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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and synthesis of new 2,4,5-triarylimidazole derivatives as selective cyclooxygenase (COX-2) inhibitors

A. Zarghi · S. Arfaei · R. Ghodsi

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Abstract A new group of 2,4,5-triarylimidazole derivatives, possessing a methyl sulfonyl pharmacophore, were synthesized and their biological activities were evaluated for cyclooxygenase-2 (COX-2) inhibitory activity. In vitro COX-1/COX-2 structure–activity relationships were determined by varying the substituents at the *para* position of C-2 phenyl ring. Among the 2,4,5-triarylimidazoles, 2-(4-hydroxy phenyl)-4-(4-methylsulfonylphenyl)-5-phenyl-1*H* imidazole (**11f**) was identified as a selective COX-2 inhibitor (COX-2 IC50 = 0.15 μM; selectivity index = 75) that was less potent than the reference drug celecoxib (COX-2 IC50 = 0.06 μM; SI = 405). A molecular modeling study where **11f** was docked in the binding site of COX-2 showed that the methylsulfonyl pharmacophore group is oriented in the vicinity of the COX-2 secondary pocket (Arg^{513}, Phe^{518}, Gly^{519}, and Val^{523}). The structure–activity data acquired indicate that COX-1/COX-2 inhibition is sensitive to the nature of the C-2 phenyl substituents.

Keywords 2,4,5-Triarylimidazoles · COX-2 inhibition · SAR

Introduction

The non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of pain, inflammation and fever. NSAIDs were discovered to act via inhibition of the cyclooxygenase (COX) enzyme, which catalyzes the first step of the biosynthesis of prostaglandins (PGs) from arachidonic acid (Fu *et al.*, 1990; Vane, 2000; Dannhardt and Kiefer, 2001). However, the gastrointestinal (GI) toxicities associated with widespread use of NSAIDs are proved to be a major problem during long-term therapy. COX activity has been found to be associated with at least two isozymes, COX-1 and COX-2. The COX isoforms are heme-containing enzymes that inhibit distinct expression roles in several physiological processes. The COX-1 isozyme is constitutively expressed in many tissues and appears to be important for protection of gastric mucosa, platelet aggregation, and renal blood flow (Smith and DeWitt, 1996). In contrast, the COX-2 isozyme is inducible and expressed by stimuli like mitogenes and oncogenes, growth factors and disorders of water–electrolyte homeostasis linking its involvement to pathological processes such as inflammation and various types of cancer (Kanaoka *et al.*, 2007; Kawamori *et al.*, 1998; Katori and Majima, 2000; Liao *et al.*, 2007). Because PGs are involved in the maintenance of GI mucosal integrity and because only COX-1 is present in the normal GI mucosa, the GI side effects of NSAIDs have been proposed to result from inhibition of COX-1 activity (Eberhart and Dubois, 1995). Thus, selective inhibition of COX-2 over COX-1 is useful for the treatment of inflammation and inflammation-associated disorders with reduced gastrointestinal toxicities when compared with NSAIDs. Recent studies have shown that COX-2 inhibition improves β-amyloid mediated suppression of memory and synaptic plasticity (Ho *et al.*, 2006). This suggests that the selective COX-2 inhibitors may prevent the progression of Alzheimer's disease by blocking the COX-2 mediated PGE2 response at synapses. Several new COX inhibitors were developed which selectively inhibit the COX-2 isoenzyme without interfering

