bonding of Tyr^{385} with the C=*O* oxygen atom could be beneficial for the acetylation of Ser^{530} in the active site of COX-2. This possibility is based on the facts that (i) Tyr^{385} hydrogen bonds to the acetyl group of aspirin, it orients the acetoxy substituent of aspirin, which it acetylates, and (ii) this H-bonding interaction increases its reactivity by stabilizing the incipient negative charge of the tetrahedral intermediate of acetylation.[30] The unsubstituted C-1 phenyl ring that is *cis* to the C-2 ethyl substituent is oriented toward the mouth of the channel comprised of Ser^{530}, Leu^{531}, and Ala^{527}. The distance between the center of the C-1 phenyl ring *cis* to the C-2 ethyl substituent and the *O*H of Ser^{530} is about 4.58 Å. The C-2 ethyl substituent is oriented towards a pocket comprised of Tyr^{355}, Arg^{120}, Leu^{531}, and Val^{349} at the mouth of the COX-2 active site. This computational study shows that the stereochemical disposition of aryl, alkyl, or acetoxy substituents about the

C=C bond controls the optimal protein–ligand binding interactions in the active site of COX-2.

Pharmacological studies were carried out to assess the in vivo anti-inflammatory (AI) and analgesic activity of some of the most potent and selective COX-2 inhibitors [**9c**,**d**, (*Z*)-**13b**] based on in vitro enzyme inhibition data (Table 1). In a carrageenan-induced rat paw edema assay model, the hex-1-ene (**9c**, ID_{50} = 2.5 mg/kg) and oct-1-ene (**9d**, ID_{50} = 1.6 mg/kg) compounds having a C-1 phenyl substituent, and the but-1-ene having a C-1 4-acetoxyphenyl moiety [(*Z*)-**13b**, ID_{50} = 4.1 mg/kg] all exhibited superior AI activity relative to the reference drug celecoxib (ID_{50} = 10.8 mg/kg). It is also noteworthy that the parent compound 1,1,2-triphenylhex-1-ene **14**, lacking the traditional $MeSO_2$ COX-2 pharmacophore, also exhibited moderate AI potency (ID_{50} = 22.3 mg/kg). In a rat model 4% NaCl-induced abdominal constriction assay, a 5 mg/kg po dose of **9c**,**d**, or (*Z*)-**13b** exhibited good analgesic activities (43–63% range), that are comparable to celecoxib, at 30 or 60 min post drug administration.

4. Conclusions

Novel acyclic 2-alkyl-1,1-diphenyl-2-(4-methylsulfonylphenyl)ethenes having a short 2-alkyl substituent, such as 1,1-diphenyl-2-(4-methylsulfonylphenyl)hex-1-ene (**9c**), exhibit optimal COX-2 inhibitory potency (IC_{50} = 0.014 μM) and selectivity (SI) > 7142]. A group of structurally related acyclic (*Z*)-1,1,2-triarylethenes (**13**) was also designed in which a C-1 4-acetoxyphenyl substituent is *cis* to a C-2 4-methylsulfonylphenyl substituent. Compounds having a C-2 hydrogen (**13a**), or ethyl (**13b**), substituent exhibited optimal COX-2 inhibitory potency (IC_{50} = 0.03–0.07 μM range) and selectivity (SI = 81–158 range). A molecular modeling study showed that the SO_2Me moiety of (*Z*)-**13b** inserts deep inside the COX-2 2°-pocket and that the C=*O* oxygen atom of the acetoxy substituent is *H*-bonding to Tyr^{385}, which could potentially enhance its ability to acetylate Ser^{530} like aspirin. The structure–activity relationships acquired show that appropriately substituted acyclic (*Z*)-olefins have the necessary geometry to provide potent and selective inhibition of the COX-2 isozyme, and to exhibit excellent AI and analgesic activities.

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. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer, where *J* (coupling constant) values are estimated in Hz. ^{13}C NMR spectra were acquired using the *J* modulated spin echo technique where methyl and methine carbons appear as positive peaks and methylene and quaternary carbon resonances appear as negative peaks. The NOE studies were performed under steady-state conditions using the Bruker NOE DIFF.AU software program (signal:noise ratio of 136 for a single pulse). $CDCl_3$ was dried using molecular sieves (type 3A, 1.6 mm pellets), treated with neutral alumina to remove acidic impurities, and degassed by passage of dry argon gas at 22 °C just prior to use. Molecular tumbling time was not altered. Elemental analysis (EA) were performed for C, H (Micro-analytical Service Laboratory, Department of Chemistry, University of Alberta). Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70–230 mesh). The starting carbonyl compounds **6a**–**c** (R^1 = Me, Et, *n*-butyl)[16–18] and **10a**–**b** (R^1 = H, Et)[22,23] were prepared using literature methods. All the 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-(methylthio)alkanophenones (6d–h)

