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Bioorganic & Medicinal Chemistry 14 (2006) 5259–5265
Bioorganic & Medicinal Chemistry

Synthesis and biological evaluation of linear phenylethynylbenzenesulfonamide regioisomers as cyclooxygenase-1/-2 (COX-1/-2) inhibitors

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Received 27 February 2006; revised 21 March 2006; accepted 29 March 2006
Available online 25 April 2006

Abstract—A group of regioisomeric phenylethynylbenzenesulfonamides possessing a COX-2 SO_2NH_2 pharmacophore at the *para*-, *meta*- or *ortho*-position of the C-1 phenyl ring, in conjunction with a C-2 substituted-phenyl (H, OMe, OH, Me, F) group, were synthesized and evaluated as inhibitors of the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isozymes. The target 1,2-diphenylacetylenes were synthesized via a palladium-catalyzed Sonogashira cross-coupling reaction. In vitro COX-1/-2 isozyme inhibition structure–activity data showed that COX-1/-2 inhibition and the COX selectivity index (SI) are sensitive to the regioisomeric placement of the COX-2 SO_2NH_2 pharmacophore where the COX-2 potency order for the benzenesulfonamide regioisomers was generally *meta* > *para* and *ortho*. Among this group of compounds, the in vitro COX-1/-2 isozyme inhibition studies identified 3-(2-phenylethynyl)benzenesulfonamide (**10a**) as a COX-2 inhibitor (COX-2 IC_{50} = 0.45 μM) with a good COX-2 selectivity (COX-2 SI = 70). In contrast, 2-[2-(3-fluorophenyl)ethynyl]benzenesulfonamide (**11c**) possessing a SO_2NH_2 COX-2 pharmacophore at the *ortho*-position of the C-1 phenyl ring exhibited COX-1 inhibition and selectivity (COX-1 IC_{50} = 3.6 μM). A molecular modeling study where **10a** was docked in the binding site of COX-2 shows that the *meta*-SO_2NH_2 COX-2 pharmacophore was inserted inside the COX-2 secondary pocket (Arg513, Phe518, Val523, and His90). Similar docking of **10a** within the COX-1 binding site shows that the *meta*-SO_2NH_2 pharmacophore is unable to interact with the respective amino acid residues in COX-1 that correspond to those near the secondary pocket in COX-2 due to the presence of the larger Ile523 in COX-1 that replaces Val523 in COX-2.

1. Introduction

The discovery of the mechanism of action of aspirin and indomethacin in the late 1970s was a critical event in the development of nonsteroidal anti-inflammatory drugs (NSAIDs).[1] Classical NSAIDs such as aspirin, ibuprofen, and indomethacin produce their beneficial anti-inflammatory activity by inhibition of cyclooxygenase (COX)-derived prostaglandin (PG) synthesis. However, a major drawback of NSAID therapy is the development of adverse side effects such as gastrointestinal (GI) toxicities that arise during their chronic use.[2] This GI toxicity has been attributed to the inhibition of cytoprotective PGs produced via the COX pathway.[3] In the early 1990s, an inducible isoform of the COX enzyme was discovered that is now called cyclooxygenase-2 (COX-2).[4] This discovery provided the rationale for development of selective COX-2 inhibitors that would be effective anti-inflammatory agents with a reduced side effect profile. Several classes of selective COX-2 inhibitors referred to as diarylheterocylces possess vicinal aryl rings attached to a central heterocyclic ring scaffold. In this regard, celecoxib (**1**) having a central five-membered pyrazole ring, and rofecoxib (**2**) having a central five-membered 2(5*H*)-furanone ring, were the first two COX-2 inhibitors approved for clinical use[5,6] (Fig. 1).

Extensive structure–activity relationship (SAR) studies for the diarylheterocycle class of compounds have shown that a SO_2NH_2, or a SO_2Me, substituent at the *para*-position of one of the aryl rings is a requirement for optimum COX-2 selectivity and potency.[7] However, the selective COX-2 inhibitors rofecoxib and valdecoxib were recently withdrawn from the market due to adverse cardiovascular side effects associated with their prolonged use.[8] Consequently, there is a current need to

Keywords: Phenylethynylbenzenesulfonamides; Cyclooxygenase-1/2 inhibitors; COX-2 pharmacophore; Anti-inflammatory and analgesic activity.
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doi:10.1016/j.bmc.2006.03.050

