Full Paper

Design, Synthesis, and Biological Evaluation of 1,5-Diaryl-1,2,4-triazole Derivatives as Selective Cyclooxygenase-2 Inhibitors

Bo Jiang[1], Yi Zeng[1], Meng-Jie Li[1], Jin-Yi Xu[1], Yong-Na Zhang[2], Qiu-Juan Wang[2], Ni-Yue Sun[3], Tao Lu[3], and Xiao-Ming Wu[1]

[1] Department of Medicinal Chemistry, College of Pharmacy, China Pharmaceutical University, Nanjing, P.R. China
[2] Department of Physiology, College of Pharmacy, China Pharmaceutical University, Nanjing, P.R. China
[3] Laboratory of Molecular Design and Drug Discovery, China Pharmaceutical University, Nanjing, P.R. China

A series of 1,5-diaryl-1,2,4-triazole derivatives were synthesized and evaluated as cyclooxygenase-2 (COX-2) inhibitors. The results of the preliminary biological assays *in vivo* showed that eight compounds **5b**, **6b**, **6c**, **7c**, **8b**, **8d**, **9c**, and **9d** have potent anti-inflammatory activity ($P < 0.01$), while compounds **6b**, **6c**, and **9c** exhibit marked potency. Compound **6c** was then selected for further investigation. In the COX inhibition assay *in vitro*, compound **6c** was identified as a potent and selective inhibitor of COX-2 (COX-2 $IC_{50} = 0.37$ μM; SI = 0.018), being equipotent to celecoxib (COX-2 $IC_{50} = 0.26$ μM; SI = 0.015). In a rat carrageenan-induced paw edema assay, **6c** exhibited moderate anti-inflammatory activity (35% inhibition of inflammation) at 2 h after administration of 15 mg/kg as an oral dose. A docking study also revealed that compound **6c** binds in the active site of COX-2 in a similar mode to that of the known selective COX-2 inhibitor SC-558.

Keywords: Anti-inflammatory activity / 1,5-Diaryl-1,2,4-triazole / NSAIDs / Selective COX-2 inhibitors

Received: September 21, 2009; Accepted: December 31, 2009

DOI 10.1002/ardp.200900227

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) remain widely prescribed drugs worldwide and have been used to treat pain, fever, and symptoms of arthritis like rheumatoid arthritis (RA), osteoarthritis (OA), etc. However, long-term use of NSAIDs has been associated with several unwanted effects such as gastrointestinal mucosal damage, bleeding and renal toxicity [1–4]. The identification of two different isoforms of the cyclooxygenase (COX) enzyme known as COX-1 and COX-2, which are key enzymes in the arachidonic acid metabolism, heralded the start of a new era for research in anti-inflammatory therapy. COX-1 is expressed in many organs and is responsible for homeostatic processes such as platelet aggregation, gastric protection, and renal function. COX-2 is an inducible isoform that is constitutively present in some organs, such as brain and kidney, but is significantly up-regulated in response to a broad range of stimuli in inflammatory condition [5, 6]. It has been shown that the biological functions of COX-1 and COX-2 are much more complicated than previously recognized. Moreover, the existence of COX-3, a variant of COX-1, has been proposed and is considered to be another target for anti-inflammatory agents [7].

Ever since the first selective COX-2 inhibitor – celecoxib – has been approved for the market by the FDA for the treatment of inflammation and the management of acute or chronic pain, diaryl-heterocycles have become the major class of selective COX-2 inhibitors, such as celecoxib, rofecoxib, parecoxib, and valdecoxib (Fig. 1), which display improved gastrointestinal safety profile compared to the traditional NSAIDs [8–12]. However, the cardiovascular risk associated with ''-coxibs'' has become a concern since rofecoxib and valdecoxib were withdrawn from the market [13]. The most plausible mechanism is the suppression of

Correspondence: Jin-Yi Xu, Department of Medicinal Chemistry, College of Pharmacy, 24 Tongjia Xiang, China Pharmaceutical University, Nanjing 210009, P.R. China.
E-mail: jinyixu@china.com
Fax: +86 25 8330-2827

Abbreviations: cyclooxygenase-1/2 (COX-1/2); non-steroidal anti-inflammatory drugs (NSAIDs); selectivity index (SI)