The alkanoyl chloride (14.4 mmol) was added drop wise to a stirred suspension of $AlCl_3$ (1.76 g, 13.2 mmol) in chloroform (10 mL). Thioanisole (1.38 g, 11.1 mmol) was added at 0 °C and the reaction was allowed to proceed with stirring for 1.5 h at 25 °C. Water (10 mL) was added slowly at 0 °C, the organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic fractions were washed with water (10 mL), the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was recrystallized from CH_2Cl_2–*n*-hexane (1:9, v/v) to afford the respective product **6d**–**h**. Some physical, spectroscopic and microanalytical data for products **6d**–**h** are listed below.

5.2. 4-(Methylthio)hexanophenone (6d)

Yield, 60%; white plates; mp 70–72 °C; IR (film): 1677 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.91 (t, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.31–1.38 [m, 4H, $(CH_2)_2$], 1.70–1.75 (m, 2H, CH_2), 2.52 (s, 3H, SCH_3), 2.91 (t, 2H, *J* = 7.3 Hz, $COCH_2$), 7.26 (d, 2H, *J* = 8.5 Hz, 4-methylthiophenyl H-3, H-5), 7.87 (d, 2H, *J* = 8.5 Hz, 4-methylthiophenyl H-2, H-6). Anal. Calcd for $C_{13}H_{18}OS$: C, 70.22; H, 8.16. Found: C, 70.03; H, 8.33.

5.3. 4-(Methylthio)heptanophenone (6e)

Yield, 96%; white needles; mp 58–60 °C; IR (film): 1667 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.81 (t, 3H, *J* = 7.0 Hz, CH_2CH_3), 1.21–1.26 [m, 6H, $(CH_2)_3$], 1.59–1.67 (m, 2H, CH_2), 2.45 (s, 3H, SCH_3), 2.84 (t, 2H, *J* = 7.3 Hz, $COCH_2$), 7.19 (d, 2H, *J* = 8.5 Hz, 4-methylthiophenyl H-3, H-5), 7.80 (d, 2H, *J* = 8.5 Hz, 4-methylthiophenyl H-2, H-6). Anal. Calcd for $C_{14}H_{20}OS$: C, 71.14; H, 8.53. Found: C, 71.07; H, 8.76.

5.4. 4-(Methylthio)octanophenone (6f)

Yield, 72%; white solid; mp 66–68 °C; IR (film): 1682 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80 (t, 3H,

$J = 7.0$ Hz, CH_2CH_3), 1.15–1.35 [m, 8H, $(CH_2)_4$], 1.60–1.70 (m, 2H, CH_2), 2.45 (s, 3H, SCH_3), 2.84 (t, 2H, $J = 7.3$ Hz, CH_2), 7.19 (d, 2H, $J = 8.5$ Hz, 4-methylthiophenyl H-3, H-5), 7.80 (d, 2H, $J = 8.5$ Hz, 4-methylthiophenyl H-2, H-6). Anal. Calcd for $C_{15}H_{22}OS$: C, 71.95; H, 8.86. Found: C, 71.48; H, 8.97.

5.5. 4-(Methylthio)decanophenone (6g)

Yield, 72%; white needles; mp 80–82 °C; IR (film): 1682 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 1.27–1.33 [m, 12H, $(CH_2)_6$], 1.67–1.74 (m, 2H, CH_2), 2.53 (s, 3H, SCH_3), 2.91 (t, 2H, $J = 7.3$ Hz, $COCH_2$), 7.27 (d, 2H, $J = 8.5$ Hz, 4-methylthiophenyl H-3, H-5), 7.88 (d, 2H, $J = 8.5$ Hz, 4-methylthiophenyl H-2, H-6). Anal. Calcd for $C_{17}H_{26}OS$: C, 73.33; H, 9.41. Found: C, 73.15; H, 9.54.

5.6. 4-(Methylthio)hexadecanophenone (6h)

Yield, 69%; white solid; mp 88–90 °C; IR (film): 1663 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 1.26–1.33 [m, 24H, $(CH_2)_{12}$], 1.69–1.74 (m, 2H, CH_2), 2.53 (s, 3H, SCH_3), 2.91 (t, 2H, $J = 7.3$ Hz, $COCH_2$), 7.27 (d, 2H, $J = 8.5$ Hz, 4-methylthiophenyl H-3, H-5), 7.87 (d, 2H, $J = 8.5$ Hz, 4-methylthiophenyl H-2, H-6). Anal. Calcd for $C_{23}H_{38}OS \cdot 1/2H_2O$: C, 74.33; H, 10.50. Found: C, 74.06; H, 10.89.

