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Synthesis and biological evaluation of a novel class of rofecoxib analogues as dual inhibitors of cyclooxygenases (COXs) and lipoxygenases (LOXs)

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Abstract—A group of 4-(4-methanesulfonylphenyl)-3-phenyl-2(5*H*)furanones possessing an acetyl, 3-oxobut-1-ynyl, [hydroxyl(or alkoxy)imino]alkyl, [hydroxyl(or alkoxy)imino]alkynyl, and *N*-alkoxy(or *N*-phenoxy)carbonyl-*N*-hydroxy-*N*-ethylamino substituents at the *para*-position of the C-3 phenyl ring of rofecoxib were synthesized. This group of compounds was designed for evaluation as dual inhibitors of cyclooxygenases (COXs) and lipoxygenases (LOXs) that exhibit in vivo anti-inflammatory and analgesic activities. In vitro COX-1/COX-2, and 5-LOX/15-LOX, isozyme inhibition structure–activity relationships identified 3-[4-(1-hydroxyimino)ethylphenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**17a**) having an optimal combination of COX-2 (COX-2 IC_{50} = 1.4 μM; COX-2 SI > 71), and 5-LOX and 15 LOX (5-LOX IC_{50} = 0.28 μM; 15-LOX IC_{50} = 0.32 μM), inhibitory effects. It was also discovered that 3-[4-(3-hydroxyiminobut-1-ynyl)phenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**18a**) possesses dual COX-2 (IC_{50} = 2.7 μM) and 5-LOX (IC_{50} = 0.30 μM) inhibitor actions. Further in vivo studies employing a rat carrageenan-induced paw edema model showed that the oxime compounds (**17a**, **18a**) were more potent anti-inflammatory agents than the 5-LOX inhibitor caffeic acid, and 15-LOX inhibitor nordihydroguaiaretic acid (NDGA), but less potent than the selective COX-2 inhibitor celecoxib. The results of this investigation showed that incorporation of a *para*-oxime moiety on the C-3 phenyl ring of rofecoxib provides a suitable template for the design of dual inhibitors of the COX and LOX enzymes.

1. Introduction

Eicosanoids, the most frequently investigated family of inflammation mediators, arise from the biotransformation of arachidonic acid (AA) via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways.[1] In this regard, prostanoids (prostaglandins, prostacyclin, and thromboxanes) are produced via the COX pathway, whereas leukotrienes (LTs) are formed by the LOX pathway. Traditional non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory effect by inhibition of the COX-1 and COX-2 isozymes.[2] Adverse side effects, in particular gastrointestinal (GI) irritation, ulcerogenicity, and renal toxicity, often limit the chronic use of NSAIDs.[3] In the late 1990s, the discovery of selective COX-2 inhibitors (see structures in Fig. 1) such as celecoxib (**1**) and rofecoxib (**2**) heralded the start of a new era for the treatment of inflammatory conditions such as rheumatoid arthritis (RA) and osteoarthritis (OA).[4] Clinical studies indicated that selective COX-2 inhibitors are effective anti-inflammatory agents with a reduced risk of undesirable GI effects compared to classical NSAIDs.[5] However, an increased risk of myocardial infarction and cardiovascular thrombotic events associated with the use of some selective COX-2 inhibitors was subsequently observed. These adverse cardiovascular effects, which are attributed to a decreased level of the vasodilatory prostacyclin (PGI_2) and an increased level of the potent platelet aggregator thromboxane A_2 (TxA_2), were primarily responsible for the recent withdrawal of rofecoxib (Vioxx®) and valdecoxib (Bextra®) from the market.[6]

Alternatively, LTs produced via the 5-LOX enzyme catalyzed pathway are known to play a role in the pathogenesis of inflammatory and allergic disorders.[1]

Keywords: Rofecoxib analogues; 3,4-Diaryl-2(5*H*)furanones; Anti-inflammatory activity; Oximes; Dual cyclooxygenase and lipoxygenase inhibitors.
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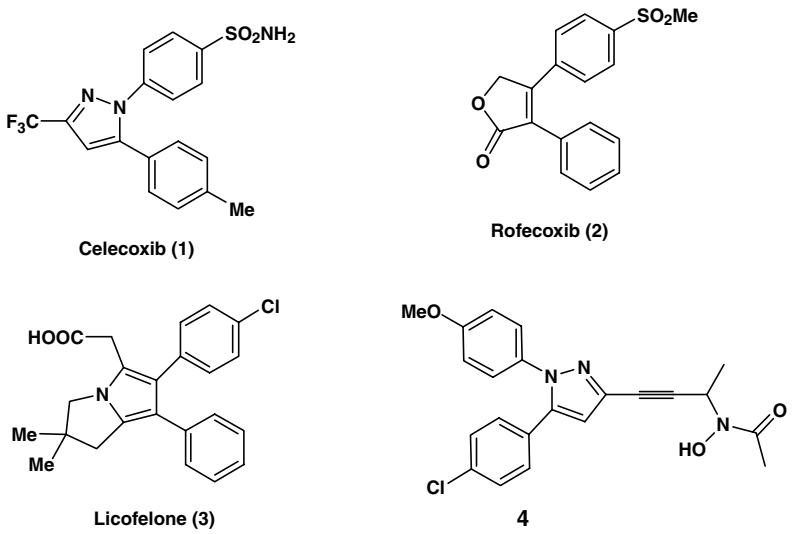


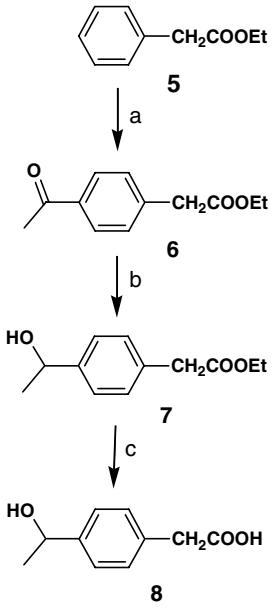
Figure 1. Some representative selective COX-2 inhibitors (**1**–**2**) and dual COX/LOX inhibitors (**3**–**4**).

The related isozyme 15-LOX is linked to cardiovascular complications since it is known to participate in oxidative modification of low-density lipoproteins (LDL) leading to the development of atherosclerosis.[7] In addition, the 5-LOX pathway is up-regulated during COX blockade. Accordingly, increased levels of LTs are potentially responsible for undesirable adverse effects such as bronchial constriction. Therefore, dual inhibitors of COXs and LOXs represent an attractive safer clinical alternative to selective COX-2 inhibitors in view of their potentially greater anti-inflammatory efficacy due to a synergistic block of both metabolic pathways in the AA cascade.[8] In this regard, the dual COX/LOX inhibitor licofelone (**3**), that has been evaluated in a Phase III clinical trial, is effective in the treatment of OA where it showed reduced GI toxicity compared to conventional NSAIDs.[9] An observation that arachidonoyl hydroxamate is a potent inhibitor of 5-LOX,[10] presumably because of chelation of the iron in the enzyme catalytic site, provided a strategy for the design of hydroxamic acid and *N*-hydroxyurea-based 5-LOX-selective inhibitors.[11] This mechanistic information was subsequently used for the rational design of dual inhibitors such as **4** which is comprised of a diarylpyrazole moiety present in selective COX-2 inhibitors such as celecoxib and an iron-chelating hydroxamic acid moiety present in 5-LOX inhibitors.[12]

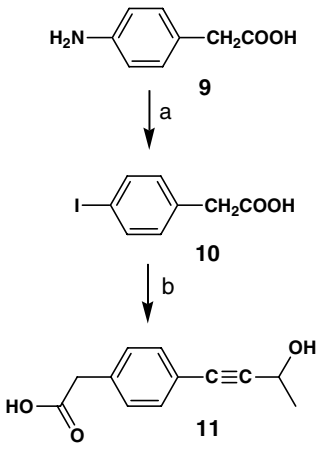
There is currently a renewed interest in the coordination chemistry of oximes that can bind a metal ion in different modes.[13] In some recent studies, it was shown that *N*-hydroxycarbamates exhibit effective 5-LOX-inhibitory activities.[14] It was therefore of interest to design a new class of hybrid compounds for evaluation as dual COX/LOX inhibitors, in which the selective COX-2 inhibitor rofecoxib was coupled to an oxime group or an *N*-hydroxycarbamate moiety. Accordingly, we now describe herein the synthesis and biological evaluation of a group of hybrid 4-(4-methanesulfonylphenyl)-3-phenyl-2(5*H*)furanone derivatives.