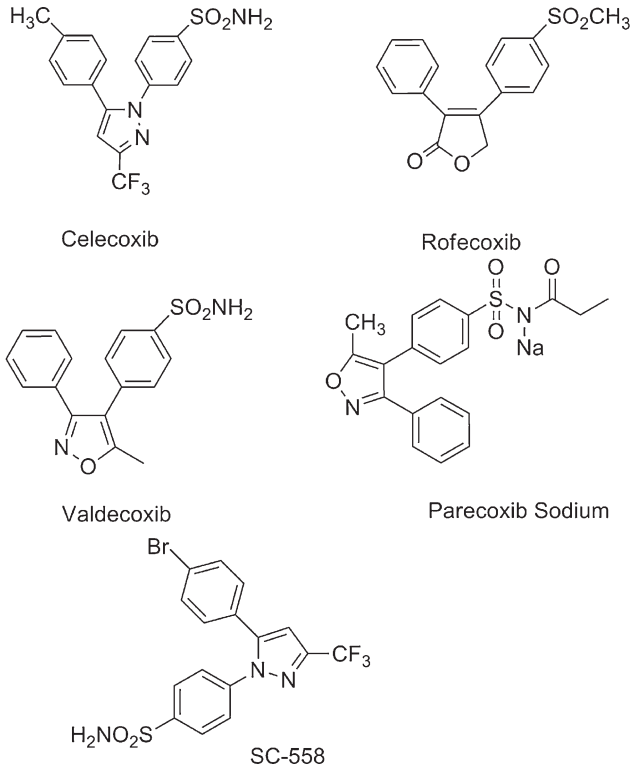


Figure 1. Selective COX-2 inhibitors.

COX-2-derived prostacyclin and to some degree prostaglandin E_2, while leaving the platelet COX-1-derived synthesis of prothrombotic thromboxanes unchanged, thereby resulting in an imbalance among the eicosanoids [14, 15]. Nonetheless, the concern that COX-2 inhibitors potentiate cardiovascular events differ among agents, and only with large doses of the available COX-2 inhibitors do such threats exist [16, 17]. Besides, the clinical use of selective COX-2 inhibitors depends on the patients' individual diversity [18]. To date celecoxib is still available on the market and current evidence suggests that celecoxib is an important therapeutic option because of its lower cardiovascular toxicity potential compared to NSAIDs and other -coxibs [19, 20]. Recent achievements have also highlighted potential applications of selective COX-2 inhibitors in treatments of cancers [21] and neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease [22].

Many efforts have been made to develop selective COX-2 inhibitors, and we became interested in exploring new surrogates for the pyrazole moiety of celecoxib. While searching for replacements of pyrazole with its bioisostere 1,2,4-triazole, we have noticed a report about 1,5-diaryl-1,2,4-triazoles displaying certain anti-inflammatory activities, but without identifying the mechanism of the compounds [23]. The results led us to propose that such 1,2,4-triazole-

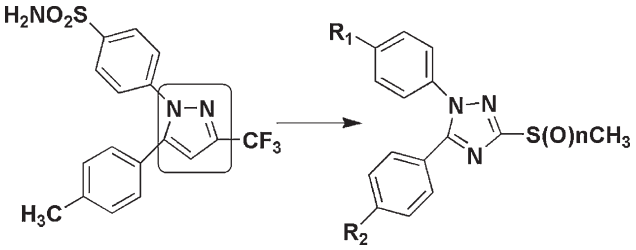


Figure 2. Structures for celecoxib and 1,5-diaryl-1,2,4-triazoles.

scaffold compounds should be capable of inhibiting the enzymatic activity of COX-2. Using celecoxib as a lead compound, a series of novel diaryl-1,2,4-triazole derivatives was designed and synthesized, and preliminary pharmacological assays *in vivo* showed that some compounds have certain anti-inflammatory effects [24]. Based on the above-mentioned results, different substituents of electron-withdrawing groups were introduced to the *para*-position of the N-1 phenyl ring. Herein, we wish to report the synthesis and biological evaluation of a series of 1,5-diaryl-1,2,4-triazole derivatives and their structures which are shown in Fig. 2.