5.7. General procedure for the synthesis of 1,1-diphenyl-2-(4-methylsulfonylphenyl)alkyl-1-enes (9a–g)

$TiCl_4$ (1.83 mL, 13 mmol) was added dropwise to a stirred suspension of Zn powder (1.7 g, 26.5 mmol) in dry THF (30 mL), under Ar, at −10 °C, and after the addition was completed the reaction mixture was refluxed for 2 h. A solution of the respective 4-(methylthio)alkanophenone (**6a**–**g**, 3.3 mmol) and benzophenone (**7**, 0.61 g, 3.3 mmol) in THF (65 mL) were added to a cooled suspension of the titanium reagent at 0 °C, and the reaction mixture was refluxed for 2.5 h. After cooling to 25 °C, the reaction mixture was poured into a 10% aqueous K_2CO_3 solution (100 mL), this mixture was stirred vigorously for 5 min, and the dispersed insoluble material was removed by vacuum filtration through a pad of Celite 545. The organic layer was separated and the aqueous layer was extracted with EtOAc (3×50 mL). The combined organic fractions were washed with water (10 mL), the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo to afford the respective 4-methylthiophenyl olefinic intermediate **8a**–**g**, which was dissolved in THF–MeOH (1:1, v/v; 10 mL). A solution of Oxone® (potassium peroxymonosulfate) (4.06 g, 6.6 mmol) in water (20 mL) was added drop wise at 0 °C, 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 fractions were washed with water (10 mL). The organic fraction was dried (Na_2SO_4), and the residue obtained was purified by silica gel flash column chromatography using *n*-hexane–EtOAc (2:1, v/v) as eluant to afford the respective 4-methylsulfonylphenyl olefin **9a**–**g**. The physical, spectroscopic, and microanalytical data for products **9a**–**g** are listed below.

5.8. 1,1-Diphenyl-2-(4-methylsulfonylphenyl)prop-1-ene (9a)

Yield, 62%; white solid; mp 154–156 °C; IR (film): 1143, 1316 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.16 (s, 3H, CH_3), 3.02 (s, 3H, SO_2CH_3), 6.85–6.88 (m, 2H, phenyl hydrogens), 7.04–7.07 (m, 3H, phenyl hydrogens), 7.23–7.38 (m, 7H, phenyl hydrogens and 4-methylsulfonylphenyl H-2, H-6), 7.72 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{22}H_{20}O_2S \cdot 1/3H_2O$: C, 74.54; H, 5.83. Found: C, 74.31; H, 5.62.

5.9. 1,1-Diphenyl-2-(4-methylsulfonylphenyl)but-1-ene (9b)

Yield, 70%; white solid; mp 124–126 °C; IR (film): 1155, 1334 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.95 (t, 3H, $J = 7.3$ Hz, CH_2CH_3), 2.53 (q, 2H, $J = 7.3$ Hz, CH_2CH_3), 3.03 (s, 3H, SO_2CH_3), 6.86–6.88 (m, 2H, phenyl hydrogens), 7.01–7.16 (m, 3H, phenyl hydrogens), 7.24–7.32 (m, 7H, phenyl hydrogens and 4-methylsulfonylphenyl H-2, H-6), 7.73 (d, 2H, $J = 8.4$ Hz, 4-methylsulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 13.5 (CH_2CH_3), 28.8 (CH_2CH_3), 44.5 (SO_2CH_3), 126.4, 126.9, 127.0, 127.6, 128.2, 129.2, 130.5, 130.6 (C_{arom}–H), 137.9, 140.2, 141.1, 141.9, 142.6, 148.6 (C_{arom}–C; C_{olefin}–C; C_{arom}–S). Anal. Calcd for $C_{23}H_{22}O_2S \cdot 1/4H_2O$: C, 75.26; H, 6.13. Found: C, 75.48; H, 6.01.

5.10. 1,1-Diphenyl-2-(4-methylsulfonylphenyl)hex-1-ene (9c)

Yield, 72%; white solid; mp 79–81 °C; IR (film): 1153, 1327 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.79 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 1.24–1.27 [m, 4H, $(CH_2)_2$], 2.48 (t, 2H, $J = 7.3$ Hz, C=C–CH_2), 3.03 (s, 3H, SO_2CH_3), 6.84–6.87 (m, 2H, phenyl hydrogens), 7.02–7.04 (m, 3H, phenyl hydrogens), 7.23–7.31 (m, 7H, phenyl hydrogens and 4-methylsulfonylphenyl H-2, H-6), 7.72 (d, 2H, $J = 8.4$ Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{25}H_{26}O_2S \cdot 1/8H_2O$: C, 76.44; H, 6.68. Found: C, 76.28; H, 6.86.

