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Synthesis, cyclooxygenase inhibitory effects, and molecular modeling study of 4-aryl-5-(4-(methylsulfonyl)phenyl)-2-alkylthio and -2-alkylsulfonyl-1*H*-imidazole derivatives

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ABSTRACT

A series of 4-aryl-5-(4-(methylsulfonyl)phenyl)-2-alkylthio and 2-alkylsulfonyl-1*H*-imidazole derivatives were synthesized. All compounds were tested in human blood assay to determine COX-1 and COX-2 inhibitory potency and selectivity. Among the synthesized compounds, 2-alkylthio series were more potent and selective than 2-sulfonylalkyl derivatives. In molecular modeling, interaction of 2-sulfonylalkyl moiety with Arg120 in COX-1 and an extra hydrogen bond with Tyr341 in COX-2 increased the residence time of ligands in the active site in 2-sulfonylalkyl and 2-alkylthio analogs, respectively.

1. Introduction

Cyclooxygenase, the enzyme involved in the first committed step of arachidonic acid metabolism to prostaglandin H_2, has been elucidated in 1971 to be the target of aspirin and other traditional non-steroidal anti-inflammatory drugs (NSAIDs).[1] Prostaglandins and thromboxane have been implicated to have active roles in the pathophysiology of some serious medical conditions including rheumatoid arthritis,[2] osteoarthritis,[3] cancer[4] and neurodegenerative disorders.[5] Since the discovery of the existence of two distinct isoforms of cyclooxygenase (COX-1 and COX-2) in 1991, several research programs have been devoted to investigate their specific roles in aforementioned disorders and design selective inhibitors of each isoform.

COX-1 is generally considered as a constitutional enzyme responsible for physiologic roles. On the other hand, COX-2 is occasionally an inducible enzyme, which increases during inflammatory conditions. Traditional NSAIDs like indomethacin inhibit both isoforms and are associated with gastrointestinal (GI) side effects.[6] In order to circumvent this drawback of NSAIDs, a new generation of COX-2 selective inhibitors with diaryl-heterocycle scaffold containing a para sulfonylmethyl or sulfamoyl moiety on one of vicinal aromatic rings have been developed as exemplified by celecoxib (Celebrex) (**1**) (Fig. 1). Despite their better GI safety profile,[7] another class related issue has been arisen in chronic use of COX-2 selective inhibitors. Some of the most popular members of these drugs such as rofecoxib (Vioxx) and valdecoxib (Bextra) have been voluntarily withdrawn from the market as a consequence of fatal cardiovascular side effects.[8] It has been implicated that COX-2 inhibition leads to a decrease in prostacyclin (PGI_2) level, a lipid mediator with some cardioprotective effects, while leaves COX-1 in platelets activated shifting PG metabolism toward thromboxane A_2 (TxA_2) synthesis. It seems that this imbalance in PGI_2/TxA_2 proportion is the reason why chronic use of COX-2 selective inhibitors results in myocardial infarction (MI) in some patients.[9–11]

As could be comprehended, design and synthesis of new cyclooxygenase inhibitors would still merit consideration in order to increase the richness of structural repertoire which contain some safer lead compounds being discovered. Some diaryl-heterocycles bearing a 6-alkylthio substituted lactone (pyrane-2-one) as central

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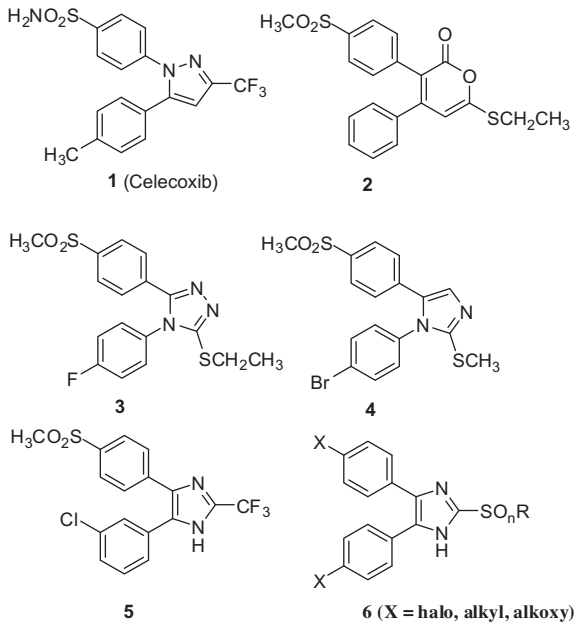


Figure 1. Representative examples of some diaryl-heterocycle COX-2 selective inhibitors. Anti-inflammatory effect has been only reported for compound **6**.

ring have been reported possessing great selectivity toward COX-2 (**2**).[12] We have synthesized 3-alkylthio-4,5-diaryl-1,2,4-triazole (**3**)[13] and some 2-alkylthio substituted 1,5-diaryl-imidazole analogs (**4**)[14] exhibiting good COX-2 selectivity and anti-inflammatory effects. Also some 4,5-diaryl-imidazoles (**5**) bearing a 2-fluoromethyl moiety on central ring have been synthesized and shown to have promising potency and selectivity.[15] Compounds with 4,5-diaryl-2-(substituted thio or sulfonyl)-imidazole structures lacking the sulfonylmethyl or sulfamoyl moieties (**6**) have been already reported to have anti-inflammatory effects in animal models. The structure–activity relationships of these compounds were discussed in three parts: (a) the sulfur oxidation; (b) the sulfur substituent; (c) the aryl substituents. Sulfone moiety and fluorinated alkyl substituents generally led to the most active analgesic and antiarthritic derivatives. Although *p*-methoxy substituents in phenyl rings were favorable in analgesic activity, the greater antiarthritic potency was obtained with para halogen-substitution.[16,17]

Considering structural features of above mentioned compounds, some 4,5-diaryl-imidazoles bearing a sulfonylmethyl pharmacophore on one of phenyl rings and also containing a 2-alkylthio or 2-sulfonylalkyl on imidazole ring were synthesized with the aim of investigating the effect of these groups on COX selectivity and potency. A human whole blood assay was performed to reveal their inhibitory effects toward COX isoforms. Docking study was also accomplished to interpret selectivity pattern among synthesized structures.

2. Results and discussion

2.1. Chemistry

For preparing unsymmetrical benzoins, methyl phenyl sulfide was reacted with appropriate phenyl acetic acid derivative. Subsequently, the products were subjected to bromination using bromine in glacial acetic acid to obtain related α-bromodesoxybenzoins according to previous reports.[18] Further, α-bromodesoxybenzoins were refluxed with sodium methoxide in methanol and finally the reaction was quenched with cold 10% hydrochloric acid to obtain benzoin **7a–c**[19] (Scheme 1).