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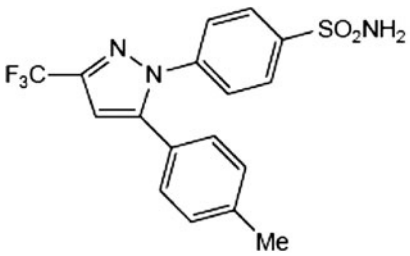
with COX-1 enzymatic activity (Penning *et al.*, 1997; Prasit *et al.*, 1999; Talley *et al.*, 2000a, b; Riendeau *et al.*, 2002; Zarghi *et al.*, 2007, 2009). These selective COX-2 inhibitors mainly belong to a class of diarylheterocycles that possess two vicinal diaryl substitution attached to a central hetero or carbocyclic ring system (see structures **1–6** in Chart 1). The recent withdrawal of some diaryl-heterocyclic selective COX-2 inhibitors such as rofecoxib, and valdecoxib due to their adverse cardiovascular side effects (Dogné *et al.*, 2005) clearly delineates the need to explore and evaluate new structural ring templates having selective COX-2 inhibitory activity. As part of our ongoing program to design new types of selective COX-2 inhibitors, we now describe the design, synthesis, cyclooxygenase inhibitory, and docking studies of a new group of 2,4,5-triarylimidazole derivatives having an imidazole cental ring scaffold and different substituents at the *para* position of C-2 phenyl ring, in order to investigate the effect of these substituents on the inhibition of COX-2 activity.

Results and discussions

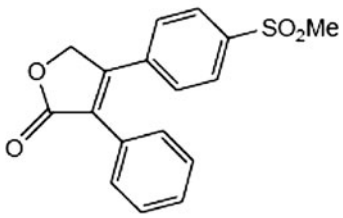
Chemistry

As shown in Scheme 1, the target 2,4,5-triarylimidazole derivatives were synthesized from 1,2-diketone **9** and appropriate aldehyde **10** in the presence of NH_4OAc under microwave irradiation (Wolkenberg *et al.*, 2004). 1,2-Diketone **9** was prepared as previously reported method (Singh *et al.*, 2004). Accordingly, 1-(4-(methylthio)phenyl)-2-phenylethanone **7** was prepared by a Friedel–Craft reaction of phenylacetylchloride and thioanisole using $AlCl_3$ as catalyst. Consequently, 1-(4-(methylthio)phenyl)-2-phenylethanone **7** was oxidized to 1-(4-(methylsulfonyl)phenyl)-2-phenylethanone **8** using oxone in THF-H_2O media. Then, oxidation of **8** using selenium dioxide gave (4-(methylsulfonyl)phenyl)-2-phenyl ethanone-1,2-dione **9.** The purity of all products was determined by thin layer chromatography several solvent systems of different polarity. All compounds were pure and

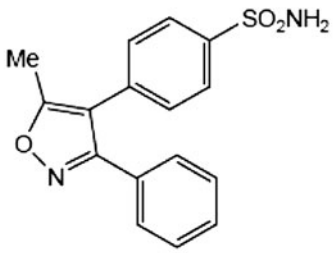
Chart 1 Representative examples of selective COX-2 inhibitors



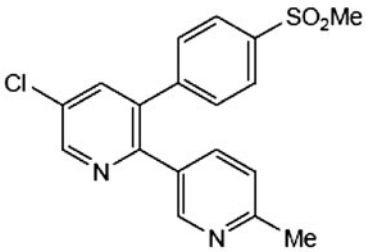
Celecoxib (**1**)



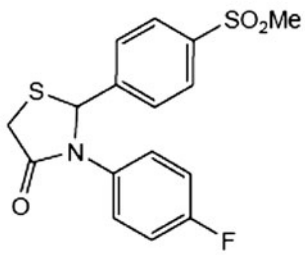
Rofecoxib (**2**)



Valdecoxib (**3**)



Etoricoxib (**4**)



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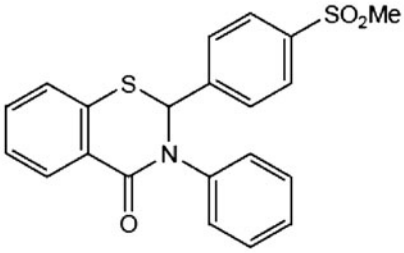