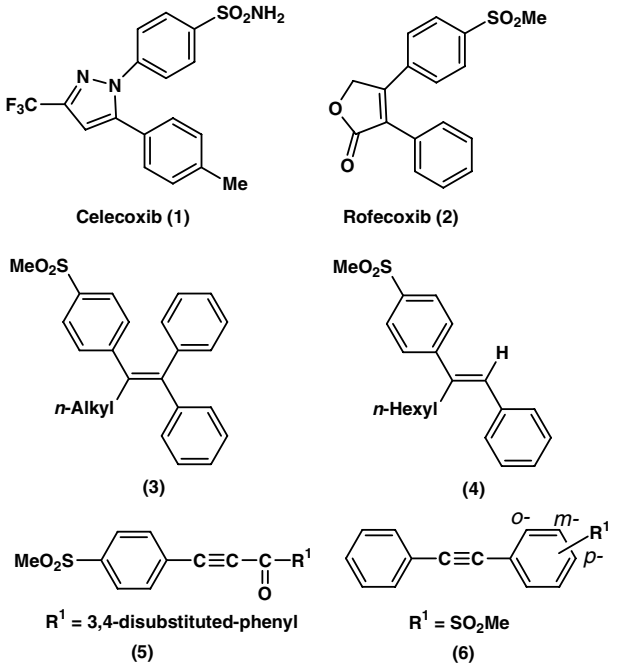
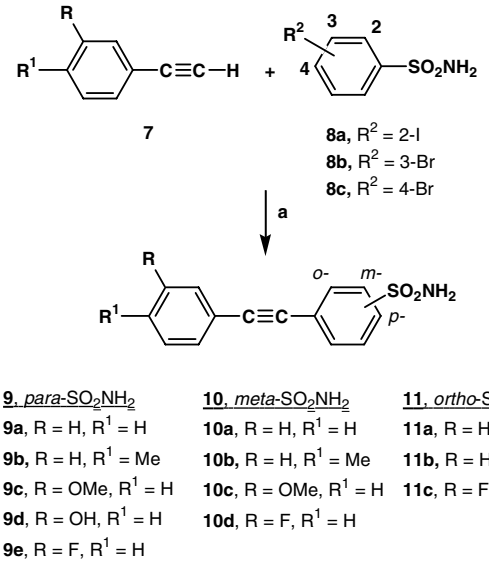


Figure 1. Some representative selective cyclooxygenase-2 (COX-2) inhibitors.

develop safer anti-inflammatory agents based on alternative templates. Studies in our drug design program indicate that acyclic triaryl olefins (**3**), 1,2-diaryl (*E*)-olefins (**4**), and acetylenic ketones (**5**), lacking a traditional central heterocyclic or carbocyclic ring template, are potent selective COX-2 inhibitors with clinically relevant anti-inflammatory and analgesic activities.[9–12] It has also been reported that substituted diphenyl compounds possessing a central linear acetylene moiety are useful for the treatment of psoriatic dermatitis, epithelial cancer, and arthritic diseases.[13] In an earlier investigation, we demonstrated that substituted-1,2-diphenylacetylene regioisomers (**6**) are effective COX-1/-2 inhibitors that exhibit in vivo anti-inflammatory activities.[14] As a part of our ongoing program, we now describe the design, synthesis, in vitro COX-1 and COX-2 inhibitory activities, and in vivo anti-inflammatory activity for a class of regioisomeric 1,2-diphenylacetylenes possessing a SO_2NH_2 COX-2 pharmacophore at the *para*-, *meta*- or *ortho*-position of one of the phenyl rings.

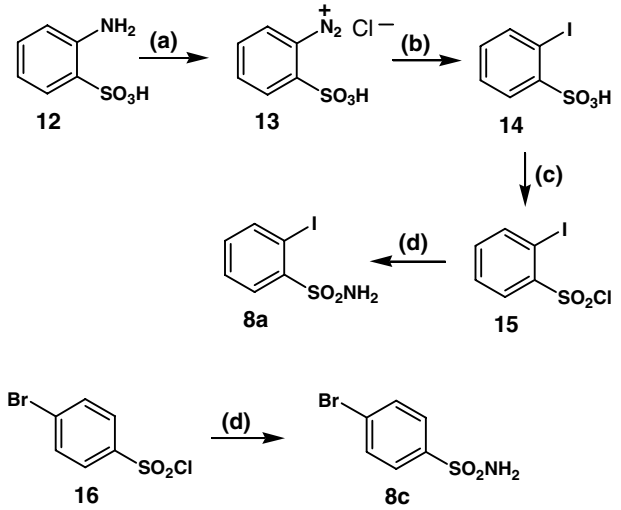
2. Chemistry

The 1,2-diphenylacetylene regioisomers (**9a–e**, **10a–d**, and **11a–c**) possessing a SO_2NH_2 substituent at the *para*-, *meta*- or *ortho*-position of the C-1 phenyl ring were prepared in good yield (52–82%) using a palladium-catalyzed Sonogashira cross-coupling reaction (Scheme 1).[15] Phenylacetylene precursors (**7**) possessing a variety of substituents (R = H, OMe, OH, F; R^1 = H, Me), and either 2-iodobenzenesulfonamide (**8a**), 3-bromobenzenesulfonamide (**8b**), or 4-bromobenzenesulfonamide (**8c**), were employed in this cross-coupling reaction using dichlorobis(triphenylphosphine)palladium(0)/CuI as catalysts in the



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, reflux 16 h, or 50 °C for 5 h.