For preparation of compounds **10–13**, two different methods were considered. In method A, benzoins were oxidized to corresponding sulfoxides using Oxone® (potassium peroxymonosulfate).[12] Obtained products were subjected with excess ammonium thiocyanate in *n*-butanol to prepare compound **8a–c**. Subsequent alkylation of **8a–c** using alkyl iodide in basic media afforded the title compounds **10a–c** and **12a–c**. In method B, benzoins were reacted with excess ammonium thiocyanate in *n*-butanol to prepare compound **9a–c**.[20] Subsequent alkylation of **9a–c** using alkyl iodide in basic media followed by oxidation with Oxone®, afforded the title compounds **11a–c** and **13a–c** (Scheme 2).

In the ^{1}H NMR spectra of all compounds collected in DMSO-d_6 at ambient temperature, two sets of signals were observed indicating a slow equilibrium between two imidazole tautomers on the NMR time scale. This slow equilibrium in 2-(alkylthio)-1*H*-imidazole derveties (**10a–c** and **12a–c**) is more distinctive. For example when the ^{1}H NMR spectrum of **12c** was collected in DMSO-d_6, two sets of signals in nearly 3:2 ratio are detectable. However, in $CDCl_3$ the interconversion of the two isomers is faster on the NMR time scale and consecutively the resonances are averaging to a single signal. In the ^{13}C NMR of compound **12b**, most of the aromatic carbons were duplicated. For example for C_4 and $C_{3,5}$ in 4-F-Phe group, two sets of signals in 162.39, 161.83 ppm with J = 243.75 Hz and 116.39, 115.83 ppm with J = 21.25 Hz were observed, respectively. However, the equilibrium in 2-(alkylsulfonyl)-1*H*-imidazole derveties (**11a–c** and **13a–c**) is much faster so that hydrogen and carbon resonances in DMSO-d_6 are averaging to broad signals. This observation showed that the rate of imidazole ring tautomerization in 2-alkylthio analogs is slower than 2-sulfonylalkyl congeners (Fig. 2).

2.2. Inhibition of cyclooxygenase

In various structure–activity relationships (SAR) studies, diaryl-heterocycle compounds possessing two vicinal aryl moieties on the central heterocyclic ring system represent the major class of selective COX-2 inhibitors.[21] Moreover; the $-SO_2CH_3$ group at the *para*-position of the aryl rings was frequently substituted and was shown to confer optimal COX-2 selectivity and potency.[22]

In this study, we have prepared two classes of compounds possessing the typical $-SO_2CH_3$ COX-2 selectivity; pharmacophore in the *para*-position of aryl rings attached to the central 2-alkylthio or 2-sulfonylalkyl on imidazole ring. Synthesized compounds **10–13** were evaluated for their ability to inhibit COX-2 and COX-1 enzymatic activity using human whole blood assay. The potency (IC_{50} values) of test compounds was determined and compared to that of the reference molecules SC-560 (selective COX-1 inhibitor) and DuP-697 and celecoxib (selective COX-2 inhibitors). These results have been summarized in Table 1. It is noteworthy to mention that the functional selectivity assessed in whole blood assay is usually considerably less than the intrinsic affinity tested by the purified proteins.[23]

The most potent COX-2 inhibitors in this series were **10a** (IC_{50} = 0.06 μM) and **12a** (IC_{50} = 0.05 μM) compared to the reference compounds DuP-697 (IC_{50} = 0.03 μM) and celecoxib (IC_{50} = 0.87 μM). Biological assay results and structure–activity relationship analysis revealed an interesting pattern in COX-2 selectivity of synthesized compounds. Although 2-alkylthio bearing analogs (compounds in **10** & **12** series) exhibit relative COX-2 selectivity (SI nearly 3), in 2-sulfonylalkyl containing structures (**11** & **13** series) selectivity on COX-2 is almost diminished and comparable IC_{50} for both isoforms was obtained with a slight preference toward COX-2. As could be seen in the Table 1, the structure–activity relationship study of all compounds indicated that the order of COX-2 inhibitory potency according to R_1 substituent was H > F > Cl. Furthermore, increasing the size in R_2 substituent