2. Chemistry

The synthetic strategies used to prepare the target 3, 4-diaryl-2(5*H*)furanones are shown in Schemes 1–4. Friedel–Crafts acetylation of ethyl phenylacetate (**5**) gave a mixture of the *meta*- and *para*-isomers, from which the *para*-isomer (**6**) crystallized from hexanes–acetone on cooling at 0 °C.[15] Subsequent reduction and hydrolysis of ethyl 4-acetylphenylacetate (**6**) afforded the 4-(1-hydroxyl)ethylphenylacetic acid (**8**) (Scheme 1). The phenylacetic acid analogue (**11**) was prepared in high yield using a dichlorobis(triphenylphosphine)palladium(0)/CuI catalyzed Sonogashira cross-coupling reaction[16] between 4-iodophenylacetic acid (**10**) and 3-butyn-2-ol in the presence of triethylamine (see Scheme 2). The 4-iodophenylacetic acid (**10**) precursor was prepared by diazo-



Scheme 1. Reagents and conditions: (a) AcCl, $AlCl_3$, CS_2, reflux overnight; (b) $NaBH_4$, MeOH, 25 °C, 1 h; (c) K_2CO_3, MeOH, H_2O, reflux, 30 min.

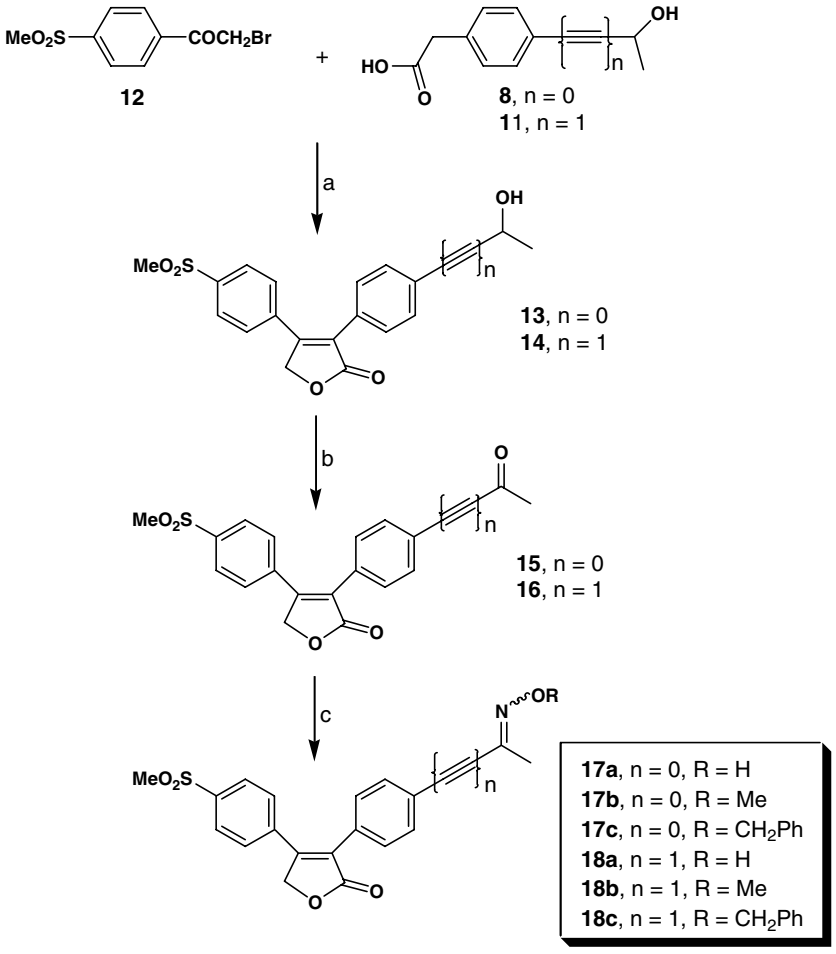


Scheme 2. Reagents and conditions: (a) i—H_2SO_4, $NaNO_2$, 0 °C, 30 min; ii—KI, 0 °C, 2.5 h; (b) 3-butyn-2-ol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 50 °C, overnight.

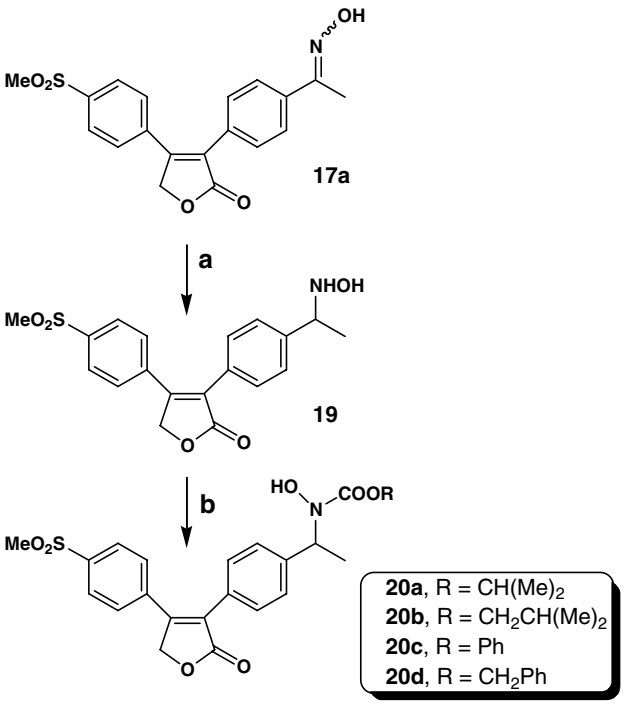
tization of commercially available 4-aminophenylacetic acid (**9**) and subsequent reaction with potassium iodide.

The target compounds (**15**–**18**) were prepared using the reaction sequence illustrated in Scheme 3. Reaction of the bromoketone (**12**) with a phenylacetic acid derivatives (**8** or **11**) proceeded via a 2-step condensation–cyclization reaction that was performed as a one-pot procedure.[17] Thus, treatment of a mixture of the bromoacetophone (**12**) and a phenylacetic acid derivative (**8** or **11**) in acetonitrile in the presence of triethylamine at 25 °C yielded the ester intermediate. Subsequent cooling to 0 °C, and addition of DBU, induced the cyclization reaction to afford the respective 3,4-diaryl-2(5*H*)furanone (**13** or **14**). Oxidation of the alcohols **13** and **14** afforded the target ketones (**15** and **16**) in 55–70% yield. Subsequent reaction of the ketones (**15** and **16**) with hydroxylamine hydrochloride, or an *O*-methyl- or *O*-benzyl derivative thereof, afforded the respective target oxime products (**17** and **18**) in 46–100% yield.

The *N*-hydroxycarbamates **20a**–**d** (R = *i*-Pr, *i*-Bu, Ph, CH_2Ph) were prepared employing the reaction sequence shown in Scheme 4. Thus, reduction of the oxime **17a** using sodium cyanoborohydride gave the intermediate hydroxylamine **19** which was treated with excess alkyl chloroformate to afford the respective *N*,*O*-bis(alkoxycarbonyl)hydroxylamine. Subsequent hydrolysis of the *N*,*O*-bis(alkoxycarbonyl)hydroxylamines in the presence of ammonia gas furnished the respective target compounds **20**.



Scheme 3. Reagents and conditions: (a) i—Et_3N, MeCN, 25 °C, 20 min; ii—DBU, 0 °C, 1 h; (b) Jones reagent, 0 °C, 1 h; (c) $H_2NOR{\cdot}HCl$, Na_2CO_3, EtOH, reflux, 1 h.



Scheme 4. Reagents and conditions: (a) $NaCNBH_3$, 12 N HCl, MeOH, THF, 25 °C, overnight; (b) i—ClCOOR (excess), Et_3N, CH_2Cl_2, 25 °C, overnight; ii—NH_3 (gas), THF, overnight.

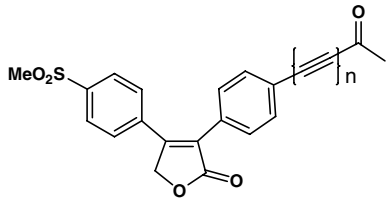
3. Results and discussion

A group of rofecoxib derivatives possessing an acetyl (**15**), 3-oxobut-1-ynyl (**16**), [hydroxy(or alkoxy)imino]alkyl (**17a–c**), [hydroxy(or alkoxy)imino]alkynyl (**18a–c**), or an *N*-alkoxy(or *N*-phenoxy)-*N*-alkylcarbonyl-*N*-hydroxy-*N*-ethylamino (**20a–d**) substituent at the *para*-position of the rofecoxib C-3 phenyl ring were evaluated in vitro to determine their COX-1, COX-2, 5-LOX, and 15-LOX inhibitory activities. In vitro COX-1 and COX-2 isozyme inhibition structure–activity studies (see data in Table 1) showed that the rofecoxib analogues possessing an additional acetyl or hydroxyiminoethyl substituent with or without an acetylene spacer (**15**, **16**, **17a**, and **18a**), are selective (COX-2 SI > 29–85), but not particularly potent (IC_{50} = 1.2–2.7 μM range), inhibitors of COX-2. Compounds having (methoxyimino)alkyl moieties at the *para*-position of the C-3 phenyl ring of rofecoxib (**17b** and **18b**) also showed modest inhibitory potency against COX-2 (**17b**, IC_{50} = 2.9 μM; **18b**, IC_{50} = 11.1 μM) with moderate COX-2 selective indexes (SI > 34) (**17b**) and >9 (**18b**). Incorporation of a bulky (benzyloxyimino)alkyl moiety (**17c** and**18c**) abolished both COX-1 and COX-2 inhibitory activity (IC_{50} values > 100 μM). On the other hand, rofecoxib analogues **20a–d** possessing an *N*-hydroxycarbamate moiety at the *para*-position of the rofecoxib C-3 phenyl ring exhibited equipotent COX-1 inhibitory activity (IC_{50}'s = 1.0 μM) in conjunction with moderate (**20a**, **20c–d**) to inactive (**20b**) COX-2 inhibition (IC_{50} values from 1.1 to >100 μM range).