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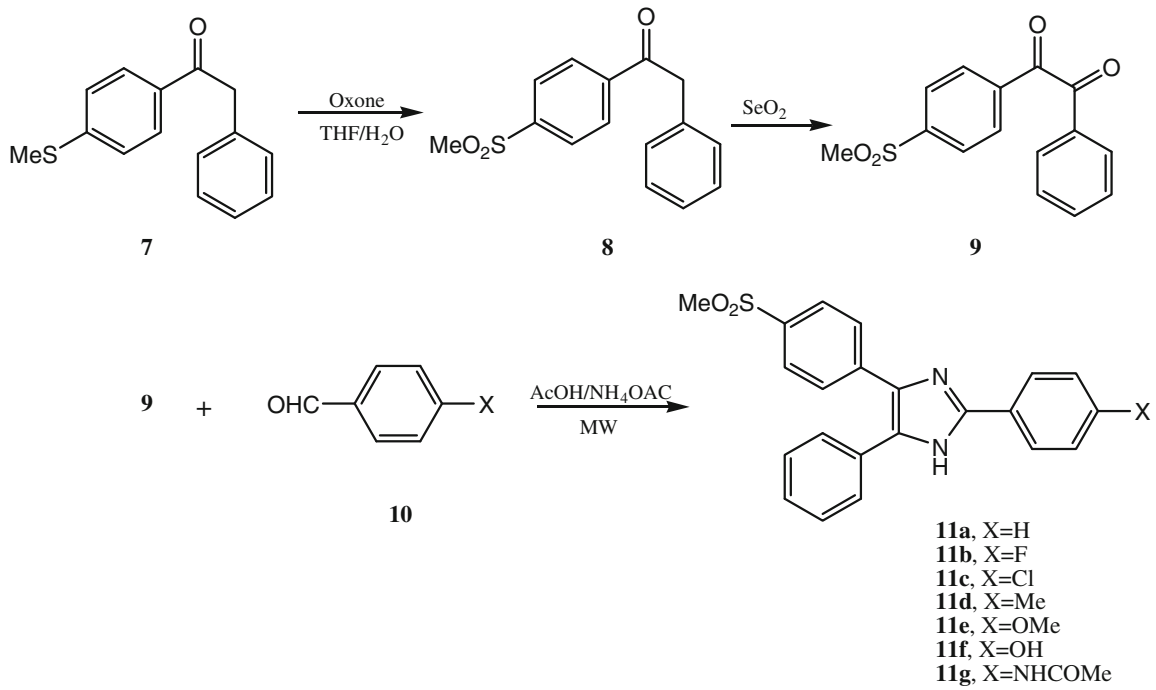


Scheme 1 Synthesis of 2,4,5-triarylimidazole derivatives

stable. The compounds were characterized by ^{1}H nuclear magnetic resonance, infrared and CHN analysis.

The physical data of final synthesized derivatives are summarized in Table 1.

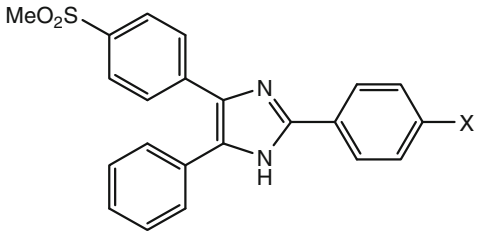
Enzyme inhibitory activity

The ability of the 2,4,5-triarylimidazole **11a–g** to inhibit the COX-1 and COX-2 isozymes was determined using chemiluminescent enzyme assays as previously described (Zarghi *et al.*, 2007). Enzyme inhibition data are given in Table 2. In vitro COX-1/COX-2 inhibition studies showed that the synthesized compounds **11a–g** were selective inhibitors of the COX-2 isozyme with IC50 values in the moderately potent 0.15 to 0.35 μM range, and COX-2 selectivity indexes (SI) in the 29–75 range. The structure–activity relationship study of these compounds indicated that the order of COX-2 selectivity was OH > F > O-Me > H, Me > NHCOMe > Cl. These results showed that the nature of substituent at *para* position of C-2 phenyl ring has an important role on selectivity and potency. According to these results, 2-(4-hydroxyphenyl)-4-(4-methylsulfonylphenyl)-5-phenyl-1*H*-imidazole **11f** was the most potent (IC50 = 0.15 μM), and selective (SI = 75), COX-2 inhibitor among the synthesized compounds. These data suggest that the compound **11f** should inhibit the synthesis of inflammatory prostaglandins via the cyclooxygenase pathway at sites of inflammation and has less ulcerogenicity due to the low COX-1 inhibitory activity.