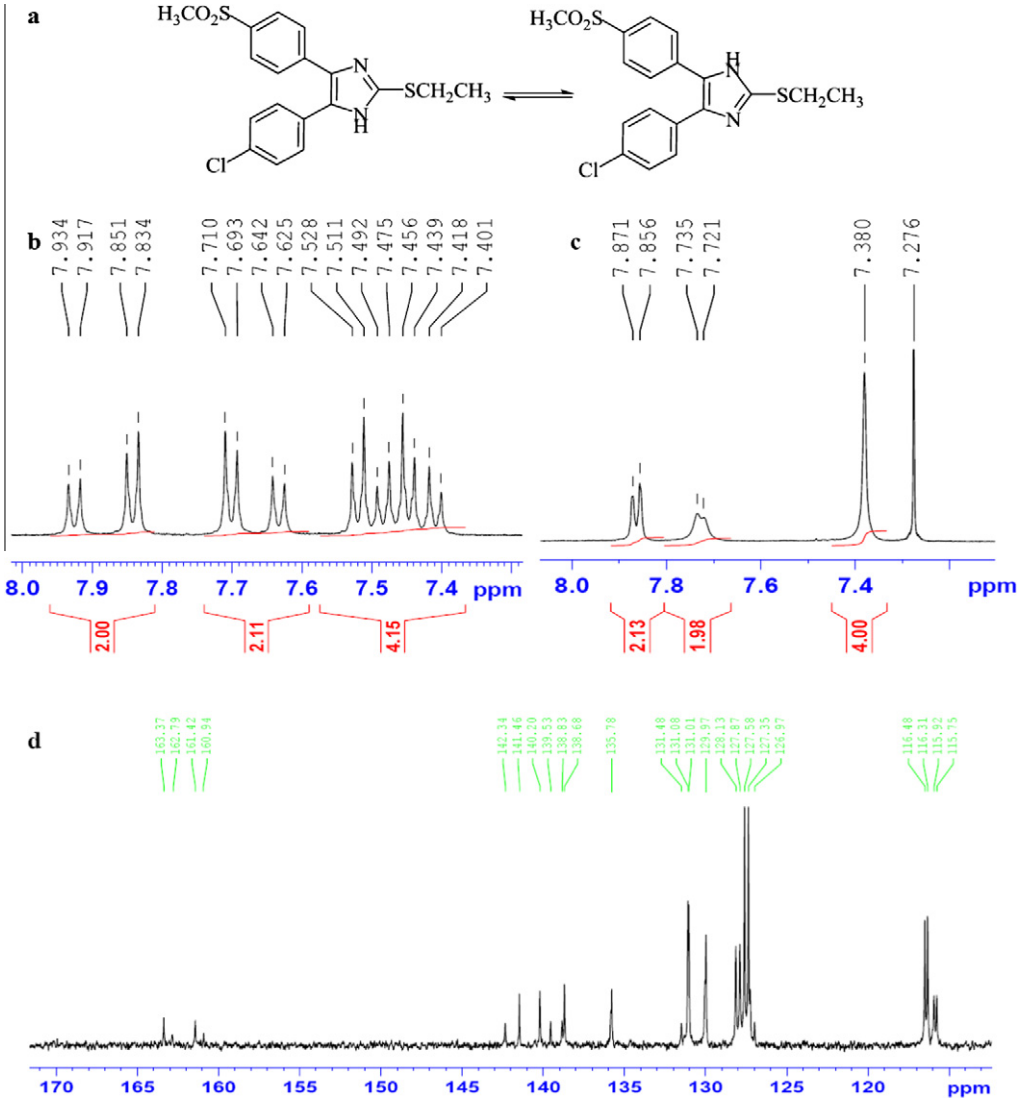


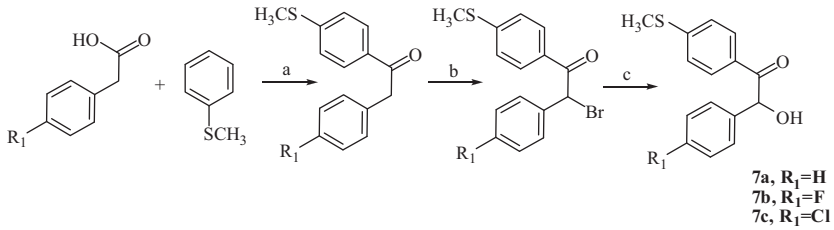
Figure 2. (a) Tautomerization in compound **12c**; (b) ^{1}H NMR spectra of **12c** in DMSO-d_6 at room temperature; (c) ^{1}H NMR spectra of **12c** in $CDCl_3$ at room temperature; (d) ^{13}C NMR of **12b** in DMSO-d_6 at room temperature.

from methyl to ethyl group, led to an increase in COX-2 selectivity. Amongst synthesized structures, **12c** which bears a para chlorophenyl group and 2-ethylthio moiety on imidazole ring is the most selective compound with SI = 3.39.

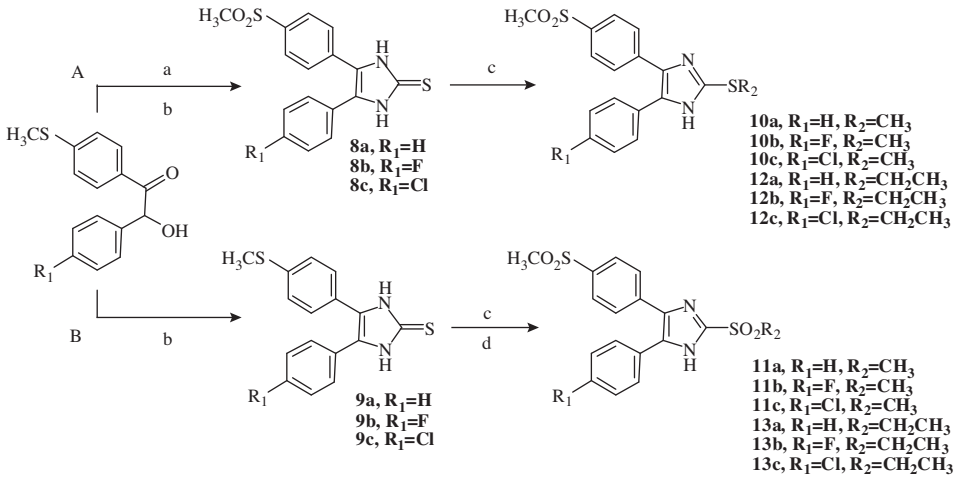
2.3. Molecular modeling study

Docking study was performed to explain the selectivity pattern obtained in biological assay at molecular level. 2-Alkylthio and 2-sulfonylalkyl imidazole analogs, as exemplified by **10b** and **11b**, respectively, were almost similarly docked in the COX-1 active site. In both compounds **10b** and **11b**, the $-SO_2CH_3$ moiety on C-4 phenyl forms two hydrogen bonds with His90 and Gln192 via each of its oxygens; the fluorophenyl group of both ligands fitted in a pocket containing catalytic amino acid Tyr385 and side chains of Trp387, Met522, Phe518 and Leu352. However, differences in the interaction of 2-alkylthio and 2-sulfonylalkyl analogs with COX-1 arise from their capability to interact with amino acids in constriction site. As could be seen in Figure 3, the 2-methylthio moiety of **10b** positioned in a hydrophobic pocket near the constriction site, which also hosted carboxylate bearing aromatic ring of NSAIDs (lined by Met113, Val116, Leu359 and Val349 lipophilic residues). In Figure 4, the 2-sulfonylmethyl moiety of **11b** oriented toward constriction site, formed an important 2.7 Å hydrogen bond with Arg120. As a result, it could be presumed that this interaction in 2-sulfonylalkyl derivates led to an increase in COX-1 potency.

Docking studies of synthesized compounds on COX-2 revealed almost similar orientation of structures in the active site. However, in 2-alkylthio series, –NH of imidazole ring oriented so that a hydrogen bond could be formed with the Tyr341 (equivalent to Tyr355 of COX-1) at the constriction site. It could be presumed that this interaction made 2-alkylthio analogs more potent and selective COX-2 inhibitors than 2-sulfonylalkyl analogs. Orientation and interactions of **12b** with COX-2 enzyme have been described in detail as an example. The SO_2CH_3 moiety on C-4 phenyl well fitted in the side pocket of COX-2 in such a way that each of its oxygens, hydrogen bonded with His75 (distance of 2.1 Å) and Gln178 (distance of 3.6 Å). The fluorophenyl group of ligand oriented toward catalytic Tyr371 (equivalent to Tyr385 of COX-1) which together with side chains of Leu338, Trp373 and Phe504 constructs a relative hydrophobic pocket. The 2-ethylthio group of **12b** hosted in a hydrophobic pocket in vicinity of constriction site which is lined by lipophilic residues of Met99, Val102, Val335, Leu345 and Leu517. In the central imidazole ring of **12b** and other 2-alkylthio

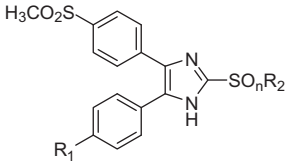


Scheme 1. Reagents and conditions: (a) H_3PO_4, $(CF_3CO)_2O$, 25 °C; (b) Br_2, glacial AcOH, rt; (c) CH_3ONa, CH_3OH, reflux, 10% HCl.