In vitro 5-LOX and 15-LOX isozyme inhibition structure–activity studies (see data in Table 1) suggested that the structural requirements for 5-LOX inhibition are more stringent than for 15-LOX inhibition. The C-3 4-(1-hydroxyimino)ethylphenyl compound **17a**, an equipotent inhibitor of 5- and 15-LOX (5-LOX IC_{50} = 0.28 μM; 15-LOX IC_{50} = 0.32 μM), was about a 10-fold more potent inhibitor of both 5- and 15-LOX compared to the reference drugs caffeic acid (5-LOX IC_{50} = 3.0 μM) and luteolin (15-LOX IC_{50} = 3.2 μM). Replacement of an ethanone oxime moiety (**17a**) with an acetyl group (**15**) led to a complete loss of 5- and 15-LOX inhibitory activity. Compounds having a methoxyiminoethyl (**17b**), or *N*-benzyloxymethyl (**17c**), moiety exhibited selective 15-LOX inhibition (15-LOX IC_{50} = 1.1–3.0 μM). In contrast, the *N*-hydroxyliminoalkynyl compound **18a** exhibited 5-LOX selectivity (5-LOX IC_{50} = 0.30 μM; 15-LOX IC_{50} > 10 μM). Replacement of the hydroxyl substituent of the oxime moiety present in **18a** by a methoxy (**18b**), or benzyloxy (**18c**), substituent abolished both 5- and 15-LOX inhibitory activity (IC_{50}values > 10 μM). The 4-(3-oxobut-1-ynyl)phenyl compound (**16**) also showed potent dual 5- and 15-LOX inhibition (5-LOX IC_{50} = 2.0 μM; 15-LOX IC_{50} = 3.4 μM). Similar in vitro 5- and 15-LOX isozyme inhibition structure–activity relationships for the *N*-hydroxycarbamate derivatives of rofecoxib (**20a–d**) showed that the alkyl(aryl)oxy moiety present in the *N*-alkyl(aryl)oxycarbonyl-*N*-hydroxylamino moiety was a determinent of LOX inhibitory activity. In this regard, the *N*-isopropyloxy compound (**20a**) did not inhibit either 5- or 15-LOX at a concentration of 10 μM, whereas the isobutyloxy (**20b**) and phenoxy (**20c**) compounds exhibited potent 15-LOX inhibitory activity that is comparable to the reference drug luteolin (15-LOX IC_{50} = 3.2 μM). In contrast, the benzyloxy derivative (**20d**) exhibited selective 5-LOX inhibitory activity comparable to the reference drug caffeic acid (5-LOX IC_{50} = 3.0 μM). The in vitro COX-1/COX-2, and 5-LOX/15-LOX, isozyme inhibition data acquired for the group of compounds **15–18**, **20** showed that 3-[4-(1-hydroxyimino)ethylphenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**17a**) exhibited an optimal combination of COX and LOX inhibition (COX-1 IC_{50} > 100 μM; COX-2 IC_{50} = 1.4 μM; COX-2 SI > 71; 5-LOX IC_{50} = 0.28 μM; 15-LOX IC_{50} = 0.32 μM). Although **17a** is a 3-fold less potent inhibitor of COX-2 than the reference drug rofecoxib, it is a 10-fold more potent inhibitor of both 5-LOX and 15-LOX compared to the reference drugs caffeic acid (5-LOX IC_{50} = 3.0 μM) and luteolin (15-LOX IC_{50} = 3.2 μM).

A molecular modeling study was performed where the most selective COX-2 inhibitor **17a** was docked in the binding site of the COX-2 isozyme (Fig. 2). This molecular modeling study reveals that **17a** binds in the center of the primary binding site such that the C-4 *para*-SO_2Me COX-2 pharmacophore is oriented in the vicinity of amino acid residues lining the COX-2 secondary (2°) pocket (His90, Gln192, Ala516, Ile517, Phe518, and Val523). One of the *O*-atoms of the SO_2Me group undergoes a dual hydrogen bonding interaction with the backbone amide hydrogens (N*H*) of both Ile517 (distance ≈ 1.96 Å) and Phe518 (distance ≈ 2.42 Å). The distance between the second *O*-atom (of the SO_2Me moiety) and the NH_2 of Gln192 is about 4.42 Å.The Me

Table 1. In vitro COX-1, COX-2, 5-LOX, and 15-LOX enzyme inhibition assay data for the rofecoxib analogues **15**, **16**, **17**, **18**, and **20**



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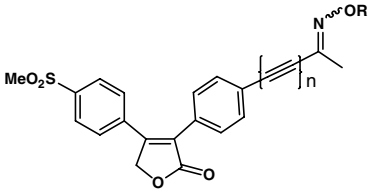

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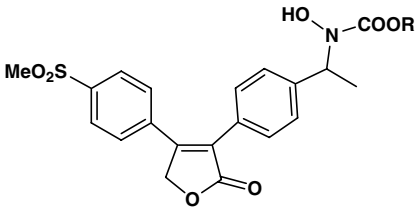


20

Compound	*n*	R	IC_{50}[a] (μM)		Selectivity index[b] (SI)	IC_{50}[a] (μM)	
			COX-1	COX-2		5-LOX	15-LOX
15	0	—	>100	1.2	>85	>10	>10
16	1	—	>100	3.5	>29	2.0	3.4
17a	0	H	>100	1.4	>71	0.28	0.32
17b	0	CH_3	>100	2.9	>33.8	>10	1.1
17c	0	CH_2Ph	>100	>100	—	>10	3.1
18a	1	H	>100	2.7	>37	0.30	>10
18b	1	CH_3	>100	11.1	>9	>10	>10
18c	1	CH_2Ph	>100	>100	—	>10	>10
20a	—	$CH(Me)_2$	1.0	6.6	0.15	>10	>10
20b	—	$CH_2CH(Me)_2$	1.0	>100	0.01	>10	3.2
20c	—	Ph	1.0	1.1	0.93	>10	3.4
20d	—	CH_2Ph	1.0	12.0	0.08	3.1	>10
Luteolin						—	3.2
Caffeic acid						3.0	—
NDGA[c]						>10	3.5
Celecoxib			33.1	0.07	472	—	—
Rofecoxib			>100	0.5	>200	—	—

[a] Values are means of two determinations acquired using an ovine COX-1 and COX-2, potato 5-LOX, and 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] NDGA, nordihydroguaiaretic acid.

group of the SO_2Me moiety is within van der Waals contact range of Ala516 (distance ≈ 3.37 Å). The C-3 *p*-C(Me)=N–OH substituted phenyl ring is oriented toward a hydrophobic pocket comprised of Phe381, Leu384, Tyr385, Trp387, and Met522. It is interesting to note that the N–O*H* [of the C(Me)=N–OH moiety] participates in a hydrogen bonding interaction with the C=O of Leu384 (distance ≈ 1.84 Å), whereas the Me substituent [of the C(Me)=N–OH moiety] is positioned within van der Waals contact range of Leu384 and Met522 (distance <5 Å) at the apex of the COX-2 binding site. It is noteworthy that the C=O of the central furanone ring forms a hydrogen bond with the O*H* of Ser530 (distance ≈ 1.91 Å), the acetylation site for aspirin. A recent study has shown the importance of Ser530 in the COX-2 inhibitory activity of rofecoxib.[18] In addition, the *O*-atom of the central furanone is separated by a distance of 5.77 Å from the NH_2 of Arg120, and about 5.67 Å from the O*H* of Tyr355, near the entrance to the COX-2 binding site.