Results and discussion

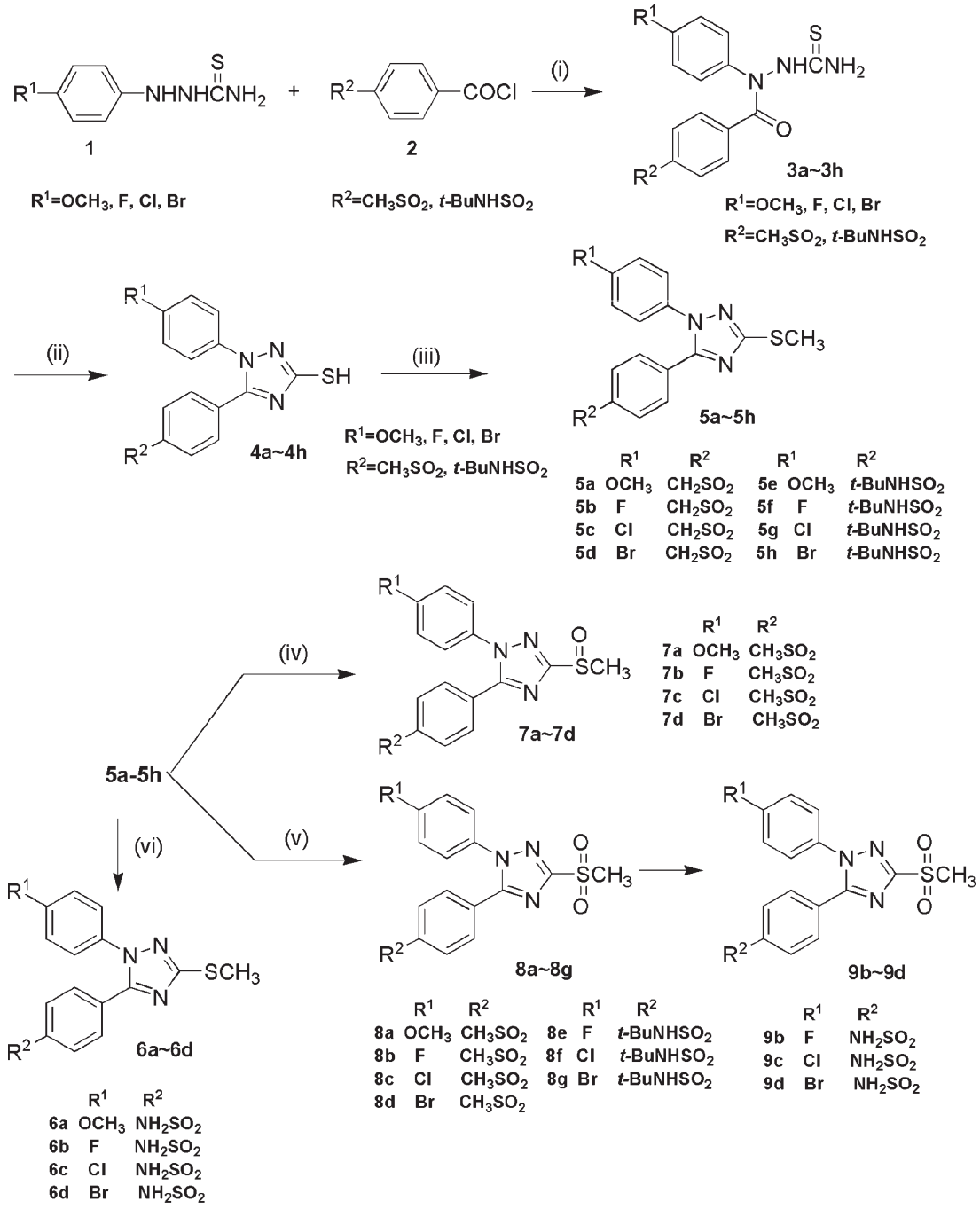
Chemistry

The synthetic routes of the target compounds are shown in the Scheme 1. *Para*-position phenylthiosemicarbazides were acylated with 4-(methylsulfonyl)benzoyl chloride to afford compounds **3a** to **3h**. Followed by cyclization of **3a–h**, compounds **4a–h** were methylated with iodomethane in good yield. Then, compounds **5a–h** were oxidated with sodium metaperiodate and 30% hydrogen peroxide to get the desired compounds **7a–d** and **8a–g**, respectively. Considering that there is a sulfonamide moiety in compounds **5e–h** and **8e–g**, which may lead to producing by-products in the reaction, we chose *tert*-butyl as a protective group and the desired compounds **6a–d** and **9b–d** were obtained through deprotection in trifluoroacetic acid and methylphenyl ether.

Pharmacology

Xylene-induced ear-edema assay in mice [25]

The pharmacological assay of xylene-induced ear edema in mice was applied. Compounds **5a–d**, **6a–d**, **7a–d**, **8a–d**, and **9b–d** were tested for their ability to inhibit ear edema compared to celecoxib after a single oral dose (Table 1). The results showed that the target compounds exhibited a broad range of anti-inflammatory activity. In general, introduction of a sulfamoyl moiety at the *para*-position of N-5 at the phenyl ring showed a more potent anti-inflammatory activity than a mesyl moiety, indicating that the sulfamoyl moiety is favorable to the *in-vivo* activity. Substituents such as methylthio



Reagents and conditions: (i) Et_3N, acetone, reflux, 2 h; (ii) 10% NaOH, MeOH, reflux, 2 h, 10% HCl; (iii) K_2CO_3 MeI, acetone, reflux, 3 h; (iv) $NaIO_4$, MeOH, reflux, 5 h; (v) 30% H_2O_2, H_2SO_4, acetic acid, 0–40°C, 3 h; (vi) CF_3COOH, $PhOCH_3$, 0°C–r. t., 24 h.