Scheme 2. Reagents and conditions: (a) 2 mmol Oxone® (potassium peroxymonosulfate), $CH_3OH/THF/H_2O$, 0 °C, 18 h; (b) NH_4SCN, *n*-butanol, reflux; (c) alkyl iodide, CH_3OH, reflux; (d) 4 mmol Oxone® (potassium peroxymonosulfate), $CH_3OH/THF/H_2O$, 0 °C, 18 h.

Table 1
IC_{50} values for the inhibition of COX-1 and COX-2 in the human whole blood assays, COX-2 selectivity index (SI), and $\Delta G_{intermolecular}$ data



Compounds	*n*	R_1	R_2	IC_{50}[a] (μM)		SI[b]	$\Delta G_{intermolecular}$[c] (kJ/mol)	
				COX-1	COX-2		COX-1	COX-2
10a	0	H	CH_3	0.16	0.06	2.69	−37	−46
10b	0	F	CH_3	0.21	0.07	3.07	−39	−46
10c	0	Cl	CH_3	0.32	0.10	3.18	−35	−47
11a	2	H	CH_3	0.34	0.28	1.22	−33	−38
11b	2	F	CH_3	0.39	0.28	1.41	−39	−38
11c	2	Cl	CH_3	0.42	0.31	1.35	−32	−42
11a	0	H	CH_2CH_3	0.17	0.05	3.30	−37	−52
12b	0	F	CH_2CH_3	0.24	0.08	2.94	−34	−53
12c	0	Cl	CH_2CH_3	0.39	0.11	3.39	−39	−56
13a	2	H	CH_2CH_3	0.36	0.24	1.52	−27	−39
13b	2	F	CH_2CH_3	0.40	0.31	1.30	−30	−39
13c	2	Cl	CH_2CH_3	0.44	0.32	1.35	−30	−38
Celecoxib				6.65	0.87	7.64		
SC-560				0.0089	—			
DuP-697				—	0.03			

[a] The in vitro test compound concentration required to produce 50% inhibition of enzymatic activity. The result (IC_{50}, l M) is the mean of three determinations acquired using the human whole blood assay 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] HYDE predicted intermolecular free energy.

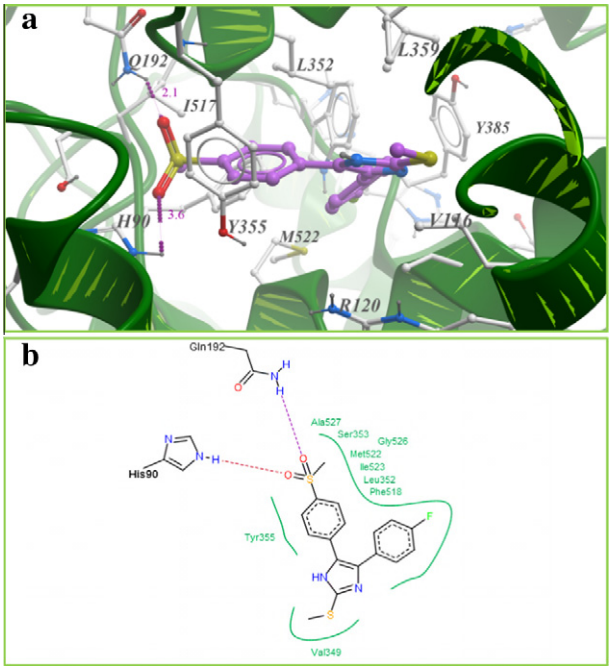


Figure 3. Compound **10b** (carbons in purple) in COX-1. (a) The sulfonylmethyl group of **4b** forms two hydrogen bonds, a 2.1 Å with Gln192 and a 3.6 Å with His90 (pictures are prepared by Molsoft ICM-browser). (b) Pose view image of **10b** orientation in COX-1.

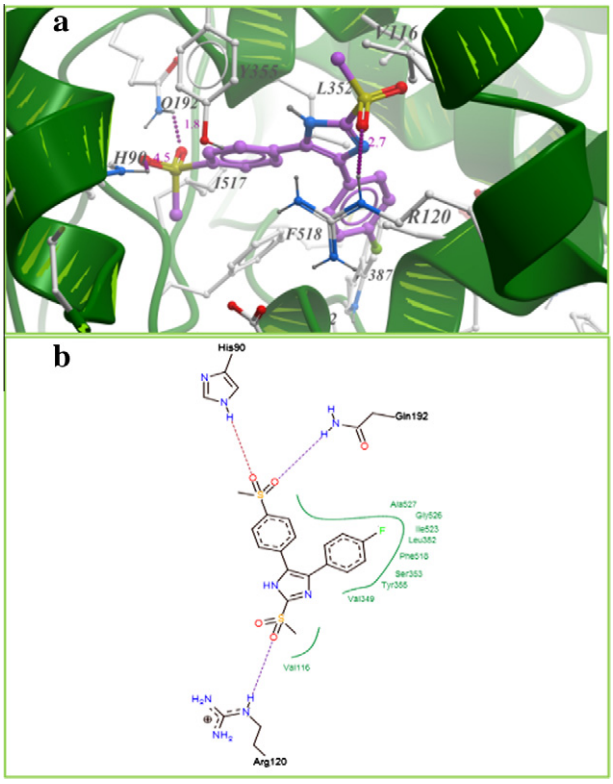


Figure 4. Compound **11b** (carbons in purple) in COX-1. (a) The sulfonylmethyl moiety on phenyl ring of **11b** forms two hydrogen bonds, a 1.8 Å with Gln192 and a 4.5 Å with His90. As could be seen one of oxygens of sulfonylmethyl moiety on imidazole participates in a 2.7 Å hydrogen bond with guadinium group of Arg120 at the constriction site. (b) Pose view image of **5b** orientation in COX-1.

analougs, –NH group forms hydrogen bond with phenolic oxygen of Tyr341 (Fig. 5).

For additional support, HYDE estimated energy of hydrogen bond between –NH imidazole of ligand and COX-2 Tyr341 was studied for all synthesized compounds in Table 2. The HYDE estimated free energy of this interaction for 2-alkylthio analogs was in the range of −4.5 to −2.0 kJ/mol. However, in 2-sulfonylalkyl congeners HYDE analysis predicted a weak interaction of this type just for **13c**. According to the HYDE data and the observation from imidazole ring tautomerization in NMR spectra, it could be postulated that hydrogen bond formation in 2-alkylthio series via –NH of imidazole ring with Tyr341 might be thermodynamically and kinetically more favorable.