A molecular modeling (docking) simulation was performed to investigate the binding interaction of the dual 5- and 15-LOX selective inhibitor, 3-[4-(1-hydroxyimino)ethylphenyl]-4-(4-methanesulfonyl-phenyl)-2(5*H*)-furanone (**17a**), within the 15-LOX binding site (Fig. 3). The C-4 *p*-$MeSO_2$-phenyl COX-2 pharmacophore is oriented toward the entrance to the 15-LOX binding site (Arg403). The Me group of the SO_2Me moiety is located within van der Waals contact range of the amino acid residues Ile400, Ala404, and Leu408 (distance < 5 Å). The interspatial distance between one of the *O*-atoms of the SO_2Me group and the NH_2 of Arg403 is about 7.79 Å. It is interesting to note that the C-3 phenyl ring possessing the C(Me)=N–OH LOX pharmacophore is oriented toward the catalytic center of 15-LOX (His361, His366, His541, and His545). The O*H* substituent present in the –C(Me)=N–O*H* moiety forms a hydrogen bond with the peptide backbone (C=O) of His361 (distance ≈ 1.72 Å), whereas the *N*-atom of the C(Me)=*N*–OH] group forms a weak hydrogen bond with the imidazole N*H* of His366 (distance ≈ 3.57 Å). In addition, the C-3 phenyl ring is involved in a π–π stacking interaction with the imidazole ring of His361. The central furanone ring is oriented toward the base of the 15-LOX binding site where it is positioned in a hydrophobic pocket comprised of Gln548, Leu549, Val594, and Gly598 (distance < 5 Å). Interestingly, the C=O of central furanone ring is hydrogen bonded to the backbone N*H* of Leu549 (distance ≈ 2.85 Å).

The COX-2, and dual 5-LOX and 15-LOX, inhibitor 3-[4-(1-hydroxyimino)ethylphenyl]-4-(4-methanesulfo-

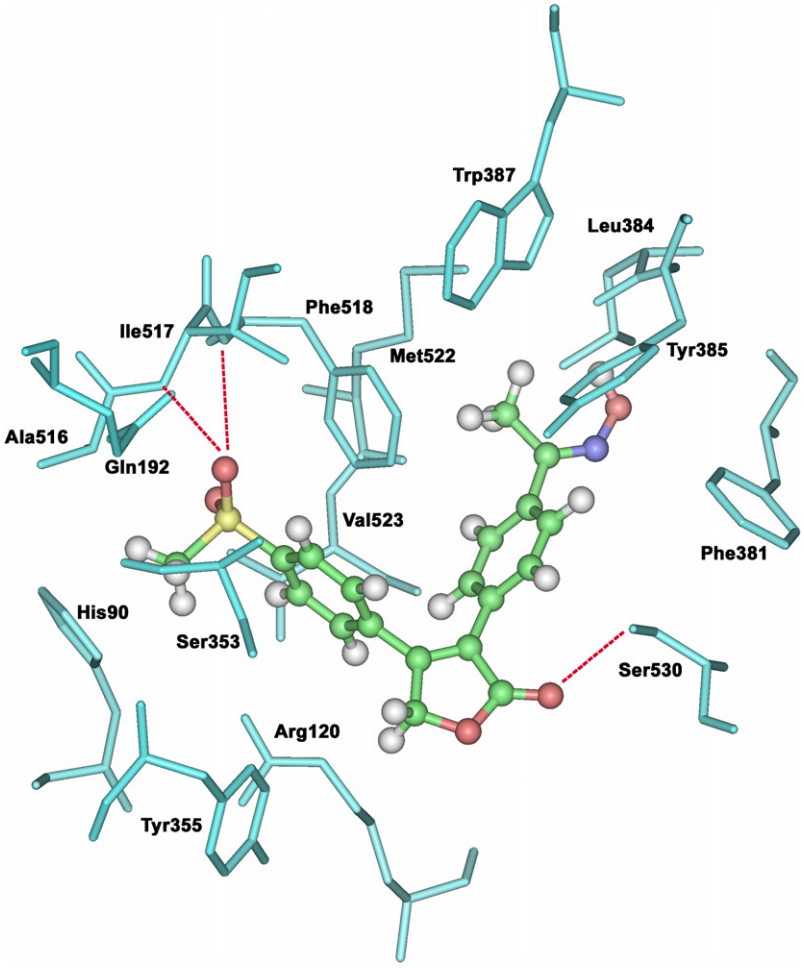


Figure 2. Docking of 3-[4-(1-hydroxyimino)ethylphenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**17a**) (ball and stick) in the active site of murine COX-2. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

nylphenyl)-2(5*H*)furanone (**17a**), and the dual COX-2/5-LOX inhibitor 3-[4-(3-hydroxyiminobut-1-ynyl)phenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**18a**), based on their desirable in vitro COX and LOX isozyme inhibition effects, 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, these two rofecoxib oxime analogues exhibited more potent anti-inflammatory activities (25–33% inhibition of inflammation for a 30 mg/kg oral dose), being superior to the respective 15-LOX inhibitor nordihydroguiaretic acid (NDGA, 15.1% inhibition for a 30 mg/kg dose), and the 5-LOX inhibitor caffeic acid (8.2% inhibition of inflammation for a 30 mg/kg oral dose), reference drugs. However, **17a** and **18a** were less potent anti-inflammatory agents than the selective COX-2 inhibitor reference drug celecoxib (ED_{50} = 10.8 mg/kg).

In a rat 4% NaCl-induced abdominal constriction (analgesic) assay, a 30 mg oral dose of the oximes **17a** and **18a** exhibited moderate analgesic activities (37–58% range) comparable 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 hybrid compounds was designed for evaluation as dual acting COX/LOX inhibitors where the selective COX-2 inhibitor rofecoxib was attached to a 5-LOX inhibitory oxime or *N*-hydroxycarbamate moiety. In vitro enzyme inhibition, and in vivo anti-inflammatory and analgesic, data acquired show that (i) incorporation of a *para*-oxime moiety on the C-3 phenyl ring of rofecoxib provides a suitable template for the design of dual acting inhibitors of COX and LOX; (ii) the oxime compound **17a** exhibits an optimal combination of COX-2/5-LOX/15-LOX inhibitory activities; (iii) the oxime compound **18a** is an effective dual inhibitor of COX-2 and 5-LOX; and (iv) dual inhibitors of COX-2 and 5-/15-LOX such as **17a** and **18a** possess oral anti-inflammatory and analgesic activities that may be clinically relevant.

5. Experimental

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet 550 Series II Magna

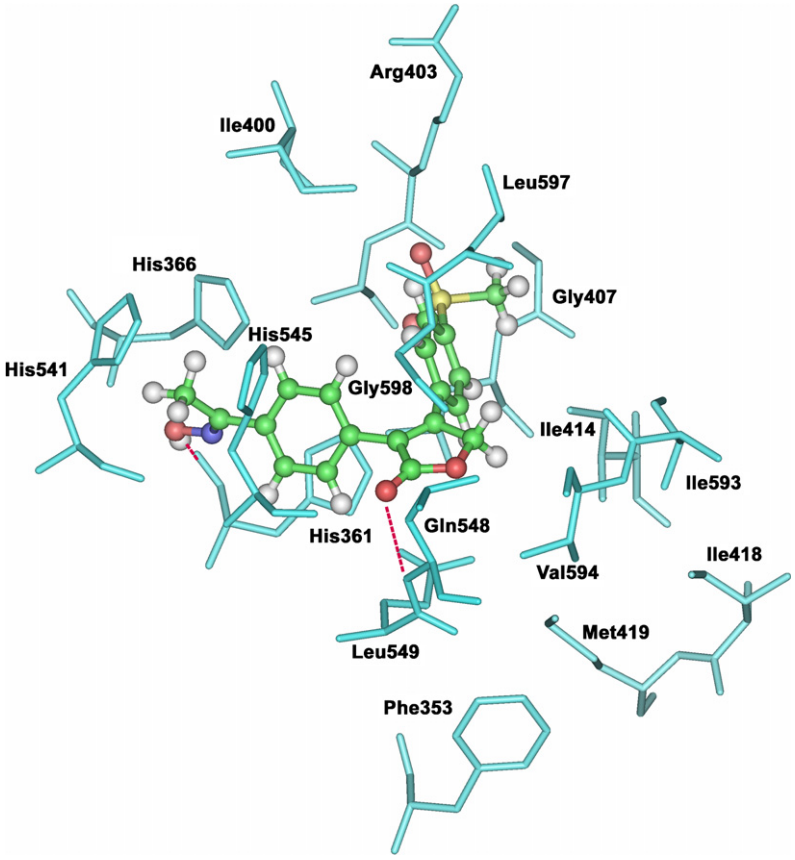
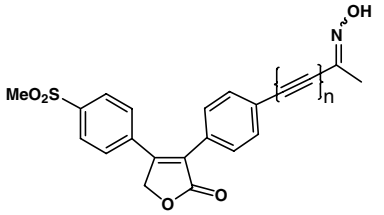


Figure 3. Docking of 3-[4-(1-hydroxyimino)ethylphenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**17a**) (ball and stick) in the active site of soyabean 15-LOX. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

Table 2. In vivo anti-inflammatory and analgesic activities for 3-[4-(1-hydroxyimino)ethylphenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**17a**) and 3-[4-(3-hydroxyiminobut-1-ynyl)phenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**18a**)



Compound	*n*	AI activity[a]	Analgesic activity[b]	
		% inhibition (3 h)	% inhibition (30 min)	% inhibition (60 min)
17a	0	33.7 ± 1.7	37.1 ± 7.6	49.0 ± 13.1
18a	1	25.5 ± 4.8	58.3 ± 10.0	52.2 ± 8.2
Caffeic acid	—	8.2 ± 2.5	47.2 ± 10.6	58.3 ± 12.8
NDGA	—	15.1 ± 1.7	45.8 ± 9.2	62.5 ± 4.8
Celecoxib	—	79.9 ± 1.9[c,d]	31.7 ± 9.6[c]	62.0 ± 7.3[c]

[a] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as means ± SEM ($n = 4$) values at 3 h following a 30 mg/kg oral dose of the test compound.
[b] Inhibitory activity in the rat 4% NaCl-induced abdominal constriction assay. The results are expressed as means ± SEM ($n = 4$–5) values following a 30 mg/kg oral dose of the test compound.
[c] Percent (%) reduction for a 50 mg/kg oral dose.
[d] $ED_{50} = 10.8$ mg/kg.

