

Full-length article

Design, synthesis and pharmacological evaluation of 4-[2-alkylthio-5(4)-(4-substitutedphenyl)imidazole-4(5)yl]benzenesulfonamides as selective COX-2 inhibitors¹Mona SALIMI², Mohammad Hossein GHAHREMANI³, Nima NADERI⁴, Mohsen AMINI⁵, Elika SALIMI⁶, Massoud AMANLOU⁵, Khosrou ABDI⁵, Raha SALEHI³, Abbas SHAFIEE^{5,7}

²Research and Development Center, Pasteur Institute of Iran, Kasas, Iran; ³Department of Pharmacology and Toxicology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran; ⁴Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran; ⁵Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran; ⁶Department of Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

Key words

cyclooxygenase-2 inhibitor; imidazole; alkylthio; celecoxib

¹ This project was supported by grants from the research council of Tehran University of Medical Sciences and Iran Chapter of TWAS (The Developing World of Academy of Sciences), and INSF (Iran National Science Foundation).

⁷ Correspondence to Dr Abbas SHAFIEE.
Phn 98-21-6640-6757.
Fax 98-21-6646-1178.
E-mail ashafiee@ams.ac.ir

Received 2006-10-23

Accepted 2007-01-07

doi: 10.1111/j.1745-7254.2007.00619.x

Abstract

Aim: To design and synthesize a series of benzenesulfonamide derivatives, 4-[2-alkylthio-5(4)-(4-substitutedphenyl)imidazole-4(5)-yl]benzenesulfonamides (4a–4j), which are intended to act as cyclooxygenase-2 (COX-2) inhibitors with good COX-2 inhibitor activity, and which will exert anti-inflammatory activities *in vivo*. **Methods:** Benzenesulfonamide derivatives were designed and synthesized through multi-step chemical reactions. All the synthesized compounds were evaluated in an *in vitro* assay. The active compound 4a–4f was selected for further evaluation in a carrageenan-induced rat paw edema model. **Results:** Docking studies showed that compound 4 bind into the primary binding site of COX-2 with the sulfonamide SO₂NH₂ moiety interacting with the secondary pocket amino acid residues. In the *in vitro* assay, compound 4 inhibited COX-2 with an inhibition concentration IC₅₀ value of 1.23–8 nmol/L, compared to celecoxib with IC₅₀ value of 1.5 nmol/L. Compound 4b and 4c had good potency and selectivity in comparison to the celecoxib. In the *in vivo* model, compound 4a–4f exhibited a moderate potency to inhibit 50% carrageenan-induced paw edema with value of 1.58–4.3 mg/kg. In the latter experiment, compound 4c was the most active compound. **Conclusion:** The anti-inflammatory effects obtained for compound 4a–4j could be due to the presence of fluorine or hydrogen substituents in the para position of the phenyl ring of these compounds.

Introduction

Non-steroidal anti-inflammatory drugs (NSAID) are among the most widely used prescriptions, primarily for the treatment of pain, bronchial asthma, allergy, and inflammation^[1]. Although modifications of established non-selective agents, such as the lengthening of the carboxyl side chain of indomethacin^[2] have been strategies for the design of cyclooxygenase-2 (COX-2) selective inhibitors^[3], the main effort has been addressed to the diarylheterocycle class^[4]. Many lead compounds reported to have selective COX-2

inhibitory activity have been clinically introduced to reduce inflammation with very little gastrointestinal GI side effects, namely, celecoxib^[5], rofecoxib^[6], valdecoxib^[7], lumiracoxib, etoricoxib^[8], and nimesulide. Also, recent studies have shown that selective COX-2 inhibitors can induce apoptosis in the colon, stomach, prostate, and breast cancer cell lines^[9–13].

Overall, these selective COX-2 inhibitors have fulfilled the hope of exhibiting a reduced risk in gastrointestinal events^[14]; however, the increased incidence of non-gastrointestinal, serious, adverse events with the COX-2 selective

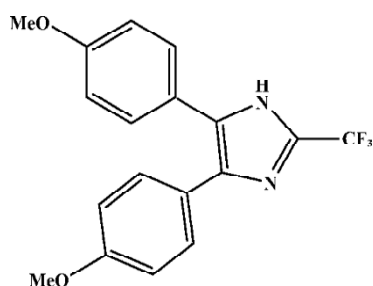


Figure 1. Structure of flunizole.

inhibitors as compared with non-selective NSAID in the Celecoxib Long-term Arthritis Safety Study and the Vioxx Gastrointestinal Outcomes Research study, remains a major concern^[15]. With all these aspects considered, developing drugs that preferentially inhibit COX-2 with moderate potency and selectivity was of interest, since the currently used, very selective COX-2 inhibitors cause unwanted side effects in a significant amount of people^[16].