5.11. 1,1-Diphenyl-2-(4-methylsulfonylphenyl)oct-1-ene (9d)

Yield, 66%; white solid; mp 104–106 °C; IR (film): 1159, 1308 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.65 (t, 3H, $J = 7.3$ Hz, CH_2CH_3), 0.96–1.08 [m, 8H, $(CH_2)_4$], 2.29 (t, 2H, $J = 7.3$ Hz, C=C–CH_2), 2.85 (s, 3H, SO_2CH_3), 6.66–6.69 (m, 2H, phenyl hydrogens), 6.84–6.86 (m, 3H, phenyl hydrogens), 7.05–7.18 (m, 7H, phenyl hydrogens and 4-methylsulfonylphenyl H-2, H-6), 7.55 (d, 2H, $J = 8.4$ Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{27}H_{30}O_2S$: C, 77.47; H, 7.22. Found: C, 77.55; H, 6.92.

5.12. 1,1-Diphenyl-2-(4-methylsulfonylphenyl)non-1-ene (9e)

Yield, 68%; white solid; mp 64–66 °C; IR (film): 1143, 1318 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 1.17–1.40 [m, 10H, $(CH_2)_5$], 2.47 (t, 2H, $J = 7.3$ Hz, C=C—CH_2), 3.02 (s, 3H, SO_2CH_3), 6.84–6.87 (m, 2H, phenyl hydrogens), 7.01–7.04 (m, 3H, phenyl hydrogens), 7.23–7.43 (m, 7H, phenyl hydrogens and 4-methylsulfonylphenyl H-2, H-6), 7.72 (d, 2H, $J = 8.4$ Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{28}H_{32}O_2S$: C, 77.74; H, 7.46. Found: C, 77.86; H, 7.41.

5.13. 1,1-Diphenyl-2-(4-methylsulfonylphenyl)undec-1-ene (9f)

Yield, 68%; white solid; mp 65–67 °C; IR (film): 1157, 1315 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 1.17–1.29 [m, 14H, $(CH_2)_7$], 2.47 (t, 2H, $J = 7.3$ Hz, C=C—CH_2), 3.03 (s, 3H, SO_2CH_3), 6.85–6.87 (m, 2H, phenyl hydrogens), 7.00–7.04 (m, 3H, phenyl hydrogens), 7.23–7.38 (m, 7H, phenyl hydrogens and 4-methylsulfonylphenyl H-2, H-6), 7.72 (d, 2H, $J = 8.4$ Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{30}H_{36}O_2S$: C, 78.22; H, 7.88. Found: C, 78.08; H, 7.91.

5.14. 1,1-Diphenyl-2-(4-methylsulfonylphenyl)heptadec-1-ene (9g)

Yield, 76%; white solid; mp 68–70 °C; IR (film): 1143, 1316 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 1.26–1.40 [m, 26H, $(CH_2)_{13}$], 2.46 (t, 2H, $J = 7.3$ Hz, C=C—CH_2), 2.69 (s, 3H, SO_2CH_3), 6.84–6.87 (m, 2H, phenyl hydrogens), 7.00–7.05 (m, 3H, phenyl hydrogens), 7.23–7.40 (m, 7H, phenyl hydrogens and 4-methylsulfonylphenyl H-2, H-6), 7.44 (d, 2H, $J = 8.4$ Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{36}H_{48}O_2S$: C, 79.36; H, 8.88. Found: C, 79.20; H, 9.16.

5.15. General procedure for the synthesis of 4-(methylsulfonyl)alkanophenones (10c–e)

A solution of Oxone® (potassium peroxymonosulfate) (4.06 g, 6.6 mmol) in water (20 mL) was added to a stirred solution of the 4-(methylthio)alkanophenone (**6c**,**d**, or **6e**, 3.3 mmol) in THF–MeOH (1:1, v/v; 10 mL) at 0 °C, and the reaction was allowed to proceed with stirring for 15 h at 25 °C. Removal of the solvent in vacuo gave a residue to which water (20 mL) was added. Extraction with EtOAc (3×30 mL), washing the combined extracts with water (10 mL), drying the organic fraction (Na_2SO_4), and removal of the solvent in vacuo gave a white solvent which was purified by recrystallization from CH_2Cl_2–*n*-hexane (1:9, v/v) to afford the respective product **10c**,**d**, or **10e**. Physical, spectroscopic, and microanalytical data products **10d**–**e** are listed below.