presence of an acid acceptor such as triethylamine. The low reactivity of 2-bromobenzenesulfonamide in the Sonogashira cross-coupling reaction prompted utilization of the more reactive 2-iodobenzenesulfonamide. Accordingly, 2-iodobenzenesulfonamide (**8a**), a precursor to the target compounds **11a–c**, was prepared by diazotization of commercially available 2-aminobenzenesulfonic acid (**12**) that was subsequently reacted with potassium iodide to afford 2-iodobenzenesulfonic acid (**14**) in 70% yield (see Scheme 2). Treatment of 2-iodobenzenesulfonic acid (**14**) with PCl_5 according to the procedure of Chau and Kice furnished 2-iodobenzenesulfonyl chloride (**15**).[16] Subsequent reaction of 2-iodobenzenesulfonyl chloride



Scheme 2. Reagents and conditions: (a) water, Na_2CO_3 to form a homogeneous solution, addition of $NaNO_2$ at 25 °C, and then concentrated HCl at 0 °C; (b) KI, water, warm to 25 °C, and then heat at reflux until nitrogen evolution ceases; (c) neutralization by titration with NaOH solution, drying the sodium sulfonate salt, and addition of PCl_5; (d) concentrated NH_4OH solution, 25 °C.

(**15**) with concentrated ammonia solution afforded **8a** in near quantitative yield.[17] 4-Bromobenzenesulfonamide (**8c**) was prepared in a similar way by reaction of commercially available 4-bromobenzenesulfonyl chloride (**16**) with concentrated ammonia solution as illustrated in Scheme 2.

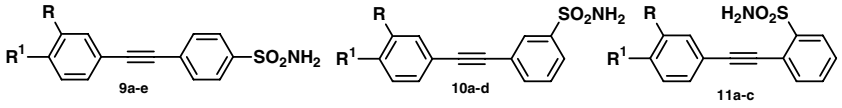
3. Results and discussion

Structure–activity relationship data for first generation COX-2 inhibitors such as celecoxib and rofecoxib identified the requirement for a SO_2NH_2 or a SO_2Me COX-2 pharmacophore with respect to optimum COX-2 inhibitory activity and selectivity.[7] Accordingly, a group of phenylethynylbenzenesulfonamide regioisomers (**9**–**11**) possessing a central linear acetylenic template were designed such that the COX-2 SO_2NH_2 pharmacophore was positioned at the *para*-, *meta*- or *ortho*-position of the C-1 phenyl ring. In addition, substituents on the C-2 phenyl ring were varied (R = H, OMe, OH, F; R^1 = H, Me) to determine the effect of positional, steric, and electronic substituent properties upon COX-1 and COX-2 inhibitory potency and selectivity. The in vitro ability of the title compounds to inhibit the COX-1 and COX-2 isozymes was carried out using an enzyme immuno assay (EIA), and the results are listed in Table 1. Structure–activity data obtained for the benzenesulfonamide regioisomers **9**–**11** indicate that COX-2 inhibitory potency and selectivity varied considerably based on the position of the COX-2 SO_2NH_2 pharmacophore and the properties of the C-2 phenyl substituents.

Among the subgroup of compounds **9a**–**e**, possessing a *para*-SO_2NH_2 COX-2 pharmacophore, compound **9e** (R = F; R^1 = H) exhibited the best combination of COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.3 μM; COX-1 IC_{50} = 3.6 μM; SI = 12), and it was a superior COX-2 inhibitor compared to the reference compound rofecoxib (IC_{50} = 0.5 μM). In comparison, a *meta*-methoxy substituent (**9c**, R = OMe; R^1 = H) produced a decrease in both COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 6.9 μM; COX-1 IC_{50} = 31.6 μM; SI = 5.0). In contrast, compound **9d** having a *meta*-hydroxy substituent (R = OH; R^1 = H) was a 10-fold more potent inhibitor of COX-1 than COX-2. A C-2 *meta*-substituted-phenyl ring is required for COX inhibitory activity since the C-2 phenyl and *para*-methylphenyl compounds (**9a**, R = H, R^1 = H; **9b**, R = Me, R^1 = H) were inactive against both COX-1 and COX-2 (IC_{50} > 100 μM). The relative COX-2 inhibitory potency order for this *para*-substituted-benzenesulfonamide subgroup of compounds **9a**–**e** was 3-F > 3-OMe ≫ 3-OH (COX-1 selective) > inactive 4-H, 4-Me.

The in vitro COX-1/-2 inhibitory activity data for compounds **10a**–**d** possessing a SO_2NH_2 COX-2 pharmacophore at the *meta*-position of the C-1 phenyl ring were highly variable. For example, the *meta*-sulfonamide compound **10a** (R = R^1 = H), unlike the inactive *para*-sulfonamide regioisomer (**9a**), exhibited good COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.45 μM; COX-1 IC_{50} = 31.6 μM; SI = 70). Related compounds having a C-2 *para*-methylphenyl (**10b**), or *meta*-methoxyphenyl (**10c**), substituent showed weak COX-2, but more potent COX-1 inhibitory activities relative to the C-2 phenyl analog (**10a**). The presence of a C-2 3-fluorophenyl substituent (**10d**, R = F, R^1 = H) maintained COX-2 selectivity (SI > 15), but caused a de-