Flunizole, an early known 4,5-diarylimidazole (Figure 1), and 4,5-diaryl-2-substituted thioimidazole, have been reported to exhibit anti-inflammatory activity^[14,17]. As a part of our ongoing research to design novel selective COX-2 inhibitors^[18–23], we describe herein, the design and biological evaluation of 4-[2-alkylthio-5(4)-(4-substitutedphenyl)imidazole-4(5)-yl]benzenesulfonamides as COX-2 inhibitors with anti-inflammatory activities.

Materials and methods

Animals Male Sprague Dawley rats, weighing 150–200 g ($n=6$), were supplied by Razi Institute, Tehran, Iran. All the animals were housed in Plexiglas cages on a 12/12 h light/dark cycle in temperature- and humidity-controlled rooms. Food was withheld 24 h before the experiments, but with free access to water. All the experiments confirmed to the guidelines of the committee on animal experiments at Tehran University of Medical Sciences (Tehran, Iran)

Chemicals and reagents All of the chemicals and reagents were purchased from Merck (KGaA, Darmstadt, Germany) and Sigma-Aldrich (St Louis, MO, USA).

Molecular modeling and chemistry Docking studies were performed using the Autodock 3.05 Package (Scripps Research Institute, La Jolla, California)^[24–26]. The coordinates of the X-ray crystal structure of selective COX-2 inhibitor S-58701 (B) bound to the murine COX-2 enzyme was obtained from the Protein Data Bank (www.rcsb.org) code 1CX2 and hydrogens were added. The ligand molecules were constructed using Chem-3D (CambridgeSoft,

Cambridge, MA) and were minimized for 500 iterations, reaching a convergence of 0.01 kcal/mol Å. The compound 4 was docked using Lamarckian genetic algorithm (LGA), where the number of GA=10, the population size=50, and the maximum number of energy evaluations is 250000. The result was analyzed using root mean square deviation (RMSD), estimated inhibition constant (K_i), and estimated free energy of binding (ΔG). The best resulting $\Delta G=-11.5$ kcal/mol was for compound 4c with RMSD 2.115 Å. The K_i was 3.72×10^{-9} .

The 4,5-diarylimidazole-2-thiones (compound 2), with substituents at the para position of one of the phenyl rings, was prepared in high yield (80%–90%) using ammonium thiocyanate and 2-oxo-1,2-diphenylethyl benzoates (compound 1) in amyl alcohol at 150–160 °C. Subsequent alkylation of compound 2 with alkyl iodide in methanol in the presence of triethylamine afforded 2-alkylthio-4,5-diarylimidazoles (compound 3, 24%–81%). The sequential chlorosulfonation of compound 3 with chlorosulfonic acid, followed by ammonia gave 4-[2-alkylthio-5(4)-(4-substitutedphenyl)imidazole-4(5)-yl]benzenesulfonamides (compound 4). The structure of compound 4 was confirmed by infrared, by proton nuclear magnetic resonance and Mass spectrometry^[23].

Biological assays

In vitro COX inhibition assay COX activity was determined by using arachidonic acid (AA) as substrate and *N,N,N,N*-tetramethylphenylenediamine (TMPD) as the cosubstrate, as previously described^[18,19,27]. The reaction mixture (200 μ L) contained 0.5 μ mol/L heme, 0.05 mmol/L TMPD, 0.1 mmol/L LAA, and 36 units of the COX-2 enzyme (57 units for COX-1) in 0.1 mol/L Tris/HCl (pH 8.1). The oxidation of the substrate, the starter of the reaction, was measured at 25 °C by monitoring the increase of absorbance at 630 nm. The inhibition of the studied compound 4^[23] was determined after pre-incubation for 5 min with the enzyme in the presence of heme, and the reaction was started by adding AA and TMPD. This mixture was incubated for another 5 min and the absorbance was measured on a strip reader. For synthesized compound 4, 10 μ L of scalar dilutions of the inhibitors in DMSO was added. Celecoxib, a potent and selective COX-2 inhibitor, was used as a reference drug. The average absorbance of all of the samples was determined. The absorbance of the test wells was normalized with background and calculated as the percentage of total activity: % test inhibition=100 (1-test abs/total activity abs) where test abs=absorbance in the test well and total activity abs=absorbance in the well without any inhibitor. The percentage of inhibition was used to calculate the inhibition concentration IC_{50} of the compound (concentration at which there was 50% inhibition).