Docking study

The orientation of the most potent and selective COX-2 inhibitor, 2-(4-hydroxy phenyl)-4-(4-methylsulfonylphenyl)-5-phenyl-1*H*-imidazole **11f** in the COX-2 active site was examined by a docking experiment (Fig. 1) (Goodsell *et al.*, 1996; Kurumbail *et al.*, 1996). This molecular modeling shows that it binds in the primary binding site such that the C-2 *p*-SO_2Me substituent inserts into the 2° pocket present in COX-2. One of the *O*-atoms of *p*-SO_2Me forms a hydrogen binding interaction with amino group of Arg^{513} (distance = 4.2 Å) whereas the other *O*-atom is close to other hydrogen of amino group of this amino acid (distance = 5.0 Å). The N*H* of the central 1*H*-imidazole ring forms hydrogen bond (distance = 4.3 Å) with the C=*O* group of Tyr348. In addition, the *OH* substituent at *para* position of C-2 phenyl ring is close to hydroxyl group of Ser530 which can form hydrogen binding interaction together and therefore, may explain the higher potency of compound **11f** compared with other derivatives. These observations together with experimental results provide a good explanation for selective inhibitory activity of **11f**.

Table 1 Physical data of the synthesized compounds



Compound	X	Color	Mp (°C)	Yield (%)	Molecular formula[a]	Molecular weight
11a	H	White	242–243	31	$C_{22}H_{18}N_2O_2S$	374.4
11b	F	Pale yellow	269–270	28	$C_{22}H_{17}N_2O_2FS$	392.4
11c	Cl	White	226–228	50	$C_{22}H_{17}N_2O_2ClS$	408.9
11d	Me	Pale yellow	234–235	34	$C_{23}H_{20}N_2O_2S$	388.5
11e	OMe	Pale yellow	230–232	54	$C_{23}H_{20}N_2O_3S$	404.5
11f	OH	Pale yellow	360	49	$C_{22}H_{18}N_2O_3S$	390.5
11g	NHCOMe	Pale yellow	340	58	$C_{24}H_{21}N_3O_3S$	431.5

[a] Satisfactory analysis for C, H, N was obtained for all the compounds within ±0.4% of the theoretical values

Experimental

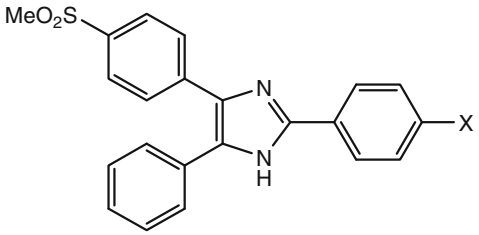
Materials

All reagents purchased from the Aldrich (USA) or Merck (Germany) Chemical Company and were used without further purifications.

General

Melting points (mp) were determined using a Thomas Hoover melting point apparatus (Philadelphia, USA). Infrared spectra were acquired on a Perkin-Elmer 1420 ratio recording spectrometer. A Bruker FT-500 MHz instrument (Brucker Biosciences, Germany) was used to acquire ^{1}H NMR spectra; chloroform-D used as solvent. Coupling constant (*J*) values are estimated in hertz (Hz) and spin multiples are given as s (singlet), d (double), t (triplet), q (quartet), m (multiplet), and br (broad). The mass spectral measurements were performed on an 6410Agilent LCMS triple quadrupole mass spectrometer (LCMS) with an electrospray ionization (ESI) interface. Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus (Perkin Elmer, Norwalk, CT, USA). The results of the elemental analyses (C,H,N) were within ± 0.4% of the calculated amounts.