Table 1. In vitro COX-1 and COX-2 inhibition data for phenylethynylbenzenesulfonamides (**9a**–**e**, **10a**–**d** and **11a**–**c**)



Compound	R	R^1	IC_{50}[a] (μM)		COX-2 SI[b]
			COX-1	COX-2	
9a	H	H	>100	>100	N/A
9b	H	Me	>100	>100	N/A
9c	OMe	H	31.6	6.8	5
9d	OH	H	3.1	32.6	—
9e	F	H	3.6	0.3	12
10a	H	H	31.6	0.45	70
10b	H	Me	1.0	3.2	—
10c	OMe	H	1.0	2.0	—
10d	F	H	>100	6.8	>15
11a	H	H	>100	>100	N/A
11b	H	Me	32.0	32.0	1
11c	F	H	3.6	>100	N/A
Aspirin	—	—	0.35	2.4	N/A
Celecoxib	—	—	33.1	0.07	472
Rofecoxib	—	—	>100	0.50	>200

[a] Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit (Catalog No. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.
[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

crease in COX-2 inhibitory potency (IC_{50} = 6.8 μM) compared to the C-2 phenyl compound **10a**. Compounds **11a–c** having a SO_2NH_2 COX-2 pharmacophore at the *ortho*-position of the C-1 phenyl ring were either inactive (**11a**, R = R^1 = H; **11c**, R = F, R^1 = H) or weak (**11b**, R = H, R^1 = Me) inhibitors of the COX-2 isozyme. In fact, the C-2 3-fluorophenyl compound **11c** was a weak (IC_{50} = 3.6 μM), but selective inhibitor of COX-1.

A molecular modeling (docking) experiment was carried out to determine the binding interactions of the most potent and selective COX-2 inhibitor 3-(2-phenylethynyl)benzenesulfonamide (**10a**, COX-2 IC_{50} = 0.45 μM; COX-1 IC_{50} = 31.6 μM; SI > 70) in the COX-2 binding site as shown in Figure 2. As per our hypothesis, modeling the most stable enzyme–ligand complex shows that the *meta*-SO_2NH_2 COX-2 pharmacophore is oriented in the vicinity of COX-2 secondary pocket where it is surrounded by amino acids Val523, Phe518, Ile517, Arg513, and His90. The S*O*$_2$ moiety (of SO_2NH_2) is positioned within van der Waals contact range of amino acids such as Phe518 and Ile517 (distance < 5 Å). The NH_2 (of SO_2NH_2) was oriented toward His90, Thr94 and Tyr355 closer to the mouth of the COX-2 active site. One of the H-atoms of the NH_2 group undergoes a hydrogen-bonding interaction with the N-atom of His90 (distance ≈ 3.57 Å). The distance between the second H-atom of the NH_2 group and the OH of Tyr355 is about 3.83 Å. The linear acetylene scaffold, which is surrounded by Leu352, Gly526, and Ala527, serves to orient the C-2 unsubstituted phenyl ring toward the apex of the COX-2 binding site, in a region comprised of Tyr385, Phe381, Tyr348, Phe205, and Val344 (distance < 5 Å). The interspacial distance between the center of the unsubstituted C-2 phenyl ring and the OH of Ser530 is about 5.44 Å.

The SO_2NH_2 and the SO_2Me pharmacophores present in celecoxib and rofecoxib, respectively, are known to induce COX-2 selectivity by insertion into the secondary pocket of the COX-2 binding site that is absent in COX-1.[7] This additional secondary pocket in COX-2 is formed due to a conformational change at Tyr355 that is attributed to the presence of Ile523 in COX-1 relative to Val523 having a smaller side chain in COX-2.[18] Accordingly, it was also of interest to perform a molecular modeling experiment wherein **10a** was docked in the COX-1 binding site (as shown in Fig. 3). The most stable enzyme–ligand complex shows that the *meta*-SO_2NH_2 COX-2 pharmacophore is unable to interact with the amino acid residues in COX-1 that correspond to the amino acid residues in the vicinity of the COX-2 secondary pocket, due to the presence of a larger Ile523 in COX-1. However, a favorable hydrogen bonding interaction was observed between the NH_2 (of SO_2NH_2) and the backbone *N*H of Ile517 (distance ≈ 2.39 Å). The benzenesulfonamide ring is oriented toward a hydrophobic pocket comprised of Leu352, Ile517, Met522, Ile523, and Gly526. Due to the presence of the bulkier Ile523 in the COX-1 binding site, the C-2 unsubstituted phenyl ring in **10a** is oriented toward the mouth of the COX-1 binding site closer to amino acid residues such as Val116, Arg120, and Tyr355. In contrast, the C-2 unsubstituted-phenyl ring of **10a** assumes a position in a hydrophobic region toward the apex of the COX-2 binding site (Fig. 2). This observation