In vivo methods The method of carrageenan-induced paw edema in rats^[28] was used to evaluate the anti-inflammatory activity. The treatment was performed 30 min before the injection of 50 μ L carrageenan 1% into the rat paw plantar surface. The foot volume was measured using a plethysmometer^[29] at 1 h intervals after the carrageenan injection for 3 h, but the activity was acknowledged only for the third hour, in which maximum edema occurred. The inflammation index was calculated as the difference between the final volume of the carrageenan injected paw (V_i) and the initial volume of the same paw before injection (V_o), that is, inflammation index (I_i)= $V_i - V_o$. The edema inhibition (%) was calculated as the percentage of the difference of I_i according to the following formula: % inhibition= $([pre\text{-}drug\ I_i] - [post\text{-}drug\ I_i]) / [pre\text{-}drug\ I_i] \times 100$.

In order to evaluate the anti-inflammatory effect of compound 4a–4f, 3 doses were used. The 50% inhibition of the compound (by definition, the dose required to reduce the carrageenan-induced paw edema to 50% of the control group) was calculated. The compound was injected intraperitoneally (ip) using the following doses: 5.1, 7.6, and 11.4 mg/kg for celecoxib (reference drug); 4.7, 7.2, and 10.7 mg/kg for compound 4a; 4.5, 6.4, and 10.3 mg/kg for compound 4b; 5.0, 7.5, and 11.3 mg/kg for compound 4c; 4.8, 7.2, and 10.9 mg/kg for compound 4d; 5.2, 7.8, and 11.8 mg/kg for compound 4e; and 5.1, 7.6, and 11.4 mg/kg for compound 4f. The rats of the control group received the same volume of DMSO according to their weight.

Statistical analysis The data were expressed as mean \pm SEM. One-way ANOVA with Tukey's *post-hoc* test was used, and $P < 0.05$ was considered statistically significant. The IC_{50} was calculated using the non-linear regression with cubic spline method.

Results

Molecular modeling and chemistry The docking study showed that compound 4 bound to the primary binding site of COX-2 with the sulfonamide SO_2NH_2 moiety interacting with the secondary pocket amino acid residues Phe⁵¹⁸, His⁹⁰, and Val⁵²³, which is comparable to S-58701 (B) (Figure 2). One of the *O*-atoms of the SO_2NH_2 substituent forms a hydrogen bond with the amide hydrogen of Phe⁵¹⁸ (2.5 Å). The N-atom of the SO_2NH_2 forms a hydrogen bond with His⁹⁰ (2.5 Å). The substituted phenyl ring lies in a hydrophobic cavity lined by Val³⁴⁹. The ethyl sulfide (EtS) substituent is oriented in the direction of the polar amino acid Arg¹²⁰, and Tyr³⁵⁵ and forms a weak hydrogen bond with them (4 Å). It is located in a hydrophobic region formed by Val¹¹⁶ and Leu⁵³¹. Also, the amino (NH) of

imidazole forms another hydrogen bond with Tyr³⁵⁵ (3 Å) (Figure 2). Considering the molecular modeling information, the synthetic reaction used for the synthesis of 4-[2-alkylthio-5(4)-(4-substitutedphenyl)imidazole-4(5)-yl]benzenesulfonamides (4a–4j) are outlined in Figure 3^[23].

In vitro assay The ability of compound 4a–4j to inhibit ovine COX-1 and COX-2 (IC_{50} values, nmol/L) was determined using a colorimetric COX (ovine) inhibitor screening assay. In this regard, compound 4a–4j exhibited a broad range of COX-2 inhibitory potency (Table 1).

In vivo evaluation The potent and selective COX-2 inhibitors emerging from the *in vitro* studies were evaluated in the acute carrageenan-induced rat paw edema. Pretreatment with celecoxib (0.02 mmol/kg) intraperitoneally resulted in a marked decrease in paw inflammation when compared to control group ($P < 0.001$). Repeated experiments with the same dose of compound 4a–4e also showed significant differences ($P < 0.001$) in anti-inflammatory effects on carrageenan hind paw edema (Figure 4). Compound 4a–4f (0.02 mmol/kg) induced protection against carrageenan-induced paw edema (Figure 5). The 50% inhibition (by definition, the dose required to reduce the carrageenan-induced paw edema to 50% of that of the control) for compound 4a–4f ranged from 1.58–4.3 mg/kg, while the 50% inhibition for the reference drug celecoxib was 2.90 mg/kg (Table 2).

Discussion

In this diarylheterocyclic class of COX-2 inhibitors, the initial modification was the insertion of sulfonamide at the para position of one of the phenyl ring and was held constant throughout of the structure-activity relationship (SAR) studies. Within the sulfonamide analogues (compound 4), modification at the alkylthio group at the C-2 position of the imidazole ring gave variable results. In the presence of smaller C-4 substituents (H, F; 4a–4d), increasing the size of alkylthio did not significantly affect the COX-2 inhibitory potency. However, in the presence of C-4 phenyl chloro substituent (4e, 4f), the size of C-2 alkylthio had an effect on COX-2 inhibition and increasing the size led to an increase in COX-2 potency.