Table 2 In vitro COX-1 and COX-2 enzyme inhibition data



Compound	X	IC_{50} (μM)[a]		COX-2 SI[b]
		COX-1	COX-2	
11a	H	9.9	0.23	43.1
11b	F	11.12	0.16	69.5
11c	Cl	10.15	0.35	29.0
11d	Me	10.40	0.24	43.3
11e	OMe	10.56	0.20	52.8
11f	OH	11.25	0.15	75.0
11g	NHCOMe	10.38	0.31	33.5
Celecoxib		24.3	0.06	405

[a] Values are mean values of two determinations acquired using an ovine COX-1/COX-2 assay kit, where 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})

Preparation of 1-(4-(methylthio)phenyl)-2-phenylethanone 7

2 ml (15.1 mmol) phenylacetyl chloride was added to a suspension of 2.1 g (15.8 mmol) AlCl3 in 25 ml dried dichloromethane under Argon atmosphere. The temperature was kept at 0–5°C. Then, 1.5 ml (12 mmol) thioanisole was added drop wise. After 2 hours, the temperature was let to approach the room temperature and stirring continued for 24 h. Then, the reaction mixture was added to crushed

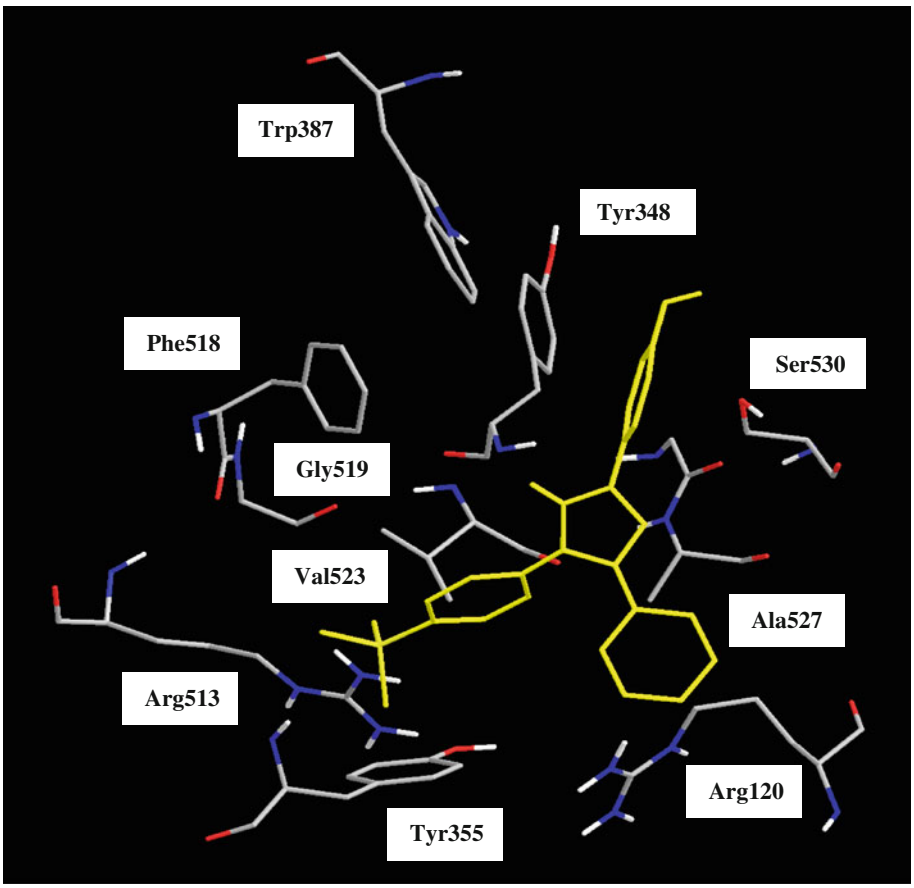


Fig. 1 Compound **11f** 2-(4-hydroxyphenyl)-4-(4-methylsulfonylphenyl)-5-phenyl-1*H*-imidazole docked in the active site of murine COX-2 isozyme

ice and extracted with dichloromethane. The organic solvent was washed with saturated $NaHCO_3$ and dried with sodium sulfate and then evaporated. The product was recrystallized in ethanol. Yield: 84%; white crystalline powder; mp: 96–97°C; IR (KBr): ν (cm^{-1}) 1675 (C=O); MS *m/z* (%): 242.2 (M^+, 10), 197.1 (10), 151.1 (100), 123.1 (20), 91.0 (20), 79.1 (20).