5.16. 4-(Methylsulfonyl)pentanophenone (10c)

Yield, 94%; white needles; mp 85–87 °C; IR (film): 1142, 1320 (SO_2), 1698 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.97 (t, 3H, $J = 7.3$ Hz, CH_2CH_3), 1.36–1.49 (m, 2H, CH_2CH_3), 1.69–1.76 (m, 2H, $COCH_2CH_2$), 3.01 (t, 2H, $J = 7.3$ Hz, $COCH_2$), 3.09 (s, 3H, SO_2CH_3), 8.05 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-3, H-5), 8.13 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-2, H-6). Anal. Calcd for $C_{12}H_{16}O_3S \cdot 1/6H_2O$: C, 59.23; H, 6.71. Found: C, 59.45; H, 6.67.

5.17. 4-(Methylsulfonyl)octanophenone (10d)

Yield, 75%; while needles; mp 105–107 °C; IR (film): 1148, 1313 (SO_2), 1691 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.89 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 1.29–1.45 [m, 8H, $(CH_2)_4CH_3$], 1.70–1.77 (m, 2H, $COCH_2CH_2$), 3.0 (t, 2H, $J = 7.3$ Hz, $COCH_2$) 3.09 (s, 3H, SO_2CH_3), 8.04 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-3, H-5), 8.13 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-2, H-6). Anal. Calcd for $C_{15}H_{22}O_3S$: C, 63.80; H, 7.85. Found: C, 63.61; H, 7.86.

5.18. 4-(Methylsulfonyl)hexadecanophenone (10e)

Yield, 65%; white plates; mp 108–110 °C; IR (film): 1143, 1327 (SO_2), 1685 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 1.26–1.40 [m, 24H, $(CH_2)_{12}CH_3$], 1.70–1.85 (m, 2H, $COCH_2CH_2$), 3.00 (t, 2H, $J = 7.3$ Hz, $COCH_2$), 3.09 (s, 3H, SO_2CH_3), 8.06 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-3, H-5), 8.13 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-2, H-6). Anal. Calcd for $C_{23}H_{38}O_3S$: C, 70.00; H, 9.71. Found: C, 69.91; H, 9.96.

5.19. 1,1-Diphenyl-2-(4-methylsulfonylphenyl)ethene (12a)

$TiCl_4$ (1.83 mL, 13 mmol) was added drop wise to a stirred suspension of Zn powder (1.7 g, 26.5 mmol) in dry THF (30 mL) under an argon atmosphere at −10 °C, and after the addition was completed this mixture was refluxed for 2 h to produce the titanium reagent. A solution of 4-(methylsulfonyl)benzaldehyde (**10a**, 0.60 g, 3.3 mmol) and benzophenone (**7**, 0.61 g, 3.3 mmol) in THF (65 mL) was added to the cooled suspension of the titanium reagent at 0 °C, and the reaction mixture was refluxed for 2.5 h. After cooling to 25 °C, the reaction mixture was poured onto a 10% aqueous K_2CO_3 solution (100 mL), this mixture was stirred vigorously for 5 min, and 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, the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo to give a residue that was purified by silica gel flash column chromatography. Elution with *n*-hexane–EtOAc (2:1, v/v) as eluant afforded **12a** (0.75 g, 70%); mp 131–133 ^{0}C; IR (film): 1155, 1313 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.02 (s, 3H, SO_2CH_3),

6.98 (s, 1H, =C*H*), 7.16–7.37 (m, 12H, phenyl hydrogens, 4-methylsulfonylphenyl H-2, H-6), 7.68 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{21}H_{18}O_2S \cdot 1/4H_2O$: C, 74.41; H, 5.45. Found: C, 74.67; H, 5.54.

5.20. (*Z*)-1-(4-Hydroxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)but-1-ene (12b)

Reaction of 4-(methylsulfonyl)propanophenone (**10b**) and 4-hydroxybenzophenone (**11**) with the titanium reagent, following the procedure described for the synthesis and silica gel flash column purification of **12a**, gave **12b** in 60% yield as a white solid; mp 188–190 °C; IR (film): 1148, 1320 (SO_2), 3381 (OH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (t, 3H, $J = 7.3$ Hz, CH_2CH_3), 2.50 (q, 2H, $J = 7.3$ Hz, CH_2CH_3), 3.05 (s, 3H, SO_2CH_3), 4.56 (br s, 1H, O*H*), 6.49 (d, 2H, $J = 8.5$ Hz, 4-hydroxyphenyl H-3, H-5), 6.70 (d, 2H, $J = 8.5$ Hz, 4-hydroxyphenyl H-2, H-6), 7.10–7.39 (m, 7H, phenyl hydrogens, 4-methylsulfonylphenyl H-2, H-6), 7.74 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{23}H_{22}O_3S \cdot 1/4H_2O$: C, 72.12; H, 5.87. Found: C, 72.05; H, 5.86.