There was, however, some sensitivity to the electronic property at the 4-position of this aromatic ring, particularly with regards to COX-2 potency. In the EtS-substituted compound, 4a, 4c, and 4e, the analogs with an electron-withdrawing group (4c, 4e), tended to increase COX-2 potency. In contrast, electron-donating group had poor COX-2 activity. Therefore, methyl (4g, 4h) and methoxy (4i, 4j) substituents all worked poorly in this regard. These results suggested that the electronic property of the substituents at

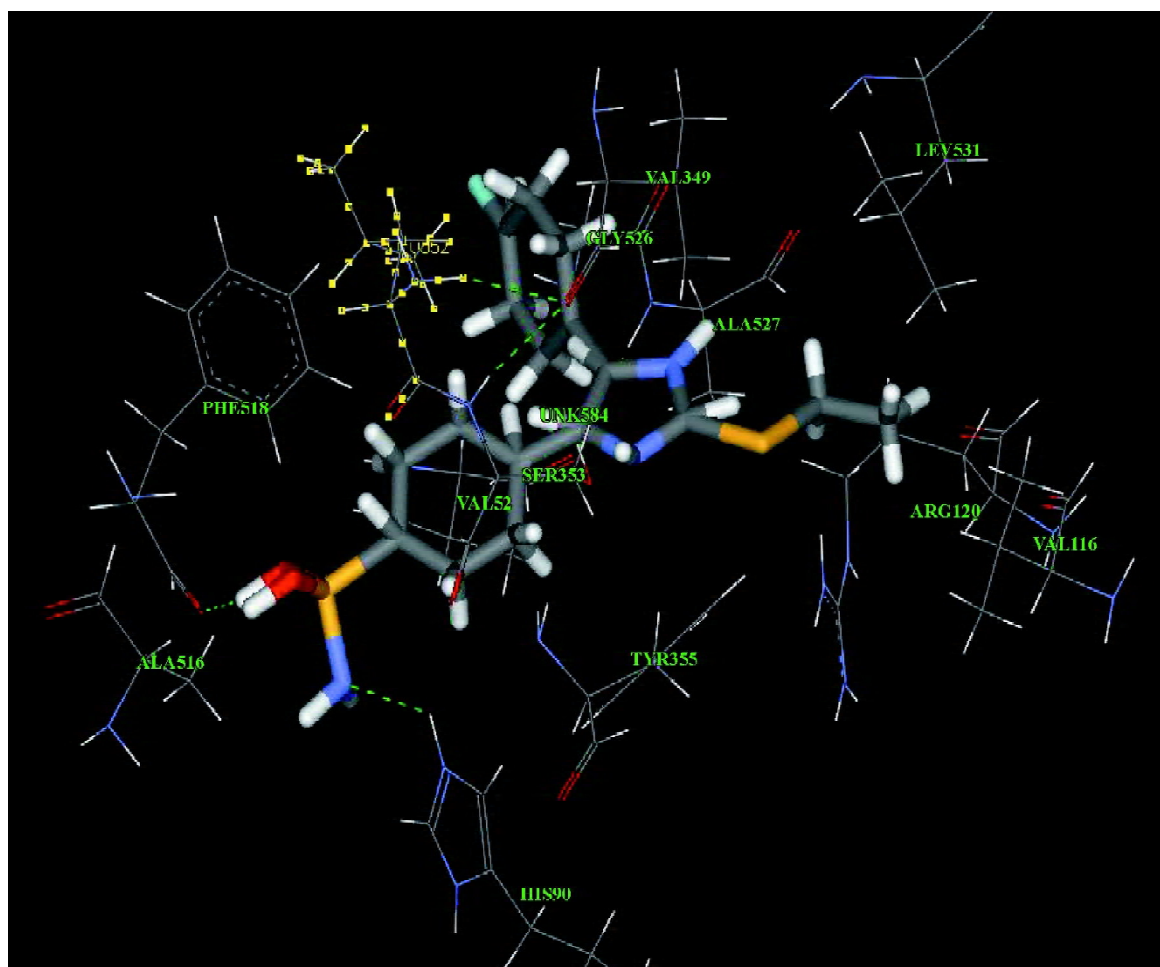


Figure 2. Docking of compound 4c (ball and stick) in the active site of murine COX-2.