Preparation of 1-(4-(methylsulfonyl)phenyl)-2-phenylethanone **8**

1 g (4.1 mmol) of **8** was dissolved in 20 ml THF, and 6 g oxone in THF/water was added. The mixture was stirred at room temperature for 12 h. After evaporation of THF (30 ml), the residue was extracted with chloroform. The organic solvent was washed with saturated $NaHCO_3$ and dried with sodium sulfate and then evaporated. The product was recrystallized in ethanol to obtain white crystalline powder. mp: 169–170°C; IR (KBr): ν (cm^{-1}) 1695 (C=O), 1300, 1160 (SO2); ^{1}H NMR *(CDCl3)*: δ 3.14 (s, 3H, SO_2Me), 4.36 (s, 2H, CH_2), 7.29–7.41 (m, 5H, phenyl), 8.07 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, J = 8.51 Hz), 8.20 (d, 2H, 4-methyl sulfonylphenyl H_3 and H_5, J = 8.51 Hz); MS *m/z* (%): 274.2 (M^+, 5), 183.1(45), 151.9 (20), 121.0 (60), 91.0 (100), 76.1 (70).

Preparation of 1-(4-(methylsulfonyl)phenyl)-2-phenylethane-1,2-dione **9**

8 g (72 mmol) selenium dioxide was dissolved in a mixture of 96 ml dioxane and 4 ml water by heating. Then it was cooled to room temperature and 4 g (14.6 mmol) of **8** in THF was added to above-mentioned solution and refluxed overnight. The selenium was filtered off and the filtrate was poured to crushed ice and extracted with ethyl acetate. The organic phase was washed with water and dried with sodium sulfate and then evaporated. The obtained product was crystallized in ethyl acetate-hexane. mp: 118–120°C; IR (KBr): ν (cm^{-1}) 1670 (C=O), 1300, 1155 (SO_2); ^{1}H NMR ($CDCl_3$): δ 3.15 (s, 3H, SO_2Me), 7.57–7.75 (m, 3H, phenyl H_3–H_5), 8.02 (d, 2H, phenyl H_2 and H_6, J = 8.08 Hz), 8.15 (d, 2H, 4-methylsulfonyl phenyl H_2 and H_6, J = 8.50 Hz), 8.22 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, J = 8.50 Hz); MS *m/z* (%): 288.2 (M^+, 5), 183.1 (100), 165.2 (20), 121.0 (80), 105.1 (90), 91.1 (50), 76.1 (30).

General procedure for preparation of 2-(4-substitutedphenyl)-4-(4-methylsulfonyl phenyl)-5-phenyl-1*H*-imidazole **11a–g**

Equivalent amounts of diketone **9** and appropriate aldehyde **10** along with 2 g ammonium acetate in 6 ml glacial acetic acid were placed in microwave reactor for 10 min, while the power was set at 180 W. After cooling, the solution was neutralized with aqueous ammonia in which the product precipitated immediately. The precipitate was filtered and washed with water and recrystallized in methanol (Yields: 28–58%).

2,5-Diphenyl-4-(4-methylsulfonylphenyl)-1H-imidazole ***11a***

Yield: 31%; white crystalline powder; mp: 242–243°C; IR (KBr): ν (cm^{-1}) 3320 (NH), 1300, 1150 (SO_2), ^{1}H NMR (DMSO-D_6): δ 3.21 (s, 3H, SO_2Me), 7.38–7.53 (m, 6H, phenyl), 7.56 (d, 2H, 5-phenyl H_2 and H_6, $J = 8.16$ Hz), 7.78 (d, 2H, 4 methylsulfonylphenyl H_2 and H_6, $J = 7.41$ Hz), 7.84 (d, 2H, 2-phenyl H_2 and H_6, $J = 8.13$ Hz), 8.11 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, $J = 7.41$ Hz), 12.91(s, 1H, NH). Anal. Calcd. for $C_{22}H_{18}N_2O_2S$: C, 70.56; H, 4.84; N, 7.48. Found: C, 70.85; H, 5.02; N, 7.19.