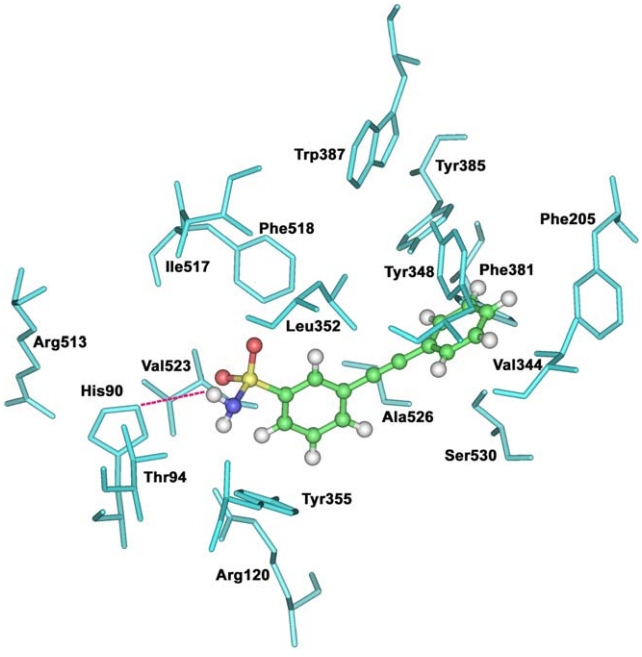


Figure 2. Docking of 3-(2-phenylethynyl)benzenesulfonamide (**10a**) (ball and stick) in the active site of murine COX-2. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

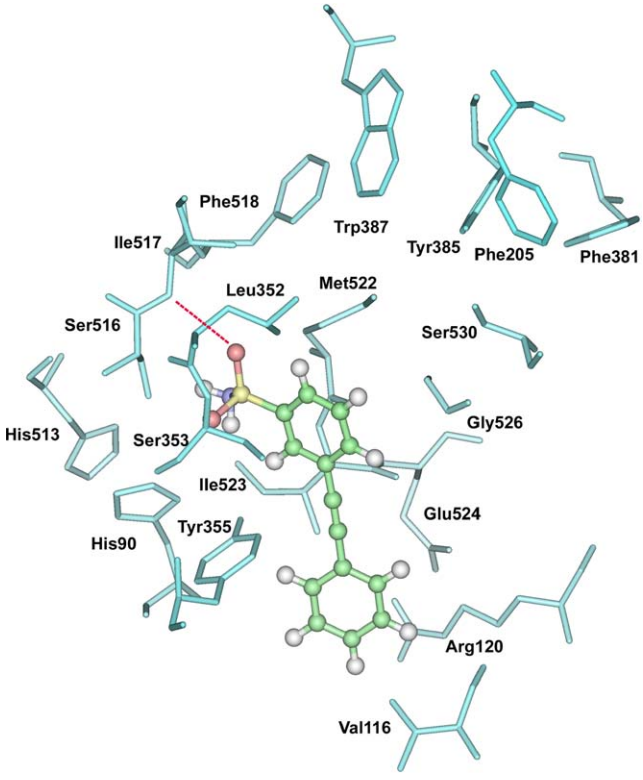
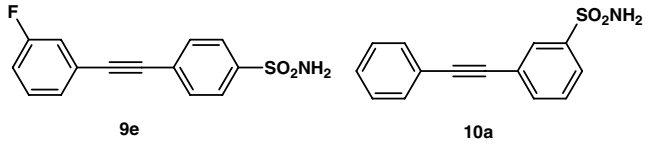


Figure 3. Docking of 3-(2-phenylethynyl)benzenesulfonamide (**10a**) (ball and stick) in the active site of ovine COX-1. Hydrogen atoms of the amino acid residues have been removed to improve clarity.

Table 2. In vivo anti-inflammatory and analgesic activities for phenylethynylbenzenesulfonamides (**9e** and **10a**)



9e **10a**

Compound	Anti-inflammatory activity[a]	Analgesic activity[b]	
	% inhibition at 3 h (75 mg/kg po dose)	% inhibition at 30 min	% inhibition at 60 min
9e	23.3 ± 2.5	41.7 ± 5.9	29.0 ± 10.6
10a	17.6 ± 0.7	58.3 ± 15.3	83.3 ± 8.2
Aspirin	25.2 ± 3.3[c,d]	—	—
Celecoxib	79.9 ± 1.9[c,e]	41.1 ± 5.9[c]	28.8 ± 10.6[c]

[a] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as means ± SEM ($n = 4$) following a 75 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 ($n = 4$) following a 75 mg/kg oral dose of the test compound.
[c] 50 mg/kg oral dose.
[d] ED_{50} = 129.0 mg/kg oral dose.
[e] ED_{50} = 10.8 mg/kg oral dose.

explains the weak binding affinity of **10a** toward COX-1 (COX-1 IC_{50} = 31.6 μM) compared to COX-2 (COX-2 IC_{50} = 0.45 μM).