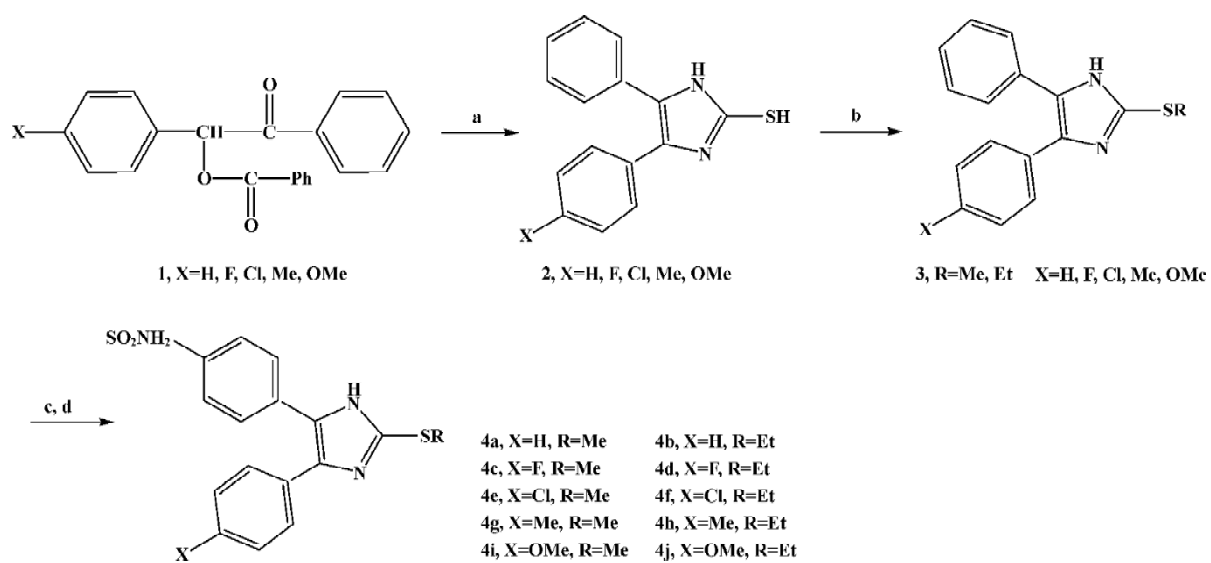
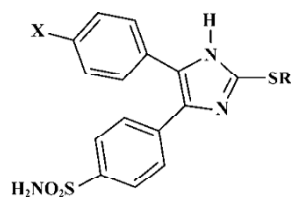


Figure 3. Reagents and conditions: (a) amyl alcohol, ammonium thiocyanate, reflux, 5 h; (b) methanol, triethylamine, methyl or ethyl iodide, 25 °C, 24 h; (c) ClSO₃H, 0 °C, 5 h; (d) methanol, NH₃(aq), 25 °C, 24 h.

Table 1. *In vitro* inhibition of COX-1 and COX-2 by 4-[2-alkylthio-5(4)-(4-substitutedphenyl) imidazole-4(5)yl]benzenesulfonamides (4a–4j). ^aValues are the means of 3 determinations acquired using the colorimetric screening assay. Each value represents the Mean±SEM. ^bSI, selectivity index.



Compound	X	R	COX-1 inhibition IC ₅₀ , nmol/L ^a	COX-2 inhibition IC ₅₀ , nmol/L	IC ₅₀ COX-1/COX-2 SI ^b
4a	H	Et	2.00±0.02	1.50±0.03	1.3
4b	H	Me	>20	1.55±0.02	13.3
4c	F	Et	4.47±0.01	1.23±0.03	3.6
4d	F	Me	2.95±0.02	1.35±0.03	2.18
4e	Cl	Et	2.98±0.02	1.25±0.02	2.38
4f	Cl	Me	2.00±0.02	2.00±0.03	1
4g	Me	Et	>10	>1.5	-
4h	Me	Me	>10	>2.5	-
4i	OMe	Et	>29	>7.0	-
4j	OMe	Me	>25	>8.0	-
Celecoxib			2.95±0.02	1.50±0.02	1.96