2-(4-Fluorophenyl)-4-(4-methylsulfonylphenyl)-5-phenyl-1H-imidazole ***11b***

Yield: 28%; pale yellow crystalline powder; mp: 269–270°C; IR (KBr): ν (cm^{-1}) 3290 (NH), 1310, 1155 (SO_2), ^{1}H NMR (DMSO-D_6): δ 2.90 (s, 3H, SO_2Me), 6.98 (t, 2H, 4-fluorophenyl H_3 and H_5), 7.23 (m, 3H, phenyl H_3–H_5), 7.56 (d, 2H, phenyl H_2 and H_6, $J = 7.80$ Hz), 7.63 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, $J = 6.80$ Hz), 7.71 (d, 2H, 4-fluorophenyl H_2 and H_6, $J = 7.84$ Hz), 7.97 (d, 2H, 4-methyl sulfonylphenyl H_3 and H_5, $J = 6.80$ Hz), 9.78 (s, 1H, NH). Anal. Calcd. for$C_{22}H_{17}N_2O_2FS$: C, 67.33; H, 4.37; N, 7.14. Found: C, 67.65; H, 4.70; N, 7.01.

2-(4-Chlorophenyl)-4-(4-methylsulfonylphenyl)-5-phenyl-1H-imidazole ***11c***

Yield: 50%; white crystalline powder; mp: 226–228°C; IR (KBr): ν (cm^{-1}) 3290 (NH), 1290, 1150 (SO_2), ^{1}H NMR (DMSO-D_6): δ 3.21 (s, 3H, SO_2Me), 7.31–7.54 (m, 5H, phenyl H_3–H_5 and 4-chlorophenyl H_3 and H_5), 7.58 (d, 2H, phenyl H_2 and H_6, $J = 8.39$ Hz), 7.79 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, $J = 8.51$ Hz), 7.84 (d, 2H, 4-chlorophenyl H_2 and H_6, $J = 8.42$ Hz), 8.11 (d, 2H, 4-methylsulfonylphenyl H_3 and H_5, $J = 8.51$ Hz), 12.95 (s, 1H, NH). Anal. Calcd. for $C_{22}H_{17}N_2O_2ClS$: C, 64.62; H, 4.19; N, 6.85. Found: C, 64.85; H, 4.30; N, 6.71.

2-(4-Methylphenyl)-4-(4-methylsulfonylphenyl)-5-phenyl-1H-imidazole ***11d***

Yield: 34%; pale yellow crystalline powder; mp: 234–235°C; IR (KBr): ν (cm^{-1}) 3320 (NH), 1285, 1145 (SO_2), ^{1}H NMR (DMSO-D_6): δ 2.37 (s, 3H, Me), 3.20 (s, 3H, SO_2Me), 7.31 (d, 2H, 4-methylphenyl H_3 and H_5, $J = 7.97$ Hz), 7.35–7.55 (m, 5H, phenyl),7.75 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, $J = 8.09$ Hz), 7.84 (d, 2H, 4-methylphenyl H_2 and H_6, $J = 8.47$ Hz), 8.01 (d, 2H, 4-methylsulfonyl phenyl H_3 and H_5, $J = 8.09$ Hz), 12.82 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{20}N_2O_2S$: C, 71.11; H, 5.19; N, 7.21. Found: C, 71.46; H, 5.32; N, 7.25.