FT-IR spectrometer. ^{1}H NMR spectra were measured on a Bruker AM-300 spectrometer in $CDCl_3$ or $CDCl_3$ + DMSO-$d_6$with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz. Spin multiples are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Microanalyses were performed for C, H, N (MicroAnalytical Service Laboratory, Department of Chemistry, University of Alberta) and were within ±0.4% of theoretical values. Nominal mass, positive polarity, electro-

spray spectra were acquired using a Waters Micromass ZQ mass spectrometer. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70–230 mesh). Celecoxib and rofecoxib were synthesized according to the literature procedures.[4a,19] Luteolin, caffeic acid, and nordihydroguaiaretic acid (NDGA) were purchased from Cayman Chemicals Inc. Ann Arbor. Ethyl 4-(1-hydroxyethyl)phenylacetate (**7**)[20] and 4-(1-hydroxyethyl)phenylacetic acid (**8**)[21] have been reported previously. Bromoketone **12** was prepared according to a literature method.[22] Other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification. Male Sprague–Dawley rats, used in the anti-inflammatory and 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. Ethyl 4-acetylphenylacetate (6)

Ethyl phenylacetate (6.5 g, 40 mmol), and then acetyl chloride (3.8 ml, 53 mmol), was added slowly to a suspension of anhydrous $AlCl_3$ powder (12 g, 90 mmol) in CS_2 (25 mL) at 0 °C with stirring. The temperature was slowly raised and finally the reaction was allowed to proceed at reflux overnight. The reaction mixture was cooled to 25 °C, poured into a mixture of ice and water, and the subsequent mixture was extracted with chloroform (3 × 50 mL). The combined extracts were washed with water (2 × 50 mL) and dried (Na_2SO_4). Removal of the solvent in vacuo gave a dark brown oil which was purified by silica gel column chromatography eluting with hexanes–acetone (5:1, v/v) to furnish a mixture of the *para*- and *meta*-isomers. This mixture, which partially solidified on standing in a refrigerator, was recrystallized from hexanes–acetone to give the *para*-isomer (2.64 g, 32%) as white needles; mp 58–59 °C (lit.[15] 64–65 °C). IR (film): 1736 (COO), 1676 (C=O), 1615, 1474 (aromatic) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.26 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 2.60 (s, 3H, $COCH_3$), 3.68 (s, 2H, CH_2), 4.17 (q, $J = 7.0$ Hz, 2H, CH_2CH_3), 7.39 (d, $J = 8.5$ Hz, 2H, phenyl H-2, H-6), 7.93 (d, $J = 8.5$ Hz, 2H, phenyl H-3, H-5).

5.2. Ethyl 4-(1-hydroxy)ethylphenylacetate (7)

Sodium borohydride (380 mg, 10 mmol) was added to a solution of ethyl 4-acetylphenylacetate (**6**, 412 mg, 2 mmol) in methanol (20 mL), the reaction was allowed to proceed at 25 °C for 1 h with stirring, and the solvent was removed in vacuo. Ethyl acetate (50 mL) and water (80 mL) were added, and the mixture was extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined, washed with brine (3 × 50 mL), and the organic fraction was dried (Na_2SO_4). Removal of the solvent in vacuo gave **7** as a colorless oil (366 mg, 88%). IR (film): 3422 (*OH*), 1743 (*CO*O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.26 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 1.49 (d, $J = 6.4$ Hz, 3H, $CHCH_3$), 3.61 (s, 2H, CH_2), 4.15 (q, $J = 7.0$ Hz, 2H, CH_2CH_3), 4.91 (q, $J = 6.4$ Hz, 1H, $CHCH_3$), 7.27 (d, $J = 8.2$ Hz, 2H, phenyl H-3, H-5), 7.35 (d, $J = 8.2$ Hz, 2H, phenyl H-2, H-6).

5.3. 4-(1-Hydroxy)ethylphenylacetic acid (8)

A solution of K_2CO_3 (704 mg, 5.1 mmol) in water (10 mL) was added to a stirred solution of ethyl 4-(1-hydroxyl)ethylphenylacetate (**7**, 353 mg, 1.7 mmol) in methanol (10 mL). The reaction was allowed to proceed at reflux for 30 min, the reaction mixture was cooled to 25 °C, and the solvent was removed in vacuo. Ethyl acetate (30 mL) and water (20 mL) were added, and the mixture was acidified to pH 3 using an aqueous solution of HCl (5% w/v) prior to extraction with ethyl acetate (3 × 30 mL). The organic extracts were combined, washed with brine, and the organic fraction was dried (Na_2SO_4). Removal of the solvent under vacuum furnished **8** as a white solid (219 mg, 87%); mp 115–116 °C. IR (film): 3422 (OH), 2515–3106 (CO*OH*), 1709 (*CO*OH) cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6; 5:1, v/v) δ 1.41 (d, $J = 6.0$ Hz, 3H, $CHCH_3$), 3.53 (s, 2H, CH_2), 4.81 (q, $J = 6.0$ Hz, 1H, $CHCH_3$), 7.21 (d, $J = 8.0$ Hz, 2H, phenyl H-3, H-5), 7.29 (d, $J = 8.0$ Hz, 2H, phenyl H-2, H-6).

5.4. 4-Iodophenylacetic acid (10)

A solution of sodium nitrite (828 mg, 12 mmol) in water (3 mL) was added slowly with stirring at 0 °C to a solution of 4-aminophenylacetic acid (1.51 g, 10 mmol) in water (15 mL) and sulfuric acid (2 mL), and the reaction mixture was stirred for 30 min prior to the addition of a cooled solution of KI (3.32 g, 20 mmol) in water (12 mL). The reaction was allowed to proceed for an additional 2.5 h at 0 °C, the dark brown mixture was extracted with ethyl acetate (3 × 50 mL), the combined organic extracts were washed successively with 5% HCl (2 × 20 mL), and then saturated sodium thiosulfate solution (2 × 50 mL), the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography using hexanes–acetone (3:1, v/v) as eluent to afford **10** (1.81 g, 69%) as a dull white powder; mp 138–140 °C (lit.[23] 138–140 °C). IR (film): 2589–3227 (CO*OH*), 1696 (*CO*OH) cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.60 (s, 2H, CH_2), 7.04 (d, $J = 8.2$ Hz, 2H, phenyl H-2, H-6), 7.67 (d, $J = 8.2$ Hz, 2H, phenyl H-3, H-5).

5.5. 4-(3-Hydroxybut-1-ynyl)phenylacetic acid (11)

Cuprous iodide (15 mg, 0.08 mmol) and dichlorobis(triphenylphosphine)palladium (27 mg, 0.04 mmol) were added to a solution of a 4-iodophenylacetic acid (**10**, 338 mg, 1.29 mmol) and 3-butyn-2-ol (135 mg, 1.93 mmol) in Et_3N (8 mL). The reaction was allowed to proceed overnight with stirring at 50 °C under an argon atmosphere, cooled to 25 °C, and then Et_3N was removed under reduced pressure. The residue obtained was purified by silica gel column chromatography using hexanes–acetone (2:1, v/v) as the eluent to furnish **11** (240 mg, 91%) as a pale yellow powder; mp 122–124 °C. IR (film): 3380 (OH), 2520–3020 (CO*OH*), 1703 (*CO*OH) cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6; 5:1, v/v) δ 1.44 (d, $J = 6.7$ Hz, 3H, $CHCH_3$), 3.50 (s, 2H, CH_2), 4.63 (q, $J = 6.7$ Hz, 1H, $CHCH_3$), 7.15 (d, $J = 7.0$ Hz, 2H, phenyl H-3, H-5), 7.28 (d, $J = 7.0$ Hz, 2H, phenyl H-2, H-6).