As discussed in the literature, diaryl-heterocycles like celecoxib inhibit COX-2 with time dependent kinetics while COX-1 inhibition takes place time independently and this is the reason why these structures inhibit COX-2 selectively. It seems that interaction with Arg120 (Arg106 in COX-2) and Tyr355 (Tyr341 in COX-2) in the constriction site is important for time dependent inhibition.[24–27] Limongelli et al. through a metadynamics-based approach explained time dependent inhibition mechanism of COX-2 by SC-558, a celecoxib analog. They have revealed that this ligand takes an alternative mode of orientation from the original one through interaction with the active site. While SC-558 firstly interacts with Arg499 in the side pocket of COX-2 through its sulfamoyl moiety then reorients in the active site so that the sulfamoyl moiety forms hydrogen bond with Arg106 and Tyr341 in the constriction site. Because of the smaller space available in COX-1 active site, this reorientation for SC-558 is not possible. This extra interaction has been proposed to be the reason for this observation that SC-558 and its analogs inhibit COX-2 time dependently while COX-1 inhibition takes place through time independent kinetics.[28] After all it has been speculated that our molecular modeling study reveals some similar results with aforementioned study. For 2-alkylthio analogs, which exhibit COX-2 selectivity, in addition to possibility of reorientation in

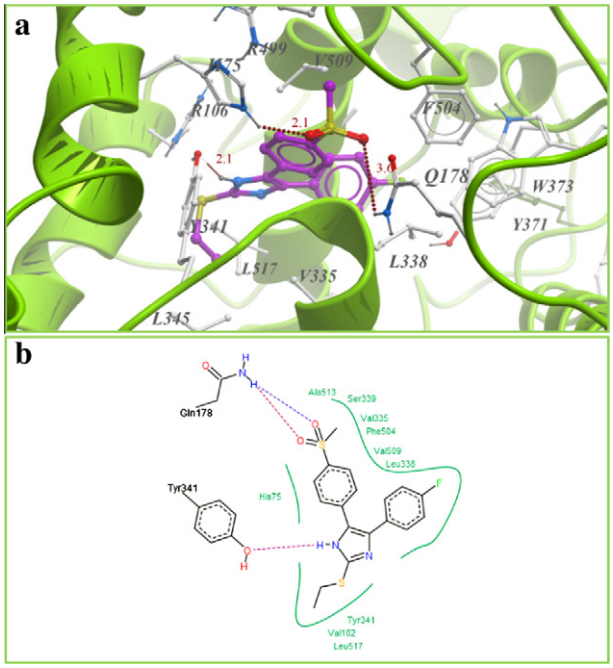


Figure 5. Compound **12b** (carbons in purple) in COX-2. (a) The sulfonylmethyl group of **12b** forms two hydrogen bonds, a 3.6 Å with Gln178 and a 2.1 Å with His75 at the side pocket of COX-2. As could be seen another hydrogen bonding via –NH of imidazole ring distanced 2.1 Å to the Tyr341 (equivalent to Tyr355 of COX-1) at the constriction site has occurred. (b) Pose view image of **12b** orientation in COX-2.

Table 2
HYDE estimated energy of hydrogen bond between –NH imidazole of ligands and COX-2 Tyr341

	Compounds											
	10a	**10b**	**10c**	**11a**	**11b**	**11c**	**12a**	**12b**	**12c**	**13a**	**13b**	**13c**
ΔG (kJ/mol)	−3.8	−4.5	−2.0	—	—	—	−4.0	−4.0	−3.2	—	—	−1.5

the COX-2 active site, an extra hydrogen bond with Tyr341 has been predicted that could increase the residence time of ligands in the active site. 2-Sulfonylalkyl analogs inhibit both isoforms almost similarly. The reason for this pattern of selectivity for **11** and **13** series could be explained through molecular modeling. While these compounds have structural determinants to inhibit COX-2 time dependently, the extra sulfonyl group on imidazole ring makes the difference through COX-1 inhibition. Molecular modeling showed that 2-sulfonylalkyl moiety of these series interacts with Arg120 in COX-1 and this interaction could increase the residence time of ligand in the active site. Because of this extra hydrogen bonding, time dependent kinetic of COX-1 inhibition by 2-sulfonylalkyl analogs would be a strong possibility. As proposed in literature, NSAIDs like naproxen which inhibit COX-1 time dependently[29] have safer cardiovascular profile and even reveal some cardioprotective effects.[10] All in all it demands further investigation to reveal COX inhibition kinetics for synthesized structures and also their cardiovascular safety profile.

3. Conclusions

A series of 4-aryl-5-(4-(methylsulfonyl)phenyl)-2-alkylthio and 2-alkylsulfonyl-1*H*-imidazole derivatives were synthesized and their inhibitory potency against cycloxygenase was determined. In synthesized compounds, 2-alkylthio derivatives are more potent and selective than 2-sulfonylalkyl derivatives. Molecular modeling showed that 2-sulfonylalkyl series interact with Arg120 in COX-1 active site and this interaction could increase the residence time of ligand in the active site. In 2-alkylthio series, which exhibit COX-2 selectivity, in addition to possibility of reorientation in the COX-2 active site, an extra hydrogen bond with Tyr341 could increase the residence time of ligands in the active site and consequently augments COX-2 selectivity.

4. Experimental section

4.1. Chemistry

^{1}H NMR spectra were recorded on a 500 MHz Bruker spectrometer using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) as internal standard. Infrared spectra were acquired on a Nicolet Magna 550-FT spectrometer. IR spectra of solids were recorded in KBr and the absorption band was given in wave numbers v in cm^{-1}. Elemental microanalyses were within ±0.4 of the theoretical values for C, H and N.

4.1.1. 5-(4-(Methylsulfonyl)phenyl)-2-(methylthio)-4-phenyl-1*H*-imidazole (10a)

Yield, 78%; mp 121–124 °C; IR (KBr, cm^{-1}): v 3298 (NH), 1280, 1137 (SO_2); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.63 (s, 3H, SCH_3), 3.20 and 3.25 (two br s, 3H, SO_2CH_3), 7.26–7.52 (m, 5H, phenyl), 7.62 and 7.71 (two d, J = 8 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.81 and 7.90 (two d, J = 8 Hz, 2H, $H_{3,5}$-methylsulfonylphenyl), 12.70 and 12.73 (two br s, 1H, NH). Anal. Calcd for $C_{17}H_{16}N_2O_2S_2$: C, 59.28; H, 4.68; N, 8.13. Found: C, 59.46; H, 4.41; N, 8.32.