5.21. General procedure for the synthesis of 1-(4-acetoxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)alkenes (13a–e)

$TiCl_4$ (1.83 mL, 13 mmol) was added drop wise to a stirred suspension of Zn powder (1.7 g, 26.5 mmol) in dry THF (30 mL) under an argon atmosphere at −10 °C, and this mixture was heated at reflux for 2 h to produce the titanium reagent. A cooled suspension of this titanium reagent was added to a solution of the respective 4-(methylsulfonyl)alkanophenone [4-(methylsulfonyl)benzaldehyde, **10a**, $R^1 = H$; 4-(methylsulfonyl)propanophenone, **10b**, $R^1 = Et$; 4-(methylsulfonyl)pentanophenone, **10c**, $R^1 = n$-Bu; 4-(methylsulfonyl)octanophenone, **10d**, $R^1 = n$-heptyl; or 4-(methylsulfonyl)hexadecanophenone, **10e**, R^1 = pentadecyl] (3.3 mmol) and 4-hydroxybenzophenone (**11**, R^2 = 4-HO-C_6H_4-; 0.65 g, 3.3 mmol) in THF (65 mL) at 0 °C, and the reaction was allowed to proceed at reflux for 2.5 h. After cooling to 25 °C, the reaction mixture was poured into a 10% aqueous K_2CO_3 solution (100 mL), this mixture was stirred vigorously for 5 min, and the dispersed insoluble material was removed by vacuum filtration through a Celite 545 pad. The organic fraction was separated, the aqueous layer was extracted with EtOAc (3 × 50 mL), and the combined organic fractions were dried (Na_2SO_4). Removal of the solvent in vacuo afforded the respective 4-hydroxyphenyl olefinic intermediate (**12**, R^1 as indicated above; R^2 = OH), which was dissolved in ether (10 mL), and triethylamine (0.5 g, 5.0 mmol) was added. Acetyl chloride (0.39 g, 5.0 mmol) was added drop wise at 0 °C, and the reaction was allowed to proceed at 25 °C for 1.5 h prior to quenching with water (20 mL). The organic layer was separated, the aqueous layer was extracted with EtOAc (3 × 30 mL), the combined organic fractions were washed with water (10 mL), and the organic fraction was dried (Na_2SO_4). Removal of the solvent in vacuo gave a residue that was purified by silica gel flash column chromatography using *n*-hexane–EtOAc (3:1, v/v) as eluant to afford the respective product **13a**–**e**. Physical, spectroscopic and microanalytical data for products **13a**–**e** are listed below.

5.22. (*E*,*Z*)-1-(4-Acetoxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)ethene (13a)

Yield, 67%; isolated as a mixture of (*E*) and (*Z*) isomers (the isomeric ratio = 1:6.4 as determined from the integrals for the CH_3SO_2 resonances at δ 3.03 and 3.02, respectively); mp 158–160 °C; IR (film): 1153, 1309 (SO_2), 1768 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) for (*Z*)-**13a**: δ 2.32 (s, 3H, $COCH_3$), 3.02 (s, 3H, SO_2CH_3), 6.96 (s, 1H, =C*H*), 7.06 (d, 2H, $J = 8.5$ Hz, 4-acetoxyphenyl H-3, H-5), 7.15 (d, 2H, $J = 8.5$ Hz, 4-acetoxyphenyl H-2, H-6), 7.27–7.38 (m, 7H, phenyl hydrogens, and 4-methylsulfonylphenyl H-2, H-6), 7.68 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{23}H_{20}O_4S$: C, 70.39; H, 5.14. Found: C, 70.14; H, 5.25.

5.23. (*Z*)-1-(4-Acetoxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)but-1-ene (13b)

Yield, 72%; colorless crystals; mp 140–142 °C; IR (film): 1153, 1318 (SO_2), 1759 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.93 (t, 3H, $J = 7.3$ Hz, CH_2CH_3), 2.23 (s, 3H, $COCH_3$), 2.52 (q, 2H, $J = 7.3$ Hz, CH_2CH_3), 3.04 (s, 3H, SO_2CH_3), 6.77 (d, 2H, $J = 8.5$ Hz, 4-acetoxyphenyl H-3, H-5), 6.85 (d, 2H, $J = 8.5$ Hz, 4-acetoxyphenyl H-2, H-6), 7.23–7.39 (m, 7H, phenyl hydrogens, and 4-methylsulfonylphenyl H-2, H-6), 7.75 (d, 2H, $J = 8.5$ Hz, 4-methylsulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 13.4 (CH_2C_3), 21.1 (COC_3), 29.7 (C_2CH_3), 44.4 (SO_2C_3), 120.6, 126.9, 127.1, 128.2, 129.1, 130.5, 131.4 (C_{arom}–H), 138.0, 139.3, 140.1, 140.7, 142.2, 148.3, 148.9 (C_{arom}–C, C_{olefin}–C, C_{arom}–O, C_{arom}–S), 169.2 (*C*=O). Anal. Calcd for $C_{25}H_{24}O_4S$: C, 71.40; H, 5.75. Found: C, 71.30; H, 5.89.