5.6. General procedure for the synthesis of 4-(4-methanesulfonylphenyl)-3-(4-substituted-phenyl)-2(5*H*)furanones (13 and 14)

Et_3N (0.38 mL, 2.75 mmol) was added to a solution of the bromoketone (**12**, 692 mg, 2.5 mmol) and a 4-substituted-phenylacetic acid (**8** or **11**) in acetonitrile (30 mL). This solution was stirred at 25 °C for 30 min, cooled to 0 °C, and then 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.56 mL, 3.75 mmol) was added. The reaction temperature was maintained at 0 °C for an additional 50 min with stirring, and the reaction mixture was acidified with 5% HCl (w/v) until a color change from dark brown to light yellow was observed. Ice water (30 mL) was added, stirring was continued for a few minutes, and the mixture was extracted with EtOAc (3×30 mL). The combined extracts were washed with brine (30 mL), dried (Na_2SO_4), and the solvent from the organic fractions was removed in vacuo. The residue obtained was subjected to silica gel column chromatography eluting with chloroform–acetone (8:1, v/v) to provide the respective title compound.

5.6.1. 3-[4-(1-Hydroxy)ethylphenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (13). Yield, 26%; pale yellow foam; mp 64–66 °C. IR (film): 3489 (OH), 1743 (furanone CO), 1300, 1152 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.52 (d, $J = 6.4$ Hz, 3H, CHCH_3), 3.08 (s, 3H, SO_2CH_3), 4.95 (q, $J = 6.4$ Hz, 1H, CHCH$_3$), 5.20 (s, 2H, furanone CH_2), 7.37–7.44 (AA′BB′ system, $J = 8.5$ Hz, 4H, 4-(1-hydroxyl)ethylphenyl H-2, H-3, H-5, H-6), 7.53 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.93 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) *m*/*z*: Calcd for $C_{19}H_{18}O_5S$ (MH^+): 359.09. Found: 358.98.

5.6.2. 3-[4-(3-Hydroxybut-1-ynyl)phenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (14). Yield, 66%; pale yellow foam; mp 96–98 °C. IR (film): 3481 (OH), 2220 (C ≡ C), 1754 (furanone CO), 1318, 1145 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.56 (d, $J = 6.7$ Hz, 3H, CHCH_3), 3.08 (s, 3H, SO_2CH_3), 4.77 (q, $J = 6.7$ Hz, 1H, CHCH$_3$), 5.19 (s, 2H, furanone CH_2), 7.36 (d, $J = 8.2$ Hz, 2H, 4-(3-hydroxybut-1-ynyl)phenyl H-2, H-6), 7.45 (d, $J = 8.2$ Hz, 2H, 4-(3-hydroxybut-1-ynyl)phenyl H-3, H-5), 7.50 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.93 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) *m*/*z*: Calcd for $C_{21}H_{18}O_5S$ (MH^+): 383.09. Found: 382.91.

5.7. General procedure for the synthesis of 3-[4-acetyl and 4-(3-oxobut-1-ynyl)phenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanones (15 and 16)

Jones reagent (0.68 mL, 1.8 mmol) was added dropwise to a solution of a 2(5*H*)furanone (**13** or **14**, 0.36 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. EtOAc (100 mL) was added, the resulting mixture was washed with brine (2×30 mL), the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo. Purification of the residue obtained via silica gel column chromatography using dichloromethane–acetone (9:1, v/v) as eluent gave the respective product **15** or **16**. Physical, spectral, and microanalytical data for **15** and **16** are listed below.

5.7.1. 3-(4-Acetylphenyl)-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (15). Yield, 70%; pale yellow crystals; mp 196–197 °C. IR (film): 1756 (furanone CO), 1313, 1159 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.62 (s, 3H, COCH_3), 3.09 (s, 3H, SO_2CH_3), 5.23 (s, 2H, furanone CH_2), 7.52 and 7.53 (two d, $J = 8.2$ Hz, 2H each, 4-methanesulfonylphenyl H-2, H-6, 4-acetylphenyl H-2, H-6), 7.95 and 7.97 (two d, $J = 8.2$ Hz, 2H each, 4-methanesulfonylphenyl H-3, H-5, 4-acetylphenyl H-3, H-5); MS (ES+) *m*/*z*: Calcd for $C_{19}H_{16}O_5S$ (MH^+): 357.07. Found: 356.93; Anal. Calcd for $C_{19}H_{16}O_5S$: C, 64.03; H, 4.53. Found: C, 64.08; H, 4.47.

5.7.2. 4-(4-Methanesulfonylphenyl)-3-[4-(3-oxobut-1-ynyl)phenyl]-2(5*H*)furanone (16). Yield, 55%; pale yellow foam; mp 88–90 °C. IR (film): 2200 (C ≡ C), 1745 (furanone CO), 1682 (CO), 1327, 1154 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H, COCH_3), 3.09 (s, 3H, SO_2CH_3), 5.22 (s, 2H, furanone CH_2), 7.45 (d, $J = 8.5$ Hz, 2H, 4-(3-oxobut-1-ynyl)phenyl H-2, H-6), 7.51 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.60 (d, $J = 8.5$ Hz, 2H, 4-(3-oxobut-1-ynyl)phenyl H-3, H-5), 7.96 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) *m*/*z*: Calcd for $C_{21}H_{16}O_5S$ (MH^+): 381.07. Found: 380.99; Anal. Calcd for $C_{21}H_{16}O_5S$: C, 66.30; H, 4.24. Found: C, 66.60; H, 4.30.

5.8. General procedure for the synthesis of 3-(4-alkyloxyiminoalkylphenyl)-4-(4-methanesulfonylphenyl)-2(5*H*)furanones (17 and 18)

Sodium carbonate (255 mg, 2.4 mmol) was added to a stirred solution of the 3-(4-acetyl or 4-acetylethynylphenyl)-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**15** or **16**, 0.3 mmol) and the respective hydroxylamine hydrochloride ($H_2NOR{\cdot}HCl$, R = H, Me, CH_2Ph; 2.4 mmol) in anhydrous ethanol (10 mL). The mixture was refluxed for 1 h, cooled to 25 °C, and H_2O (60 mL) was added prior to extraction with EtOAc (3×50 mL). The combined extracts were washed successively with 5% HCl (30 mL) and water (30 mL), the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo. The crude product was purified by silica gel column chromatography employing dichloromethane–acetone (10:1, v/v) as eluent to furnish the respective title compounds. Physical, spectral, and microanalytical data for **17a–c** and **18a–c** are listed below.

5.8.1. 3-[4-(1-Hydroxyimino)ethylphenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (17a). Yield, 46.5%; pale yellow solid; mp 214–216 °C. IR (film): 3361 (OH), 1750 (furanone CO), 1320, 1145 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.33 (s, 3H, N=C–CH_3), 3.09 (s, 3H, SO_2CH_3), 5.21 (s, 2H, furanone CH_2), 7.45 (d, $J = 8.5$ Hz, 2H, (1-hydroxyimino)ethylphenyl H-2, H-6), 7.52 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.67 (d, $J = 8.5$ Hz, 2H, (1-hydroxyimino)ethylphenyl H-3, H-5), 7.95 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) *m*/*z*:

Calcd for $C_{19}H_{17}NO_5S$ (MH^+): 372.09. Found: 371.90; Anal. Calcd for $C_{19}H_{17}NO_5S$: C, 61.44; H, 4.61; N, 3.77. Found: C, 61.62; H, 4.58; N, 4.00.

5.8.2. 4-(4-Methanesulfonylphenyl)-3-[4-(1-methoxyimino)ethylphenyl]-2(5*H*)furanone (17b). Yield, 61.5%; yellow oil. IR (film): 1750 (furanone CO), 1326, 1152 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.24 (s, 3H, N=C–CH_3), 3.09 (s, 3H, SO_2CH_3), 4.01 (s, 3H, OCH_3), 5.20 (s, 2H, furanone CH_2), 7.43 (d, J = 8.5 Hz, 2H, 4-(1-methoxyimino)ethylphenyl H-2, H-6), 7.52 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.69 (d, J = 8.5 Hz, 2H, 4-(1-methoxyimino)ethylphenyl H-3, H-5), 7.94 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) m/z: Calcd for $C_{20}H_{19}NO_5S$ (MH^+): 386.10. Found: 385.98; Anal. Calcd for $C_{20}H_{19}NO_5S{\cdot}1/4H_2O$: C, 61.60; H, 5.04; N, 3.59. Found: C, 61.87; H, 4.94; N, 3.72.