In vivo anti-inflammatory and analgesic activities were determined for compounds **9e** and **10a** that showed superior in vitro COX-2 inhibitory potency and selectivity properties. In a carrageenan-induced rat paw edema assay **9e** produced a 23% reduction in inflammation at 3 h postdrug administration for a 75 mg/kg oral dose (see data in Table 2). In comparison, the *meta*-benzenesulfonamide compound **10a** produced a 17% decrease in inflammation at 3 h postdrug administration for a 75 mg/kg oral dose. Thus, **9 e** and **10a** are weaker anti-inflammatory agents than the reference drug celecoxib (80% inhibition of inflammation at 3 h postdrug administration for a 50 mg/kg oral dose). In a rat 4% NaCl-induced abdominal constriction (analgesic) assay, the *meta*-benzenesulfonamide **10a** exhibited superior analgesic activity (58 and 83% inhibition of writhing at 30 and 60 min postdrug administration) for a 75 mg/kg oral dose compared to the *para*-benzenesulfonamide **9e** (41 and 29% inhibition of writhing at 30 and 60 min postdrug administration).

4. Conclusions

The structure–activity relationships acquired indicate that (i) a linear acetylene spacer between two vicinal phenyl rings is a suitable template for the design of new COX inhibitors, (ii) the position of the sulfonamide (SO_2NH_2) pharmacophore on the C-1 phenyl ring is a determinant of COX-2 activity where the relative potency profile is generally *meta* > *ortho* and *para*, (iii) in the group of *meta*-benzenesulfonamides (**10**), incorporation of a C-2 3-methoxyphenyl or 3-fluorophenyl substituent decreases COX-2 potency and selectivity, and (iv) 3-(2-phenylethynyl)benzenesulfonamide (**10a**) exhibited potent and selective in vitro COX-2 inhibition (COX-2 IC_{50} = 0.45 μM; COX-1 IC_{50} = 31.6 μM; SI > 70), moderate in vivo anti-inflammatory activity, and good analgesic activity.

5. Experimental

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. ^{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 Hz. Spin multiples are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Microanalyses were performed for C, H, and N (MicroAnalytical Service Laboratory, Department of Chemistry, University of Alberta) and were within ±0.4% of theoretical values. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70–230 mesh). All 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.

6. General procedure for the synthesis of 2-, 3-, and 4-(substituted-phenylethynyl)benzenesulfonamides (9–11)

Triethylamine (7 mL), dichlorobis(triphenylphosphine)palladium (42 mg, 0.06 mmol), and then cuprous iodide (5.8 mg, 0.03 mmol) were added slowly with stirring to a solution of either 2-iodobenzenesulfonamide (**8a**, 290 mg, 1 mmol), 3- bromobenzenesulfonamide (**8b**, 240 mg, 1 mmol) or 4-bromobenzenesulfonamide (**8c**, 240 mg, 1 mmol), and a phenylacetylene (**7**, R = H, OMe, OH, F; R^1 = H, Me; 1 mmol) in THF

(3 mL) under an argon atmosphere at 25 °C. The reaction was allowed to proceed for 16 h with stirring at 80 °C (for reactions using **8b** or **8c**), or at 50 °C and a reaction time of 5 h for reactions using 2-iodobenzenesulfonamide (**8a**). The reaction mixture was cooled to 25 °C, the solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography using ethyl acetate/hexanes (1:4, v/v) as eluent to afford the respective title compounds (**9**, **10** or **11**). Some physical and spectroscopic data for **9a–e**, **10a–d**, and **11a–c** are listed below.

6.1. 4-(2-Phenylethynyl)benzenesulfonamide (9a)

Yield, 65%; white crystals; mp 214–216 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 6.50 (s, 2H, SO_2NH_2), 7.21–7.24 (m, 3H, phenyl H-3, H-4, H-5), 7.35–7.40 (m, 2H, phenyl H-2, H-6), 7.46 (d, J = 8.2 Hz, 2H, benzenesulfonamide H-3, H-5), 7.75 (d, J = 8.2 Hz, 2H, benzenesulfonamide H-2, H-6). Anal. Calcd for $C_{14}H_{11}NO_2S$: C, 65.35; H, 4.31; N, 5.44. Found: C, 65.28; H, 4.10; N, 5.33.

6.2. 4-[2-(4-Methylphenyl)ethynyl]benzenesulfonamide (9b)

Yield, 52%; yellow solid; mp 229–231 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 2.16 (s, 3H, CH_3), 6.56 (s, 2H, SO_2NH_2), 6.96 (d, J = 7.9 Hz, 2H, 4-methylphenyl H-3, H-5), 7.20 (d, J = 7.9 Hz, 4-methylphenyl H-2, H-6), 7.37 (d, J = 8.2 Hz, 2H, benzenesulfonamide H-3, H-5), 7.67 (d, J = 8.2 Hz, 2H, benzenesulfonamide H-2, H-6). Anal. Calcd for $C_{15}H_{13}NO_2S$: C, 66.40; H, 4.83; N, 5.16. Found: C, 66.24; H, 4.76; N, 5.15.