4.1.2. 4-(4-Fluorophenyl)-5-(4-(methylsulfonyl)phenyl)-2-(methylthio)-1*H*-imidazole (10b)

Yield, 74%; mp 131–133 °C; IR (KBr, cm^{-1}): v 3278 (NH), 1280, 1142 (SO_2); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.63 (s, 3H, SCH_3), 3.19 and 3.25 (two br s, 3H, SO_2CH_3), 7.19 and 7.31(two dd, J = 8.5 Hz, J = 8.5 Hz, 2H, $H_{3,5}$-fluorophenyl), 7.43–7.54 (m, 2H, $H_{2,6}$-fluorophenyl), 7.61 and 7.69 (two d, J = 8 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.82 and 7.91 (two d, J = 8 Hz, 2H, $H_{3,5}$-methylsulfonylphenyl), 12.70 and 12.73 (two br s, 1H, NH). Anal. Calcd for $C_{17}H_{15}FN_2O_2S_2$: C, 56.34; H, 4.17; N, 7.73. Found: C, 56.52; H, 4.35; N, 7.43.

4.1.3. 4-(4-Chlorophenyl)-5-(4-(methylsulfonyl)phenyl)-2-(methylthio)-1*H*-imidazole (10c)

Yield, 78%; mp 121–123 °C; IR (KBr, cm^{-1}): v 3308 (NH), 1285, 1137 (SO_2); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.63 (s, 3H, SCH_3), 3.20 and 3.25 (two br s, 3H, SO_2CH_3), 7.36–7.56 (m, 4H, chlorophenyl), 7.63 and 7.70 (two d, J = 7.5 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.84 and 7.93 (two d, J = 7.5 Hz, 2H, $H_{3,5}$-methylsulfonylphenyl), 12.77 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{15}ClN_2O_2S_2$: C, 53.89; H, 3.99; N, 7.39. Found: C, 53.64; H, 3.71; N, 7.62.

4.1.4. 2-(Methylsulfonyl)-5-(4-(methylsulfonyl)phenyl)-4-phenyl-1*H*-imidazole (11a)

Yield, 68%; mp 218–221 °C; IR (KBr, cm^{-1}): v 3230 (NH), 1285, 1137 (SO_2); ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.22 (br s, 3H, SO_2CH_3), 3.45 (s, 3H, SO_2CH_3), 7.31–7.57 (m, 5H, phenyl), 7.71 (d, J = 8 Hz , 2H, $H_{2,6}$-methylsulfonylphenyl), 7.87 and 7.97 (two d, J = 8 Hz, 2H, $H_{3,5}$-methylsulfonylphenyl), 14.25 and 14.27 (two br s, 1H, NH). Anal. Calcd for $C_{17}H_{16}N_2O_4S_2$: C, 54.24; H, 4.28; N, 7.44. Found: C, 54.46; H, 4.42; N, 7.24.

4.1.5. 4-(4-Fluorophenyl)-2-(methylsulfonyl)-5-(4-(methylsulfonyl)phenyl)-1*H*-imidazole (11b)

Yield, 67%; mp 227–229 °C; IR (KBr, cm^{-1}): v 3211 (NH), 1280, 1142 (SO_2); ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.23 (br s, 3H, SO_2CH_3), 3.44 (s, 3H, SO_2CH_3), 7.17–7.40 (m, 2H, $H_{3,5}$-fluorophenyl), 7.41–7.61 (br s, 2H, $H_{2,6}$-fluorophenyl), 7.70 (d, J = 8.5 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.80–7.99 (m, 2H, $H_{3,5}$-methylsulfonylphenyl), 14.28 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{15}FN_2O_4S_2$: C, 51.76; H, 3.83; N, 7.10. Found: C, 51.39; H, 3.61; N, 7.34.

4.1.6. 4-(4-Chlorophenyl)-2-(methylsulfonyl)-5-(4-(methylsulfonyl)phenyl)-1*H*-imidazole (11c)

Yield, 68%; mp 233–235 °C; IR (KBr, cm^{-1}): v 3196 (NH), 1285, 1132 (SO_2); ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.25 (br s, 3H, SO_2CH_3), 3.44 (s, 3H, SO_2CH_3), 7.41–7.61 (m, 4H, chlorophenyl), 7.71 (d, J = 8 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.93 (d, J = 8 Hz, 2H, $H_{3,5}$-methylsulfonylphenyl), 14.33 (br s, 1H, NH). Anal. Calcd for $C_{17}H_{15}ClN_2O_4S_2$: C, 49.69; H, 3.68; N, 6.82. Found: C, 49.83; H, 3.46; N, 6.69.

4.1.7. 2-(Ethylthio)-5-(4-(methylsulfonyl)phenyl)-4-phenyl-1*H*-imidazole (12a)

Yield, 78%; mp 157–158 °C; IR (KBr, cm^{-1}): v 3247 (NH), 1296, 1142 (SO_2); ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.33 (t, J = 7.5 Hz, 3H,

SCH_2CH_3), 3.14 (q, J = 7.5 Hz, 2H, SCH_2CH_3), 3.20 and 3.25 (two br s, 3H, SO_2CH_3), 7.26–7.52 (m, 5H, phenyl), 7.63 and 7.71 (two d, J = 8 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.81 and 7.91 (two d, J = 8 Hz, 2H, $H_{3,5}$-methylsulfonylphenyl), 12.74 and 12.76 (two br s, 1H, NH). Anal. Calcd for $C_{18}H_{18}N_2O_2S_2$: C, 60.31; H, 5.06; N, 7.81. Found: C, 60.58; H, 5.27; N, 7.67.

4.1.8. 2-(Ethylthio)-4-(4-fluorophenyl)-5-(4-(methylsulfonyl) phenyl)-1*H*-imidazole (12b)

Yield, 73%; mp 82–84 °C; IR (KBr, cm^{-1}): ν 3277 (NH), 1306, 1137 (SO_2); ^1H NMR (500 MHz, DMSO-d_6): δ 1.33 (t, J = 7.5 Hz, 3H, SCH_2CH_3), 3.14 (q, J = 7.5 Hz, 2H, SCH_2CH_3), 3.19 and 3.25 (two s, 3H, SO_2CH_3), 7.19 and 7.30(two dd, J = 9 Hz, J = 9 Hz, 2H, $H_{3,5}$-fluorophenyl), 7.44–7.54 (m, 2H, $H_{2,6}$-fluorophenyl), 7.61 and 7.69 (two d, J = 8.5 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.82 and 7.91 (two d, J = 8.5 Hz, 2H, $H_{3,5}$-methylsulfonylphenyl), 12.70 and 12.73 (two br s, 1H, NH). Anal. Calcd for $C_{18}H_{17}FN_2O_2S_2$: C, 57.43; H, 4.55; N, 7.44. Found: C, 57.67; H, 4.36; N, 7.61.