5.8.3. 3-[4-(1-Benzyloxyimino)ethylphenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (17c). Yield, 65%; yellow oil. IR (film): 1756 (furanone CO), 1320, 1152 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.28 (s, 3H, CNCH_3), 3.08 (s, 3H, SO_2CH_3), 5.20 (s, 2H, furanone CH_2), 5.26 (s, 2H, benzyl CH_2), 7.29–7.42 (m, 7H, 4-(1-benzyloxyimino)ethylphenyl H-2, H-6, benzyl phenyl hydrogens), 7.52 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.68 (d, J = 8.5 Hz, 2H, 4-(1-benzyloxyimino)ethylphenyl H-3, H-5), 7.93 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) m/z: Calcd for $C_{26}H_{23}NO_5S$ (MH^+): 462.13. Found: 462.01; Anal. Calcd for $C_{26}H_{23}NO_5S$: C, 67.66; H, 5.02; N, 3.03. Found: C, 67.52; H, 5.01; N, 3.25.

5.8.4. 3-[4-(3-Hydroxyiminobut-1-ynyl)phenyl]-4-(4- methanesulfonylphenyl)-2(5*H*)furanone (18a). Yield, 100%; pale yellow powder; mp 160–162 °C. IR (film): 3325 (OH), 2252 (C ≡ C), 1750 (furanone CO), 1307, 1150 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.50 (s, 3H, N=C–CH_3), 3.09 (s, 3H, SO_2CH_3), 5.22 (s, 2H, furanone CH_2), 6.33 (br s, 1H, OH), 7.50 and 7.57 (two d, J = 8.5 Hz, 2H each, 4-methanesulfonylphenyl H-2, H-6, 4-(3-hydroxyiminobut-1-ynyl)phenyl H-2, H-6), 7.81 (d, J = 8.5 Hz, 2H, 4-(3-hydroxyiminobut-1-ynyl)phenyl H-3, H-5), 7.95 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) m/z: Calcd for $C_{21}H_{17}NO_5S$ (MH^+): 396.09. Found: 395.90; Anal. Calcd for $C_{21}H_{17}NO_5S$: C, 63.79; H, 4.33; N, 3.54. Found: C, 63.40; H, 4.37; N, 3.54.

5.8.5. 4-(4-Methanesulfonylphenyl)-3-[4-(3-methoxyiminobut-1-ynyl)phenyl]-2(5*H*)furanone (18b). Yield, 88%; yellow oil. IR (film): 2220 (C ≡ C), 1756 (furanone CO), 1313, 1145 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.10 and 2.14 (two s, 3H total, N=C–CH_3), 3.09 (s, 3H, SO_2CH_3), 3.98, 4.00 (s, 3H, OCH_3), 5.20 (s, 2H, furanone CH_2), 7.38–7.57 (m, 6H, 4-methanesulfonylphenyl H-2, H-6, 4-(3-methoxyiminobut-1-ynyl)phenyl H-2, H-3, H-5, H-6), 7.95 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) m/z: Calcd for $C_{22}H_{19}NO_5S$ (MH^+): 410.11. Found: 409.98; Anal. Calcd for $C_{22}H_{19}NO_5S$: C, 64.53; H, 4.68; N, 3.42. Found: C, 64.38; H, 4.71; N, 3.53.

5.8.6. 3-[4-(3-Benzyloxyiminobut-1-ynyl)phenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (18c). Yield, 85.5%; pale yellow syrup. IR (film): 2226 (C ≡ C), 1750 (furanone CO), 1313, 1159 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.14 and 2.15 (two s, 3H total, N=C–CH_3), 3.08 (s, 3H, SO_2CH_3), 5.20 (s, 2H, furanone CH_2), 5.21 and 5.23 (two s, 2H total, benzyl CH_2), 7.30–7.56 (m, 11H, 4-methanesulfonylphenyl H-2, H-6, 4-(3-benzyloxyiminobut-1-ynyl)phenyl hydrogen, benzyl phenyl hydrogen), 7.95 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) m/z: Calcd for $C_{28}H_{23}NO_5S$ (MH^+): 486.14. Found: 485.96; Anal. Calcd for $C_{28}H_{23}NO_5S{\cdot}1/2H_2O$: C, 68.00; H, 4.89; N, 2.83. Found: C, 68.25; H, 4.73; N, 3.11.

5.9. General procedure for the synthesis of *N*-alkoxycarbonyl-*N*-hydroxylamines (20)

$NaBH_3CN$ (3.0 g, 47.6 mmol) and a trace of methyl orange were added to a solution of 3-[4-(1-hydroxyimino)ethylphenyl]-4-(4-methanesulfonylphenyl)-2(5*H*)furanone (**17a**, 2.4 g, 6.5 mmol) in methanol (500 mL) and THF (100 mL). The reaction mixture was stirred at 25 °C for 5 min prior to the addition of 12 N HCl that was added dropwise until the color remained pink. The reaction mixture was maintained at 25 °C for 12 h with stirring. After removal of the organic solvents, water was added (200 mL), the mixture was acidified to pH 3 using 5% w/v HCl, and then washed with EtOAc (3 × 150 mL). The aqueous fraction was basified to pH 9 with sodium carbonate, extracted with EtOAc (3 × 150 mL), the combined extracts were dried (Na_2SO_4), and the organic solvent was removed in vacuo to provide the hydroxylamine **19** (1.5 g, 72%) as a white foam which was used immediately without further purification for the preparation of the *N*-hydroxycarbamates (**20**).

Under an argon atmosphere, the hydroxylamine (**19**, 150 mg, 0.4 mmol) obtained above was dissolved in anhydrous dichloromethane (8 mL), Et_3N (0.09 mL, 0.52 mmol) and an alkyl chloroformate (2.0 mmol) were added. This reaction was allowed to proceed with stirring at 25 °C for 12 h, and the organic solvent was removed in vacuo. The residue was dissolved in THF (20 mL), this solution was stirred under a stream of gaseous ammonia for 30 min at 25 °C prior to capping the reaction flask, and the reaction was allowed to proceed at 25 °C for 12–16 h with stirring. The THF solvent was removed in vacuo, and the residue was purified by silica gel column chromatography eluting with chloroform–acetone to furnish the respective *N*-hydroxycarbamate (**20a–d**). Physical and spectral data for **20a–d** are listed below.

5.9.1. *N*-Isopropyloxycarbonyl-*N*-{1-[4-(4-methanesulfonylphenyl)-2(5*H*)furanon-3-yl]phenyl}ethylhydroxylamine (20a). Yield, 18%; pale yellow foam; mp 83–85 °C. IR (film): 3368 (OH), 1750 (furanone CO), 1689 (carbamate CO), 1306, 1145 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.27 and 1.31 [two d, J = 6.4 Hz, 6H total, $CH(CH_3)_2$], 1.63 (d, J = 7.0 Hz, 3H, $CHCH_3$), 3.09 (s, 3H, SO_2CH_3), 5.00 [septet, J = 6.4 Hz, 1H, $CH(CH_3)_2$], 5.20 (s, 2H, furanone CH_2), 5.30 (q, J = 7.0 Hz, 1H, $CHCH_3$), 7.38 and 7.43 (AA′BB′ system, J = 8.0 Hz, 4H total, disubstituted-

phenyl hydrogens), 7.53 (d, $J = 7.9$ Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.94 (d, $J = 7.9$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) *m/z*: Calcd for $C_{23}H_{25}NO_7S$ (MH^+): 460.14. Found: 460.01.

5.9.2. *N*-Isobutyloxycarbonyl-*N*-{1-[4-(4-methanesulfonylphenyl)-2(5*H*)furanon-3-yl]phenyl}ethylhydroxylamine (20b). Yield, 13%; pale yellow powder; mp 97–99 °C. IR (film): 3355 (OH), 1757 (furanone CO), 1703 (carbamate CO), 1315, 1142 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.92 (d, $J = 6.7$ Hz, 6H, $CH(Me)_2$), 1.64 (d, $J = 7.0$ Hz, 3H, $CHMe$), 1.87–2.01 [m, 1H, $CH_2CH(Me)_2$], 3.09 (s, 3H, SO_2CH_3), 3.96 (d, $J = 6.7$ Hz, 2H, CH_2CH), 5.19 (s, 2H, furanone CH_2), 5.31 (q, $J = 7.0$ Hz, 1H, $CHCH_3$), 7.37 and 7.43 (AA′BB′ system, $J = 8.5$ Hz, 4H total, disubstituted-phenyl hydrogens), 7.53 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.93 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) *m/z*: Calcd for $C_{24}H_{27}NO_7S$ (MH^+): 474.15. Found: 474.07; Anal. Calcd for $C_{24}H_{27}NO_7S$: C, 60.87; H, 5.75; N, 2.96. Found: C, 60.99; H, 5.78; N, 2.99.