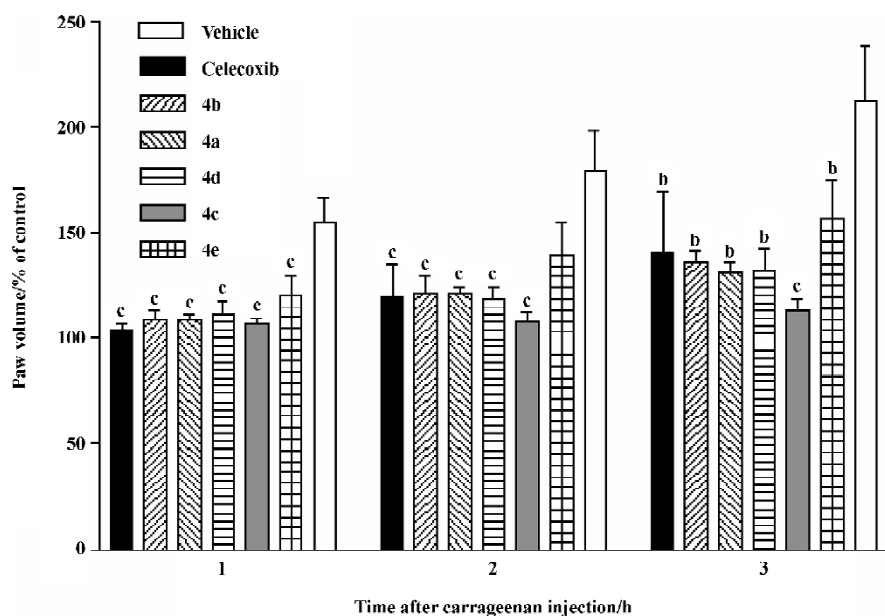


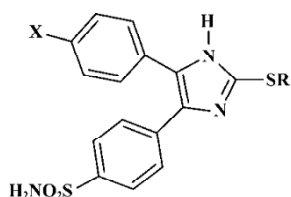
Figure 4. Time course of anti-inflammatory effect of different celecoxib derivatives (0.02 mmol/kg) on carrageenan-induced paw edema in rats. Carrageenan 1% (50 µg) was injected into the plantar surface of the right hind paw, 30 min after ip injection of the drugs. Increases in paw volume and edema were measured before and 1, 2, and 3 h after the carrageenan injection. Values are presented as Mean±SEM (n=6). ^bP<0.05, ^cP<0.01 vs the vehicle group.

the para position of the phenyl ring can influence the COX-2 inhibitory activity.

In the *in vivo* studies, the fluorine and hydrogen analogs (4a–4d) showed a good inhibition on edema (Table 2). The other derivative, compound 4e and 4f, despite good COX-2

potency, was moderately active *in vivo*. In general, *in vivo* data prove our SAR of compound 4a–4j. Its potency is greatly influenced by the substitution pattern and shows that para fluorine or hydrogen besides the SO₂NH₂ pharmacophore represents a series of anti-inflammatory agents, which pref-

Table 2. *In vivo* evaluation of compound 4a–4f. *n*=6, 95% confidence limits in parentheses, ^b*P*<0.05.



Compound	X	R	50% inhibition ^b mg/kg
4a	H	Et	2.80 (±0.50)
4b	H	Me	2.90 (±0.55)
4c	F	Et	1.58 (±0.80)
4d	F	Me	2.18 (±0.43)
4e	Cl	Et	4.30 (±1.88)
4f	Cl	Me	3.85 (±1.19)
Celecoxib			2.90 (±0.53)

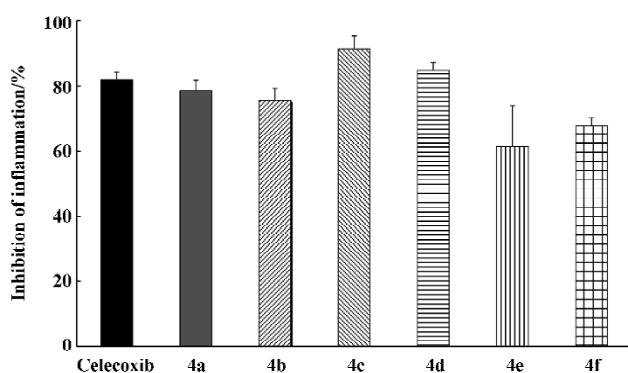


Figure 5. Anti-inflammatory effect of different doses of celecoxib derivatives (0.02 mmol/kg) on reducing carrageenan-induced paw edema. The drugs were administered 0.5 h before carrageenan injection. Edema was measured 3 h after the carrageenan injection. Each point is presented as mean±SEM (*n*=6).

erentially inhibit COX-2 with moderate potency and selectivity.

The potent *in vitro* and *in vivo* COX-2 inhibition exhibited by compound 4c is consistent with observations from a molecular modeling experiment where compound 4c was docked in the active site of the COX-2 enzyme. Molecular modeling studies show that the critical difference between the binding sites for COX-1 and COX-2 is at position 523 where COX-2 has the amino acid residue Val in place of the bulkier Ile in COX-1. This difference produces a secondary