4.1.9. 4-(4-Chlorophenyl)-2-(ethylthio)-5-(4-(methylsulfonyl) phenyl)-1*H*-imidazole (12c)

Yield, 75%; mp 92–94 °C; IR (KBr, cm^{-1}): ν 3262 (NH), 1311, 1147 (SO_2); ^1H NMR (500 MHz, DMSO-d_6): δ 1.33 (t, J = 7 Hz, 3H, SCH_2CH_3), 3.14 (q, J = 7 Hz, 2H, SCH_2CH_3), 3.20 and 3.26 (two s, 3H, SO_2CH_3), 7.41 and 7.44 (two d, J = 8.5 Hz, 2H, chlorophenyl), 7.48 and 7.52 (two d, J = 8.5 Hz, 2H, chlorophenyl), 7.63 and 7.70 (two d, J = 8.5 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.84 and 7.93 (two d, J = 8.5 Hz, 2H, $H_{3,5}$-methylsulfonylphenyl), 12.76 and 12.78 (two br s, 1H, NH). ^1H NMR (500 MHz, $CDCl_3$): δ 1.43 (t, J = 7 Hz, 3H, SCH_2CH_3), 3.08 (s, 3H, SO_2CH_3), 3.20 (q, J = 7 Hz, 2H, SCH_2CH_3), 7.38 (br s, 4H, chlorophenyl), 7.73 (d, J = 7.5 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.86 (d, J = 7.5 Hz, 2H, $H_{3,5}$-methylsulfonylphenyl), 12.76 and 12.78 (two br s, 1H, NH) Anal. Calcd for $C_{18}H_{17}ClN_2O_2S_2$: C, 55.02; H, 4.36; N, 7.13. Found: C, 55.28; H, 4.43; N, 7.29.

4.1.10. 2-(Ethylsulfonyl)-5-(4-(methylsulfonyl)phenyl)-4-phenyl-1*H*-imidazole (13a)

Yield, 67%; mp 248–250 °C; IR (KBr, cm^{-1}): ν 3180 (NH), 1291, 1142 (SO_2); ^1H NMR (500 MHz, DMSO-d_6): δ 1.27 (t, J = 7.5 Hz, 3H, $SO_2CH_2CH_3$), 3.22 (br s, 3H, SO_2CH_3), 3.52 (q, J = 7.5 Hz, 2H, $SO_2CH_2CH_3$), 7.26–7.59 (m, 5H, phenyl), 7.71 (d, J = 8 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.82–7.03 (m, 2H, $H_{3,5}$-methylsulfonylphenyl), 14.27 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{18}N_2O_4S_2$: C, 55.37; H, 4.65; N, 7.17. Found: C, 55.62; H, 4.87; N, 7.34.

4.1.11. 2-(Ethylsulfonyl)-4-(4-fluorophenyl)-5-(4-(methylsulfonyl)phenyl)-1*H*-imidazole (13b)

Yield, 63%; mp 225–228 °C; IR (KBr, cm^{-1}): ν 3211 (NH), 1285, 1137 (SO_2); ^1H NMR (500 MHz, DMSO-d_6): 1.26 (t, J = 7 Hz, 3H, $SO_2CH_2CH_3$), 3.28 (br s, 3H, SO_2CH_3), 3.52 (q, J = 7 Hz, 2H, $SO_2CH_2CH_3$), 7.17–7.41 (m, 2H, $H_{3,5}$-fluorophenyl), 7.43–7.62 (m, 2H, $H_{2,6}$-fluorophenyl), 7.70 (d, J = 8 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.81–8.03 (m, 2H, $H_{3,5}$-methylsulfonylphenyl), 14.29 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{17}FN_2O_4S_2$: C, 52.93; H, 4.19; N, 6.86. Found: C, 52.76; H, 4.31; N, 6.58.

4.1.12. 4-(4-Chlorophenyl)-2-(ethylsulfonyl)-5-(4-(methylsulfonyl)phenyl)-1*H*-imidazoleimidazole (13c)

Yield, 76%; mp 243–246 °C; IR (KBr, cm^{-1}): ν 3206 (NH), 1275, 1132 (SO_2); ^1H NMR (500 MHz, DMSO-d_6): δ 1.26 (t, J = 7.5 Hz, 3H, $SO_2CH_2CH_3$), 3.25 (br s, 3H, SO_2CH_3), 3.52 (q, J = 7.5 Hz, 2H, $SO_2CH_2CH_3$), 7.41–7.63 (m, 4H, chlorophenyl), 7.71 (d, J = 8 Hz 2 Hz, 2H, $H_{2,6}$-methylsulfonylphenyl), 7.84–8.03 (m, 2H, $H_{3,5}$-methylsulfonylphenyl), 14.35 (br s, 1H, NH). Anal. Calcd for $C_{18}H_{17}ClN_2O_4S_2$: C, 50.88; H, 4.03; N, 6.59. Found: C, 50.65; H, 4.21; N, 6.39.

4.2. Molecular modeling

The molecular geometry of all compounds was fully minimized by MMFF94 force field using ChemBio3D Ultra 12.0 (Cambridgesoft), setting the terminal condition as the RMS of potential energy smaller than 0.0001 kcal $Å^{-1}$ mol^{-1}. The crystal structures of cyclooxygenase-2 in complex with celecoxib (entery code 3LN1) and cyclooxygenase-1 also complexed with celecoxib (entery code 3KK6) were retrieved from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The FlexX program interfaced with LeadIT 2.1.2 (BioSolveIT GmbH, Sankt Augustin, Germany) was used to dock all compounds. FlexX is an automated docking program, which considers ligand conformational flexibility by an incremental fragment placing method.[30] The active site for docking was determined as all atoms within 7 Å radius of the cocrystallized ligand. Validation of the docking procedure was accomplished by energy minimization of celecoxib structure by the above mentioned method and the optimized geometry structure was redocked in the active site of COX-2 and COX-1 which resulted in predicted docking poses with RMSD 0.723 and 0.975, respectively, within the best scored poses.

For evaluation of the ligands affinity toward docked receptor, the HYDE assessment facility of LeadIT software was implemented to report the free energy of binding (ΔG) and ligand efficiency of some of the best dock scored poses. HYDE is an empirical scoring function, which assesses protein–ligand complex by considering hydrogen bond interactions and also hydrophobic and desolvation effects and provides estimation for the binding affinity.[31,32]