5.9.3. *N*-Phenoxycarbonyl-*N*-{1-[4-(4-methanesulfonylphenyl)-2(5*H*)furanon-3-yl]phenyl}ethylhydroxylamine (20c). Yield, 21%; white foam; mp 128–130 °C. IR (film): 3348 (OH), 1750 (furanone CO), 1703 (carbamate CO), 1313, 1152 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.70 (d, $J = 7.0$ Hz, 3H, $CHCH_3$), 3.07 (s, 3H, SO_2CH_3), 5.19 (s, 2H, furanone CH_2), 5.45 (q, $J = 6.7$ Hz, 1H, $CHCH_3$), 7.22 (t, $J = 7.7$ Hz, 1H, phenyl H-4), 7.33–7.39 (m, 4H, disubstituted-phenyl hydrogens), 7.47–7.50 (m, 4H, 4-methanesulfonylphenyl H-2, H-6, phenyl H-3, H-5), 7.92 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) *m/z*: Calcd for $C_{26}H_{23}NO_7S$ (MH^+): 494.13. Found: 493.88.

5.9.4. *N*-Benzyloxycarbonyl-*N*-{1-[4-(4-methanesulfonylphenyl)-2(5*H*)furanon-3-yl]phenyl}ethylhydroxylamine (20d). Yield, 23%; white foam; mp 95–96 °C. IR (film): 3388 (OH), 1750 (furanone CO), 1320, 1152 (SO_2) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.63 (d, $J = 6.7$ Hz, 3H, $CHCH_3$), 3.08 (s, 3H, SO_2CH_3), 5.19 and 5.21 (two s, 2H each, furanone CH_2, benzyl CH_2), 5.35 (q, $J = 6.7$ Hz, 1H, $CHCH_3$), 7.30–7.43 (m, 9H, disubstituted-phenyl hydrogens, benzyl phenyl hydrogens), 7.51 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.93 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); MS (ES+) *m/z*: Calcd for $C_{27}H_{25}NO_7S$ (MH^+): 508.14. Found: 507.95.

6. Molecular modeling (docking) study

Docking experiments were performed using Insight II software Version 2000.1 (Accelrys Inc.) running on a Silicon Graphic Octane 2 R14000A workstation according to a previously reported method.[24]

7. In vitro lipoxygenase (LOX) inhibition assays

The ability of the test compounds listed in Table 1 to inhibit potato 5-LOX (Catalog No. 60401, Cayman Chemical, Ann Arbor, MI, USA) and soybean 15-LOX (Catalog No. 760700, Cayman Chemical, Ann Arbor, MI, USA) (IC_{50} values, μM) was determined using an enzyme immunoassay (EIA) kit according to our previously reported method.[25]

8. In vitro cyclooxygenase (COX) inhibition assay

The ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and COX-2 (IC_{50} value, μM) was determined using an enzyme immunoassay (EIA) kit (Catalog No. 560101, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method.[26]

9. Anti-inflammatory assay

Anti-inflammatory activity was performed using a method described by Winter et al.[27]

10. Analgesic assay

Analgesic activity was determined using a 4% sodium chloride-induced abdominal constriction assay previously reported.[28]

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References and notes

1. Funk, C. D. *Science* **2001**, *294*, 1871.
2. Dannhardt, G.; KieferEur, W. *J. Med. Chem.* **2001**, *36*, 109.
3. Fosslien, E. *Ann. Clin. Lab. Sci.* **1998**, *28*, 67.
4. (a) Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347; (b) De Leval, X.; Julemont, F.; Benoit, V.; Frederich, M.; Pirotte, B.; Dogne, J. M. *Mini-Rev. Med. Chem.* **2004**, *4*, 597.
5. Cannon, G. W.; Breedveld, F. G. *Am. J. Med.* **2001**, *110*, 6S–12S.
6. (a) Vioxx® (Rofecoxib) sales suspended by Merk Sharp & Dohme-Chibret on September 30, 2004; (b) Bextra® (Valdecoxib) sales suspended by Pfizer on April 12, 2005; (c) Dogné, J.-M.; Supuran, C. T.; Pratico, D. *J. Med. Chem.* **2005**, *48*, 2251.
7. Zhao, L.; Funk, C. D. *Trends Cardiovasc. Med.* **2004**, *14*, 191.

8. (a) Fiorucci, S.; Meli, R.; Bucci, M.; Cirino, G. *Biochem. Pharmacol.* **2001**, *62*, 1433; (b) Charlier, C.; Michaux, C. *Eur. J. Med. Chem.* **2003**, *38*, 645.
9. (a) Ding, C.; Cicuttini, F. *IDrugs* **2003**, *6*, 802; (b) Alvaro-Gracia, J. M. *Rheumatology* **2004**, *43*, 121; (c) Bias, P.; Buchner, A.; Klesser, B.; Laufer, S. *Am. J. Gastroenterol.* **2004**, *99*, 611.
10. Corey, E. J.; Cashman, J. R.; Kantner, S. S.; Wright, S. W. *J. Am. Chem. Soc.* **1984**, *106*, 1503.
11. Carter, G. W.; Young, P. R.; Albert, D. H.; Bouska, J.; Dyer, R.; Bell, R. L.; Summers, J. B.; Brooks, D. W. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 929.
12. Clint, D. W.; Brooks, A. O.; Stewart, A. B.; Pramila, B.; James, D. R.; Jonathan, G. M.; Richard, A. C.; Teodozyj, K.; Jennifer, B. B.; Carmine, L.; Richard, R. H.; Peter, E. M.; George, W. C.; Randy, L. B. *J. Med. Chem.* **1995**, *38*, 4768.
13. Pombeiro, A. J. L.; Kukushkin, V. Y. In *Comprehensive Coordination Chemistry II*; McCleberty, J. A., Meyer, T. C., Eds.; Elsevier: Amsterdam, 2004; Vol. 1, p 631.
14. (a) Surman, M. D.; Mulvihill, M. J.; Miller, M. J. *J. Org. Chem.* **2002**, *67*, 4115; (b) Yatabe, T.; Kawai, Y.; Oku, T.; Tanaka, H. *Chem. Pharm. Bull.* **1998**, *46*, 966; (c) Connolly, P. J.; Wetter, S. K.; Beers, K. N.; Hamel, S. C.; Chen, R. H. K.; Wachter, M. P.; Ansell, J.; Singer, M. M.; Steber, M.; Ritchie, D. M.; Argentieri, D. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 979; (d) Lewis, T. A.; Bayless, L.; DiPesa, A. J.; Eckman, J. B.; Gillard, M.; Libertine, L.; Scannell, R. T.; Wypij, D. M.; Young, M. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1083.
15. Morgan, E. D. *Tetrahedron* **1967**, *23*, 1735.
16. Takahashi, S.; Kuroyama, Y.; Sonogashira, K.; Hagihara, N. *Synthesis* **1980**, 627.
17. Therien, M. T.; Gauthier, J. Y.; Leblanc, Y.; Leger, S.; Perrier, H.; Prasit, P.; Wang, Z. *Synthesis* **2001**, 1778.
18. Soliva, R.; Almansa, C.; Kalko, S. G.; Luque, F. J.; Orozco, M. *J. Med. Chem.* **2003**, *46*, 1372.
19. Prasit, P.; Wang, Z.; Brideau, C.; Chan, C. C.; Charleson, S.; Cromlish, W.; Ethier, D.; Evans, J. F.; Ford-Hutchinson, A. W.; Gauthier, J. Y.; Gordon, R.; Guay, J.; Gresser, M.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Leger, S.; Mancini, J.; O'Neill, G. P.; Ouellet, M.; Percival, M. D.; Perrier, H.; Riendeau, D.; Rodger, I.; Zamboni, R.; Boyce, S.; Rupniak, N.; Forrest, M.; Visco, D.; Patrick, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1773.
20. Yoon, N. M.; Kang, J. *Taehan Hwahakhoe Chi* **1975**, *19*, 360; . *Chem. Abstr.* **1976**, *84*, 73602.
21. Schmid, L.; Schultes, H.; Wichtl, M. *Monatshefte fuer Chemie* **1954**, *85*, 80; . *Chem. Abstr.* **1955**, *49*, 46102.
22. Culter, R. A.; Stenger, R. J.; Suter, C. M. *J. Am. Chem. Soc.* **1952**, *74*, 5475.
23. Patrick, T. B.; Scheibel, J. J.; Hall, W. E.; Lee, Y. H. *J. Org. Chem.* **1980**, *15*, 4492.
24. Rao, P. N. P.; Amini, H.; Li, H.; Habeeb, A. G.; Knaus, E. E. *J. Med. Chem.* **2003**, *46*, 4872.
25. Rao, P. N. P.; Chen, Q.-H.; Knaus, E. E. *J. Med. Chem.* **2006**, *49*, 1668.
26. Uddin, M. J.; Rao, P. N. P.; Knaus, E. E. *Bioorg. Med. Chem.* **2004**, *12*, 5929.
27. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.
28. Fukawa, K.; Kawano, O.; Hibi, M.; Misaka, N.; Ohba, S.; Hatanaka, Y. *J. Pharmacol. Methods* **1980**, *4*, 251.