5.24. (*E*,*Z*)-1-(4-Acetoxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)but-1-ene [(*E*,*Z*)-13b]

The title compound was synthesized as an isomeric mixture using a McMurry reductive cross-coupling reaction of 4-(methylthio)propiophenone (**6b**) and 4-hydroxybenzophenone (**11**, R^2 = OH)) to give an (*E*) and (*Z*) mixture of 1-(4-hydroxyphenyl)-1-phenyl-2-(4-methylthiophenyl)but-1-ene, that was then oxidized using Oxone® to give the corresponding 1-(4-hydroxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)but-1-ene. A subsequent acetylation of the (*E*,*Z*)-1-(4-hydroxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)but-1-ene using acetyl chloride gave the target (*E*,*Z*)-1-(4-acetoxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)but-1-ene (**13b**, isomeric ratio = 1:4) in 65% yield. The (*E*):(*Z*) ratio was calculated from the integrals for the respective CH_3SO_2 resonances at δ 3.03 and 3.04, respectively. The purification method used was identical to the procedure described above to purify (*Z*)-**13a**. The ^1H NMR spectral data for the (*Z*)-**13b** isomer is the same as that

listed above. The ^{1}H NMR spectral data for (*E*)-**13b** is listed below. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H, J = 7.2 Hz, CH_2CH_3), 2.32 (s, 3H, $COCH_3$), 2.51 (q, 2H, J = 7.2 Hz, CH_2CH_3), 3.03 (s, 3H, SO_2CH_3), 6.76 (d, 2H, J = 8.5 Hz, 4-acetoxyphenyl H-3, H-5), 6.84 (d, 2H, J = 8.5 Hz, 4-acetoxyphenyl H-2, H-6), 7.02–7.39 (m, 7H, phenyl hydrogens, 4-methylsulfonylphenyl H-2, H-6), 7.74 (d, 2H, J = 8.5 Hz, 4-methylsulfonylphenyl H-3, H-5).

5.25. (*Z*)-1-(4-Acetoxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)hex-1-ene (13c)

Yield, 68%; white plates; mp 115–117 °C; IR (film): 1150, 1315 (SO_2), 1750 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.79 (t, 3H, J = 7.0 Hz, CH_2CH_3), 1.18–1.35 [m, 4H, $(CH_2)_2CH_3$], 2.23 (s, 3H, $COCH_3$), 2.47 (t, 2H, J = 7.3 Hz, C=C—CH_2), 3.04 (s, 3H, SO_2CH_3), 6.76 (d, 2H, J = 8.5 Hz, 4-acetoxyphenyl H-3, H-5), 6.85 (d, 2H, J = 8.5 Hz, 4-acetoxyphenyl H-2, H-6), 7.21–7.39 (m, 7H, phenyl hydrogens, and 4-methylsulfonylphenyl H-2, H-6), 7.74 (d, 2H, J = 8.5 Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{27}H_{28}O_4S$: C, 72.29; H, 6.29. Found: C, 72.53; H, 6.04.

5.26. (*Z*)-1-(4-Acetoxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)non-1-ene (13d)

Yield, 67%; colorless syrup; IR (film): 1157, 1315 (SO_2), 1757 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.84 (t, 3H, J = 7.0 Hz, CH_2CH_3), 1.12–1.57 [m, 10H, $(CH_2)_5CH_3$], 2.23 (s, 3H, $COCH_3$), 2.44 (t, 2H, J = 7.3 Hz, C=C—CH_2), 3.04 (s, 3H, SO_2CH_3), 6.76 (d, 2H, J = 8.5 Hz, 4-acetoxyphenyl H-3, H-5), 6.84 (d, 2H, J = 8.5 Hz, 4-acetoxyphenyl H-2, H-6), 7.22–7.39 (m, 7H, phenyl hydrogens, 4-methylsulfonylphenyl H-2, H-6), 7.74 (d, 2H, J = 8.4 Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{30}H_{34}O_4S{\cdot}1/6H_2O$: C, 72.98; H, 6.95. Found: C, 72.94; H, 9.91.