pocket extending off the primary binding site in COX-2 that is absent in COX-1. Consequently, the combined volume of the primary binding site and the secondary pocket in COX-2 is about 25% larger than the volume of the COX-1 binding site^[30,31]. This difference in volume can be exploited to manipulate COX-2 selectivity of the diarylheterocyclic class of COX-2 inhibitors by varying the volume of the drug and the appropriate placement of substituents with varying electronic and steric properties^[32]. Designed compound 4c binds in the center of the active site with the phenylsulfonamide moiety oriented toward the secondary pocket region where it can undergo *H*-bonding via one of its SO₂ oxygen atoms and NH₂ group of the sulfonamide moiety with Phe⁵¹⁸ and His⁹⁰. Interestingly, the C-2 EtS substituent is located in a hydrophobic region, with the *S*-atom forming a weak hydrogen bond with the Tyr³⁵⁵ and Arg¹²⁰. This shows the importance of the C-2 substituent in orienting the molecule such that the sulfonamide moiety inserts into the secondary pocket of COX-2. The ring *N*-atom of the central imidazole is oriented in the direction of the polar amino acid Tyr³⁵⁵, where this *N*-atom is about 3 Å away from the NH₂ of Tyr³⁵⁵. This interaction may disrupt the salt bridge between His⁹⁰, Arg¹²⁰, and Tyr³⁵⁵ at the mouth of the COX-2 active site (Figure 2). The similarity between the *K_i* (S-8701 B) as the reference drug and the *K_i* which was calculated for compound 4c, shows that changing the structure from S-8701 B to compound 4c does not decrease the binding for the COX-2 enzyme. However, introducing the imidazole ring, ethyl sulfide SET substituent, and SO₂NH₂ pharmacophore improves the binding, which is a result of the hydrogen bonds. These observations confirm the suggested SAR for COX-2 inhibitory activity.

Our observations provide a good explanation for these results: (i) compound 4 with good COX-2 inhibitory potency and selectivity can be designed by the appropriate placement of the para *P*-SO₂NH₂ pharmacophore on the C-4 phenyl ring, in which the 2-alkylthio imidazole ring serves as a suitable central ring template; and (ii) COX-2 inhibitory potency and selectivity is sensitive to substituent electronic property at the para position of the phenyl ring where compound 4b exhibits the best combination of potency and selectivity and compound 4c exhibits better potency on COX-2, but lower selectivity compared to compound 4b.

References

- 1 Hansch C, Sammes PG, Taylor JB. The rational design, mechanistic study and therapeutic application of chemical compounds. Comprehensive medicinal chemistry; v 6. Oxford: Pergamon Press; 1990.