2-(4-Methoxyphenyl)-4-(4-methylsulfonylphenyl)-5-phenyl-1H-imidazole ***11e***

Yield: 54%; pale yellow crystalline powder; mp: 230–232°C; IR (KBr): ν (cm^{-1}) 3300 (NH), 1290, 1145 (SO_2), ^{1}H NMR (DMSO-D_6): δ 3.05 (s, 3H, SO_2Me), 3.90 (s, 3H, OMe), 7.01 (d, 2H, 4-methoxyphenyl H_3 and H_5, $J = 8.11$ Hz), 7.43–7.58 (m, 7H, phenyl and 4-methoxyphenyl H_2 and H_6), 7.80 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, $J = 7.99$ Hz), 7.90 (d, 2H, 4-methylsulfonyl phenyl H_3 and H_5, $J = 7.99$ Hz), 9.78 (s, 1H, NH). Anal. Calcd. for $C_{23}H_{20}N_2O_3S$: C, 68.30; H, 4.98; N, 6.93. Found: C, 68.02; H, 4.68; N, 6.98.

2-(4-Hydroxyphenyl)-4-(4-methylsulfonylphenyl)-5-phenyl-1H-imidazole ***11f***

Yield: 49%; pale yellow crystalline powder; mp: 360°C; IR (KBr): ν (cm^{-1}) 3550 (OH), 3300 (NH), 1290, 1150 (SO_2), ^{1}H NMR (DMSO-D_6): δ 3.21 (s, 3H, SO_2Me), 6.88 (d, 2H, 4-hydroxyphenyl H_3 and H_5, $J = 7.85$ Hz), 7.43–7.51 (m, 3H, phenyl H_3–H_5), 7.54 (d, 2H, phenyl H_2 and H_6, $J = 7.96$ Hz), 7.76 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, $J = 8.27$ Hz), 7.83 (d, 2H, 4-hydroxyphenyl H_2 and H_6, $J = 7.98$ Hz), 7.92 (d, 2H, 4-methylsulfonyl phenyl H_3 and H_5, $J = 8.27$ Hz), 9.77 (s, 1H, OH), 12.60 (s, 1H, NH). Anal. Calcd. for $C_{22}H_{18}N_2O_3S$: C, 66.67; H, 4.65; N, 7.17. Found: C, 66.92; H, 4.88; N, 6.95.

2-(4-Acetamidophenyl)-4-(4-methylsulfonylphenyl)-5-phenyl-1H-imidazole ***11g***

Yield: 58%; pale yellow crystalline powder; mp: 340°C; IR (KBr): ν (cm^{-1}) 3350, 3300 (NH), 1675 (C=O),1310, 1160 (SO_2), ^{1}H NMR (DMSO-D_6): δ 2.08 (s, 3H, COMe), 3.21 (s, 3H, SO_2Me), 7.01 (d, 2H, 4-acetamidophenyl H_3 and H_5, $J = 8.11$ Hz), 7.45–7.55 (m, 5H, phenyl), 7.70 (d, 2H, 4-methylsulfonylphenyl H_2 and H_6, $J = 8.27$ Hz), 7.84 (d, 2H, 4-acetamidophenyl H_2 and H_6, $J = 8.11$ Hz), 8.01 (d,

2H, 4-methylsulfonylphenyl H_3 and H_5, $J = 8.27$ Hz), 10.36 (s, 1H, NH), 12.75 (s, 1H, NH). Anal. Calcd. for $C_{24}H_{21}N_3O_3S$: C, 66.80; H, 4.90; N, 9.74. Found: C, 67.05; H, 4.58; N, 9.88.

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 chemiluminescent enzyme assays kit (catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method (Zarghi *et al.,* 2007).

Molecular modeling (docking) studies

Docking studies were performed using Autodock software Version 3.0. The coordinates of the X-ray crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme was obtained from the RCSB Protein Data Bank (1cx2) and hydrogens were added. The ligand molecules were constructed using the Builder module and were energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The energy minimized ligands were superimposed on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The purpose of docking is to search for favorable binding configuration between the small flexible ligands and the rigid protein. Protein residues with atoms greater than 7.5 Å from the docking box were removed for efficiency. Searching is conducted within a specified 3D docking box using annealing based on the Monte Carlo method and MMFF94 molecular mechanics force field for 8000 iterations. These docked structures were very similar to the minimized structures obtained initially. The quality of the docked structures was evaluated by measuring the intermolecular energy of the ligand-enzyme assembly (Kurumbail *et al.*, 1996).

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