4.3. Human whole blood assays for COX-2 and COX-1

The assays were carried out using previously described procedures.[33,34] Fresh blood was taken from human volunteers who had no apparent inflammatory conditions and had not taken any NSAIDs for at least 7 days prior to blood collection. For the COX-2 assay, heparinized blood samples (500 μL) were incubated with vehicle (2 μL of DMSO) or test compound (2 μL of a DMSO solution) at 37 °C for 15 min; then, blood samples were incubated in the presence of 10 μL of lipopolysaccharide (LPS; 100 μg/mL in PBS) at 37 °C for 24 h. After incubation, all blood samples were centrifuged (12,000g for 5 min); 100 μL of plasma were mixed with 400 μL of methanol to precipitate plasma proteins. The obtained supernatants were assayed for PGE_2 levels by an enzyme immunoassay kit according to the instructions of the manufacturer (Cayman Chemicals, Ann Harbor, MI, #500141). For the COX-1 assay, the aliquots of blood samples (500 μL) were immediately transferred to siliconized microcentrifuge tubes and mixed with either 2 μL of DMSO or a test compound. Following vortexing at 37 °C for 1 h, the samples were centrifuged (12,000×g, 5 min) and 100 μL of serum supernatants were mixed with 400 μL of methanol for protein precipitation. The obtained supernatants were assayed for TxB_2 levels by an enzyme immunoassay kit according to the instructions of the manufacturer (Cayman, Ann Arbor, MI, #519031). The reference molecules SC-560, celecoxib and Dup-697 were analyzed under the same experimental conditions. The IC_{50} values (the concentration of the test compound causing 50% inhibition) were calculated from the concentration inhibition response curves (duplicate determinations).

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References and notes

1. Simmons, D. L.; Botting, R. M.; Hla, T. *Pharmacol. Rev.* **2004**, *56*, 387.
2. McCoy, J. M.; Wicks, J. R.; Audoly, L. P. *J. Clin. Invest.* **2002**, *110*, 651.
3. Kojima, F.; Naraba, H.; Miyamoto, S.; Beppu, M.; Aoki, H.; Kawai, S. *Arthritis Res. Ther.* **2004**, *6*, R355.
4. Pai, R.; Soreghan, B.; Szabo, I. L.; Pavelka, M.; Baatar, D.; Tarnawski, A. S. *Nat. Med.* **2002**, *8*, 289.
5. Matousek, S. B.; Hein, A. M.; Shaftel, S. S.; Olschowka, J. A.; Kyrkanides, S.; O'Banion, M. K. *J. Neurochem.* **2010**, *114*, 247.
6. Peskar, B. M. *J. Physiol. (Paris)* **2001**, *95*, 3.
7. van der Linden, M. W.; Gaugris, S.; Kuipers, E. J.; van Herk-Sukel, M. P. P.; van den Bemt, B. J. F.; Sen, S. S.; Herings, R. M. C. *Pharmacoepidemiol. Drug Saf.* **2009**, *18*, 880.
8. Ritter, J. M.; Harding, I.; Warren, J. B. *Trends Pharmacol. Sci.* **2009**, *30*, 503.
9. Capone, M. L.; Tacconelli, S.; Di Francesco, L.; Sacchetti, A.; Sciulli, M. G.; Patrignani, P. *Prostaglandins Other Lipid Mediat.* **2007**, *82*, 85.
10. Hinz, B.; Brune, K. *Trends Pharmacol. Sci.* **2008**, *29*, 391.
11. Dogne, J. M.; Hanson, J.; Supuran, C.; Pratico, D. *Curr. Pharm. Des.* **2006**, *12*, 971.
12. Rao, P. N. P.; Amini, M.; Li, H. Y.; Habeeb, A. G.; Knaus, E. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2205.
13. Navidpour, L.; Shafaroodi, H.; Abdi, K.; Amini, M.; Ghahremani, M. H.; Dehpour, A. R.; Shafiee, A. *Bioorg. Med. Chem.* **2006**, *14*, 2507.
14. Navidpour, L.; Shadnia, H.; Shafaroodi, H.; Amini, M.; Dehpour, A. R.; Shafiee, A. *Bioorg. Med. Chem.* **1976**, *2007*, 15.
15. Barta, T. E.; Stealey, M. A.; Collins, P. W.; Weier, R. M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3443.
16. Sharpe, T. R.; Cherkofsky, S. C.; Hewes, W. E.; Smith, D. H.; Gregory, W. A.; Haber, S. B.; Leadbetter, M. R.; Whitney, J. G. *J. Med. Chem.* **1985**, *28*, 1188.
17. Niedballa, U.; Bottcher, I. U.S. Patent 4,440,776, 1984; *Chem. Abstr.* **1980**, *92*, 146771.
18. Gadad, A. K.; Palkar, M. B.; Anand, K.; Noolvi, M. N.; Boreddy, T. S.; Wagwade, J. *Bioorg. Med. Chem.* **2008**, *16*, 276.
19. Nagano, T. *J. Org. Chem.* **1957**, *22*, 817.
20. Salimi, M.; Amini, M.; Shafiee, A. *Phosphorus, Sulfur Silicon Relat. Elem.* **2005**, *180*, 1587.
21. Singh, P.; Mittal, A. *Mini-Rev. Med. Chem.* **2008**, *8*, 73.
22. Talley, J. *Prog. Med. Chem.* **1999**, *36*, 201.
23. Nies, A. S.; Gresser, M. J. *Adv. Protein Chem.* **2001**, *56*, 115.
24. Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Pak, J. Y.; Gildehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K.; Isakson, P. C.; Stallings, W. C. *Nature* **1996**, *384*, 644.
25. Selinsky, B. S.; Gupta, K.; Sharkey, C. T.; Loll, P. J. *Biochemistry* **2001**, *40*, 5172.
26. So, O. Y.; Scarafia, L. E.; Mak, A. Y.; Callan, O. H.; Swinney, D. C. *J. Biol. Chem.* **1998**, *273*, 5801.
27. Blobaum, A. L.; Marnett, L. J. *J. Med. Chem.* **2007**, *50*, 1425.
28. Limongelli, V.; Bonomi, M.; Marinelli, L.; Gervasio, F. L.; Cavalli, A.; Novellino, E.; Parrinello, M. P. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 5411.
29. Duggan, K. C.; Walters, M. J.; Musee, J.; Harp, J. M.; Kiefer, J. R.; Oates, J. A.; Marnett, L. J. *J. Biol. Chem.* **2010**, *285*, 34950.
30. Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. *J. Mol. Biol.* **1996**, *261*, 470.
31. Reulecke, I.; Lange, G.; Albrecht, J.; Klein, R.; Rarey, M. *ChemMedChem* **2008**, *3*, 885.
32. Schneider, N.; Hindle, S.; Lange, G.; Klein, R.; Albrecht, J.; Briem, H.; Beyer, K.; Claussen, H.; Gastreich, M.; Lemmen, C.; Rarey, M. *J. Comput. Aided Mol. Des.* **2012**, *26*, 701.
33. Brideau, C.; Kargman, S.; Liu, S.; Dallob, A.; Ehrich, E.; Rodger, I.; Chan, C. *Inflamm. Res.* **1996**, *45*, 68.
34. Young, J. M.; Panah, S.; Satchawatcharaphong, C.; Cheung, P. S. *Inflamm. Res.* **1996**, *45*, 246.