6.3. 4-[2-(3-Methoxyphenyl)ethynyl]benzenesulfonamide (9c)

Yield, 72%; white solid; mp 164–165 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 3.63 (s, 3H, OCH_3), 6.56 (s, 2H, SO_2NH_2), 6.73–6.77 (m, 1H, 3-methoxyphenyl H-4), 6.86 (d, J = 2.1 Hz, 1H, 3-methoxyphenyl H-2), 6.93 (d, J = 7.9 Hz, 1H, 3-methoxyphenyl H-6), 7.08 (t, J = 7.9 Hz, 1H, 3-methoxyphenyl H-5), 7.40 (d, J = 8.2 Hz, 2H, benzenesulfonamide H-3, H-5), 7.71 (d, J = 8.2 Hz, 2H, benzenesulfonamide H-2, H-6). Anal. Calcd for $C_{15}H_{13}NO_3S$: C, 62.70; H, 4.56; N, 4.87. Found: C, 62.43; H, 4.42; N, 4.71.

6.4. 4-[2-(3-Hydroxyphenyl)ethynyl]benzenesulfonamide (9d)

Yield, 70%; yellow solid; mp 233–234 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 6.52 (s, 2H, SO_2NH_2), 6.66–6.69 (m, 1H, 3-hydroxyphenyl H-4), 6.80–6.83 (m, 2H, 3-hydroxyphenyl H-2, H-6), 6.99 (t, J = 7.9 Hz, 1H, 3-hydroxyphenyl H-5), 7.41 (d, J = 8.2 Hz, 2H, benzenesulfonamide H-3, H-5), 7.71 (d, J = 8.2 Hz, 2H, benzenesulfonamide H-2, H-6), 8.92 (s, 1H, O*H*). Anal. Calcd for $C_{14}H_{11}NO_3S$: C, 61.52; H, 4.06; N, 5.12. Found: C, 61.41; H, 4.05; N, 5.02.

6.5. 4-[2-(3-Fluorophenyl)ethynyl]benzenesulfonamide (9e)

Yield, 58%; white solid; mp 176–178 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 6.40 (s, 2H, SO_2NH_2), 6.94–7.01 (m, 1H, 3-fluorophenyl H-4), 7.12 (dd, J = 8.5, 1.8 Hz, 1H, 3-fluorophenyl H-2), 7.20–7.27 (m, 2H, 3-fluorophenyl H-5, H-6), 7.51 (d, J = 8.2 Hz, 2H, benzenesulfonamide H-3, H-5), 7.81 (d, J = 8.2 Hz, 2H, benzenesulfonamide H-2, H-6). Anal. Calcd for $C_{14}H_{10}FNO_2S$: C, 61.08; H, 3.66; N, 5.09. Found: C, 60.98; H, 3.41; N, 4.90.

6.6. 3-(2-Phenylethynyl)benzenesulfonamide (10a)

Yield, 59%; yellow crystals; mp 156–157 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 6.31 (s, 2H, SO_2NH_2), 7.28–7.30 (m, 3H, phenyl H-3, H-4, H-5), 7.39–7.46 (m, 3H, phenyl H-2, H-6, benzenesulfonamide H-5), 7.56 (d, J = 7.9 Hz, 1H, benzenesulfonamide H-4), 7.78–7.81 (m, 1H, benzenesulfonamide, H-6), 8.00 (s, 1H, benzenesulfonamide H-2). Anal. Calcd for $C_{14}H_{11}NO_2S{\cdot}1/8H_2O$: C, 64.78; H, 4.37; N, 5.40. Found: C, 64.86; H, 4.07; N, 5.34.

6.7. 3-[2-(4-Methylphenyl)ethynyl]benzenesulfonamide (10b)

Yield, 68%; white solid; mp 159–160 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 2.31 (s, 3H, CH_3), 6.24 (s, 2H, SO_2NH_2), 7.10 (d, J = 7.9 Hz, 2H, 4-methylphenyl H-3, H-5), 7.33–7.42 (m, 3H, 4-methylphenyl H-2, H-6, benzenesulfonamide H-5), 7.57 (d, J = 7.9 Hz, 1H, benzenesulfonamide H-4), 7.79 (d, J = 7.9 Hz, 1H, benzenesulfonamide H-6), 8.00 (t, J = 1.2 Hz, 1H, benzenesulfonamide H-2). Anal. Calcd for $C_{15}H_{13}NO_2S$: C, 66.40; H, 4.83; N, 5.16. Found: C, 66.34; H, 5.21; N, 5.16.

6.8. 3-[2-(3-Methoxyphenyl)ethynyl]benzenesulfonamide (10c)

Yield, 61%; white crystals; mp 120–122 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 3.76 (s, 3H, OCH_3), 6.28 (s, 2H, SO_2NH_2), 6.82–6.86 (m, 1H, 3-methoxyphenyl H-4), 6.97 (s, 1H, 3-methoxyphenyl H-2), 7.04 (d, J = 7.9 Hz, 1H, 3-methoxyphenyl H-6), 7.20 (t, J = 7.9 Hz, 1H, 3-methoxyphenyl H-5), 7.41 (t, J = 7.9 Hz, 1H, benzenesulfonamide H-5), 7.58 (d, J = 7.9 Hz, 1H, benzenesulfonamide H-4), 7.80 (d, J = 7.9 Hz, 1H, benzenesulfonamide H-6), 8.01 (s, 1H, benzenesulfonamide H-2). Anal. Calcd for $C_{15}H_{13}NO_3S$: C, 62.70; H, 4.56; N, 4.87. Found: C, 62.63; H, 4.56; N, 4.80.