5.27. (*Z*)-1-(4-Acetoxyphenyl)-1-phenyl-2-(4-methylsulfonylphenyl)heptadec-1-ene (13e)

Yield, 70%; colorless oil; IR (film): 1155, 1313 (SO_2), 1753 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, J = 7.0 Hz, CH_2CH_3), 1.12–1.56 [m, 26H, $(CH_2)_{13}CH_3$], 2.23 (s, 3H, $COCH_3$), 2.45 (t, 2H, J = 7.3 Hz, C=C—CH_2), 3.04 (s, 3H, SO_2CH_3), 6.76 (d, 2H, J = 8.5 Hz, 4-acetoxyphenyl H-3, H-5), 6.84 (d, 2H, J = 8.5 Hz, 4-acetoxyphenyl H-2, H-6), 7.22–7.39 (m, 7H, phenyl hydrogens, 4-methylsulfonylphenyl H-2, H-6), 7.74 (d, 2H, J = 8.4 Hz, 4-methylsulfonylphenyl H-3, H-5). Anal. Calcd for $C_{38}H_{50}O_4S{\cdot}1/6H_2O$: C, 75.32; H, 8.30. Found: C, 75.28; H, 7.91.

5.28. 1,1,2-Triphenylhex-1-ene (14)

$TiCl_4$ (1.83 mL, 13 mmol) was added drop-wise to a stirred suspension of Zn powder (1.7 g, 26.5 mmol) in dry THF (30 mL), under Ar at −10 °C. After the addition was completed, the reaction mixture was refluxed for 2 h. A solution of valerophenone (0.54 g, 3.3 mmol) and benzophenone (0.61 g, 3.3 mmol) in THF (65 mL) was added to a cooled suspension of this titanium reagent at 0 °C, and the reaction was allowed to proceed at reflux for 2.5 h. After cooling to 25 °C, the reaction mixture was poured into 10% aqueous K_2CO_3 solution (100 mL), and after vigorous stirring for 5 min, the dispersed insoluble matters were removed by vacuum filtration using Celite 545. The organic layer was separated and the aqueous layer was extracted with EtOAc (3×50 mL). The combined organic fractions were washed with water, and the organic fraction was dried (Na_2SO_4). Purification by flash silica gel column chromatography using *n*-hexane–EtOAc (3:1, v/v) as eluent afforded the olefin **14** (0.69 g, 67%) as a white solid, mp 90–92 °C (lit. mp 91 °C);[31] ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.78 (t, 3H, J = 7.0 Hz, CH_2CH_3), 1.18–1.55 [m, 4H, $(CH_2)_2CH_3$], 2.44 (t, 2H, J = 7.0 Hz, C=C—CH_2) 6.86–7.38 (m, 15H, phenyl hydrogens).

6. Molecular modeling (docking) study

Docking experiment was 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 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 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 4*r*. 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.

7. Volume determination

The ALCHEMY 2000 program[32] was used to calculate the molecular volume (Å^3) of compounds in the Table 1 after minimization using PM3.

8. In vitro cyclooxygenase inhibition assays

The ability of the test compounds listed in the Table 1 to inhibit ovine COX-1 and COX-2 (IC_{50} values, μM) was determined using an enzyme immuno assay (EIA) kit

(catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid (AA) to PGH_2. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, is measured by enzyme immunoassay (ACE™ competitive EIA). Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (960 μL, 0.1 M Tris–HCl pH 8.0 containing 5 mM EDTA and 2 mM phenol) with either COX-1 or COX-2 (10 μL) enzyme in the presence of heme (10 μL) were added 10 μL of various concentrations of test drug solutions (0.01, 0.1, 1, 10, 50, and 100 μM in a final volume of 1 mL). These solutions were incubated for a period of 5 min at 37 °C after which 10 μL of AA (100 μM) solution were added and the COX reaction was stopped by the addition of 50 μL of 1 M HCl after 2 min. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride was measured by enzyme immunoassay. This assay is based on the competition between PGs and a PG-acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells since the concentration of PG tracer is held constant while the concentration of PGs varies. This antibody–PG complex binds to a mouse anti-rabbit monoclonal antibody that had been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's reagent, which contains the substrate to acetylcholine esterase, is added to the well. The product of this enzymatic reaction produces a distinct yellow color that absorbs at 405 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of PGs present in the well during the incubation: Absorbance α [Bound PG Tracer] $\alpha 1$/PGs. 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 measured using a carrageenan-induced rat paw edema assay described by Winter et al.[33] Briefly, four male Sprague Dawley rats weighing 100–110 g were used in each group. Test compounds suspended in water containing 1% methyl cellulose, were administered orally at different doses (0.5–30 mg/kg range) 1 h prior to a 0.05 mL subcutaneous injection of 1% carrageenan in 0.9% NaCl solution under the planter skin of the right hind paw. Control experiments were identical except that the vehicle did not contain a test compound. The volume of the injected paw was measured at 0 and 3 h using a UGO Basile 7141 Plethysmometer (Ser. No 43201) for calculation of % inhibition of inflammation.

10. Analgesic assay

Analgesic activity was determined using the 4% sodium chloride-induced writhing (abdominal constriction) assay described by Fukawa et al.[34]

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