- 2 Leblanc Y, Black WC, Chan CC, Charleson S, Delorme D, Denis D, *et al*. Synthesis and biological evaluation of both enantiomers of L-761,000 as inhibitors of cyclooxygenase. *Bioorg Med Chem Lett* 1996; 6: 731–6.
- 3 Kalgutkar AS. Selective cyclooxygenase-2 inhibitors as non-ulcerogenic anti-inflammatory agents. *Exp Opin Ther Pat* 1999; 9: 831–49.
- 4 Reitz DB, Isakson PC. Cyclooxygenase-2 inhibitors. *Curr Pharm Des* 1995; 1: 211–20.
- 5 Penning T, Talley J, Bertenshaw S, Carter J, Collins P, Docter S, *et al*. Synthesis and biological evaluation of 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: Identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazole-1-yl] benzene-sulfonamide (SC-58635, Celecoxib). *J Med Chem* 1997; 40: 1347–65.
- 6 Prasit P, Wang Z, Brideau C, Chan CC, Charleson S, Cromlish W, *et al*. The discovery of rofecoxib, [MK 966, Vioxx (R), 4-(4'-methylsulfonylphenyl)-3-phenyl-2(5H)-furanone], an orally active cyclooxygenase-2 inhibitor. *Bioorg Med Chem Lett* 1999; 9: 1773–8.
- 7 Talley JJ, Brown DL, Carter JS, Graneto MJ, Koboldt CM, Masferrer JL, *et al*. 4-[5-methyl-3-phenylisoxazol-4-yl]-benzenesulfonamide, valdecoxib: a potent and selective inhibitor of COX-2. *J Med Chem* 2000; 43: 775–7.
- 8 Riendeau D, Percival MD, Brideau C, Charleson S, Dube D, Ethier D, *et al*. Preclinical profile and comparison with other agents that selectively inhibit cyclooxygenase-2. *J Pharmacol Exp Ther* 2001; 296: 558–66.
- 9 Arico S, Pattingre S, Baury C, Gane P, Barbat A, Codogno P, *et al*. Celecoxib induces apoptosis by inhibiting 3-phosphoinositide dependent protein-kinase-1 activity in the human colon cancer. *J Biol Chem* 2002; 277: 27 613–21.
- 10 Davies G, Martin LA, Sacks N, Dowsett M. Cyclooxygenase-2 (COX-2), aromatase and breast cancer: a possible role for COX-2 inhibitors in breast cancer chemoprevention. *Ann Oncol* 2002; 13: 669–78.
- 12 Liu HX, Kirschenbaum A, Yao S, Lee R, Holland FJ, Levine CAJ. Inhibition of cyclooxygenase-2 suppresses angiogenesis and the growth of prostate cancer *in vivo*. *J Urol* 2000; 164: 820–5.
- 13 Sawaoka H, Kawano S, Tsuji S, Tsuji M, Gunawan ES, Takei Y, *et al*. Cyclooxygenase-2 inhibitors suppress the growth of gastric cancer xenografts via induction of apoptosis in nude mice. *Am J Physiol* 1998; 274: G1061–7.
- 14 Khanna IK, Weier RM, Yu Y, Xu XD, Koszyk FJ, Collins PW, *et al*. 1,2-Diarylimidazoles as potent, cyclooxygenase-2 selective, and orally active anti-inflammatory agents. *J Med Chem* 1997; 40: 1634–47.
- 15 Wright JM. The double-edged sword of COX-2 selective NSAIDs. *CMAJ* 2002; 167: 1131–7.
- 16 Kontogiorgis CA, Hadjipavlou-Litina DJ. Synthesis and anti-inflammatory activity of coumarin derivatives. *J Med Chem* 2005; 48: 6400–8.
- 17 Niedballa U, Bottcher I. Antiinflammatory 4,5-diphenyl-2-substituted-thio-imidazoles and their corresponding sulfoxides and sulfones. US patent 4 440 776. 1984 Apr 03.
- 18 Navidpour L, Shafaroodi H, Abdi KH, Amini M, Ghahremani MH, Dehpour AR, *et al*. Design, synthesis, and biological evaluation of substituted 3-alkylthio-4,5-diaryl-4*H*-1,2,4-triazoles as selective COX-2 inhibitors. *Bioorg Med Chem* 2006; 14: 2507–17.
- 19 Navidpour L, Amini M, Shafaroodi H, Abdi KH, Ghahremani MH, Dehpour AR, *et al*. Design and synthesis of new water-soluble tetrazolide derivatives of celecoxib and rofecoxib as selective cyclooxygenase-2 (COX-2) inhibitors. *Bioorg Med Chem Lett* 2006; 16: 4483–7.
- 20 Navidpour L, Karimi L, Amini M, Vosoughi M, Shafiee A. Syntheses of 5-alkylthio-1,3-diaryl-1,2,4-triazoles. *J Heterocyclic Chem* 2004; 41: 201–4.
- 21 Karimi L, Navidpour L, Amini M, Shafiee A. Synthesis of 4,5-Diaryl-1,2,3-thiadiazoles. *Phosphorus Sulfur and Silicon and the Related Elements* 2005; 180: 1593–600.
- 22 Johari Daha F, Matloubi H, Tabatabai SA, Shafiee B, Shafiee A. Synthesis of 1-(4-methylsulfonylphenyl)-5-aryl-1,2,3-triazoles and 1-(4-aminosulfonylphenyl)-5-aryl-1,2,3-triazoles. *J Heterocyclic Chem* 2005; 42: 33–7.
- 23 Salimi M, Amini M, Shafiee A. Syntheses of 2-alkylthio-(4,5-diaryl) imidazoles. *Phosphorus Sulfur Silicon* 2005; 180: 1587–92.
- 24 Goodsell DS, Olson AJ. Automated docking of substrates to proteins by simulate annealing. *Proteins: structure function and genetics* 1990; 8: 195–202.
- 25 Morris GM, Goodsell DS, Huey R, Olson AJ. Distributed automated docking of flexible ligands to proteins: Parallel applications of autodock 2.4. *J Computre-aided Mol Design* 1996; 10: 293–304.
- 26 Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, *et al*. Automated docking using Lamarckian genetic algorithm and an empirical binding free energy function. *J Comp Chem* 1998; 19: 1639–62.
- 27 Kulmacz RJ, Lands WEM. Requirements for hydroperoxide by the cyclooxygenase and peroxidase activities of prostaglandin H synthase. *Prostaglandins* 1983; 25: 531–40.
- 28 Winter CA, Riset EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc Soc Exp Biol Med* 1962; 111: 544–7.
- 29 Ahmadiani A, Fereidoni M, Semnianian S, Kamalinejad M, Saremi S. Antinociceptive and anti-inflammatory effects of sambucus ebulus rhysome extracts in rats. *J Ethnopharmacol* 1998; 61: 229–35.
- 30 Luong F, Miller A, Barnett J, Chow J, Ramesha C, Browner MF. Flexibility of the NSAIDs binding site in the structure of human cyclooxygenase-2. *Nat Struct Biol* 1996; 3: 927–33.
- 31 Gierse JK, McDonald JJ, Hauser SD, Rangwala SH, Koboldt CM, Seibert K. A single amino acid difference between cyclooxygenase-1 (COX-1) and 2- (COX-2) reverse the selectivity of COX-2 specific inhibitors. *J Biol Chem* 1996; 271: 15810–4.
- 32 Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegman RA, Pak JY, *et al*. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature* 1996; 384: 644–8.