6.9. 3-[2-(3-Fluorophenyl)ethynyl]benzenesulfonamide (10d)

Yield, 66%; yellow solid; mp 148–149 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 6.35 (s, 2H, SO_2NH_2), 6.95–7.00 (m, 1H, 3-fluorophenyl H-4), 7.01–7.13 (m, 1H, 3-fluorophenyl H-2), 7.20–7.31 (m, 2H, 3-fluorophenyl H-5, H-6), 7.41 (t, J = 7.9 Hz, 1H, benzenesulfonamide H-5), 7.57 (d, J = 7.9 Hz, 1H, benzenesulfonamide H-

4), 7.81 (d, $J = 7.9$ Hz, 1H, benzenesulfonamide H-6), 8.01 (s, 1H, benzenesulfonamide H-2). Anal. Calcd for $C_{14}H_{10}FNO_2S$: C, 61.08; H, 3.66; N, 5.09. Found: C, 60.86; H, 3.92; N, 5.03.

6.10. 2-(Phenylethynyl)benzenesulfonamide (11a)

Yield, 82%; white solid; mp 103–104 °C; ^{1}H NMR ($CDCl_3$) δ 5.23 (s, 2H, SO_2NH_2), 7.20–7.24 (m, 3H, phenyl H-3, H-4, H-5), 7.40–7.50 (m, 3H, phenyl H-2, H-6, benzenesulfonamide H-4), 7.55 (dt, $J = 7.9$ Hz, 1.2 Hz, 1H, benzenesulfonamide H-5), 7.72 (d, $J = 7.9$ Hz, 1H, benzenesulfonamide H-3), 8.07 (d, $J = 7.9$ Hz, 1H, benzenesulfonamide H-6). Anal. Calcd for $C_{14}H_{11}NO_2S$: C, 65.35; H, 4.31; N, 5.44. Found: C, 65.14; H, 4.51; N, 5.42.

6.11. 2-[2-(4-Methylphenyl)ethynyl]benzenesulfonamide (11b)

Yield, 63%; brown solid; mp 125–126 °C; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H, CH_3), 5.20 (s, 2H, SO_2NH_2), 7.22 (d, $J = 7.9$ Hz, 4-methylphenyl H-3, H-5), 7.44–7.50 (m, 3H, 4-methylphenyl H-2, H-6, benzenesulfonamide H-4), 7.52 (dt, $J = 7.9$ Hz, 1.2 Hz, 1H, benzenesulfonamide H-5), 7.69 (d, $J = 7.9$ Hz, 1H, benzenesulfonamide H-3), 8.07 (d, $J = 7.9$ Hz, 1H, benzenesulfonamide H-6). Anal. Calcd for $C_{15}H_{13}NO_2S{\cdot}1/2\ H_2O$: C, 64.18; H, 4.99; N, 4.99. Found: C, 63.92; H, 4.85; N, 4.66.

6.12. 2-[2-(3-Fluorophenyl)ethynyl]benzenesulfonamide (11c)

Yield, 70%; yellow solid; mp 98–100 °C; ^{1}H NMR ($CDCl_3$) δ 5.19 (s, 2H, SO_2NH_2), 7.12–7.16 (m, 1H, 3-fluorophenyl H-4), 7.27–7.47 (m, 3H, 3-fluorophenyl H-2, H-5, H-6), 7.50–7.59 (m, 2H, benzenesulfonamide H-4, H-5), 7.71 (d, $J = 7.9$ Hz, 1H, benzenesulfonamide H-3), 8.08 (d, $J = 7.9$ Hz, 1H, benzenesulfonamide H-6). Anal. Calcd for $C_{14}H_{10}FNO_2S$: C, 61.08; H, 3.66; N, 5.09. Found: C, 61.36; H, 3.91; N, 4.81.

6.13. Molecular modeling (docking) studies

Docking experiments were performed using InsightII software Version 2000.1 (Accelrys Inc.) running on a Silicon Graphic Octane 2 R14000A workstation according to a previously reported method.[19]

6.14. In vitro cyclooxygenase (COX) inhibition assays

The ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and COX-2 (IC_{50} value, μM) was determined using an enzyme immuno assay (EIA) kit (Catalog No. 560101, Cayman Chemical, Ann Arbor, MI, USA) according to a previously reported method.[20]

6.15. Anti-inflammatory assay

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

6.16. Analgesic assay

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

Acknowledgments

We are grateful to (i) the Canadian Institutes of Health Research (CIHR) (MOP-14712) for financial support of this research and (ii) Rx&D-HRF/CIHR for a postdoctoral fellowship (to Q.H.C.).

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