Scheme 1. The synthesis of 1,5-diaryl-1,2,4-triazole derivatives.

and sulfonyl groups at the 3-position of 1,2,4-triazoles bearing a sulfamoyl moiety at the *para*-position of N-5 at the phenyl ring contributed to good inhibitory potency. It was interesting to note that introduction of a weak electron-withdrawing group (F, Cl, or Br) at the *para*-position of the N-1 phenyl ring provided potent anti-inflammatory activity (**6b**, **6c**, **8d**, **9c**), especially the compound possessing Cl (**6c**) exhibited more a potent anti-inflammatory (80%) activity than the reference

Table 1. Inhibitory effect of target compounds on xylene-induced ear edema in mice.

Compound	*m* (mg)	Inhibition (%)	Thickness (mm)	Inhibition (%)
Model	11.5 ± 1.8		0.448 ± 0.140	
Celecoxib	4.04 ± 3.05#	64.9	0.160 ± 0.098#	64.3
5a	8.58 ± 1.70§	25.4	0.188 ± 0.033#	58.0
5b	7.98 ± 3.10#	30.6	0.206 ± 0.030#	54.0
5c	9.88 ± 1.90	14.1	0.300 ± 0.034	33.0
5d	6.16 ± 4.42§	46.4	0.178 ± 0.080#	60.3
6a	9.38 ± 1.47	18.4	0.284 ± 0.033§	36.6
6b	2.88 ± 1.52#	75.0	0.172 ± 0.041#	61.6
6c	2.30 ± 1.67#	80.0	0.076 ± 0.041#	83.0
6d	8.92 ± 1.45§	22.4	0.258 ± 0.025§	42.4
7a	8.48 ± 4.62	26.3	0.260 ± 0.035§	42.0
7b	9.12 ± 6.29	20.7	0.340 ± 0.047	24.1
7c	6.72 ± 2.09#	41.6	0.176 ± 0.017#	60.7
7d	5.94 ± 2.20#	48.4	0.234 ± 0.009§	47.8
8a	7.94 ± 1.36#	31.0	0.196 ± 0.013#	56.3
8b	7.44 ± 3.10§	35.3	0.178 ± 0.144#	60.3
8c	8.16 ± 2.34§	29.0	0.202 ± 0.063§	54.9
8d	3.36 ± 3.22#	70.8	0.182 ± 0.064#	59.4
9b	8.28 ± 3.27	28.0	0.304 ± 0.011	32.1
9c	2.62 ± 1.97#	77.2	0.184 ± 0.074#	59.0
9d	5.22 ± 3.79#	54.6	0.196 ± 0.044#	56.3

$n = 10$, $\bar{x} \pm S$; § $p < 0.05$, # $p < 0.01$.

Table 2. *In-vitro* COX-1 and COX-2 inhibitory activity data for **6c**.

Compound	% inhibition (1 μM)§		IC_{50} (μM)#		Selectivity Index& (SI)
	COX-1	COX-2	COX-1	COX-2	
Celecoxib	41.3	59.4	16.3$	0.26d	0.015
6c	40.7	63.5	20	0.37	0.018

§ The results are expressed as the mean %-inhibition value at a concentration of 1 μM of the test compound. The result is the mean of four determinations; # the test-compound concentration required to produce 50% inhibition of COX-1 or COX-2 *in vitro*. The result (IC_{50}, in μM) is the mean of four determinations; & COX-2 selectivity index (IC_{50} COX-2/IC_{50} COX-1) *in vitro*; $ see [27].

Table 3. The effect of compound **6c** on carrageenan-induced paw edema in rats.

Compound	Dose& (mg/kg)	Extent of swelling (mL × 10) and inhibition (%) at different times				PGE_2 ($\times 10^2$)
		1 h	2 h	3 h	4 h	
Model	–	1.35 ± 0.44	1.97 ± 0.47	2.67 ± 0.92	3.18 ± 1.01	7.3 ± 2.4
Celecoxib	15.0	0.80 ± 0.26# (40.7)	1.16 ± 0.29# (41.1)	1.62 ± 0.38# (39.3)	2.05 ± 0.33# (35.5)	4.1 ± 1.2#
6c	30.0	0.83 ± 0.27# (38.5)	1.18 ± 0.23# (40.1)	1.67 ± 0.44# (37.5)	2.15 ± 0.57§ (32.4)	4.1 ± 1.0#
	15.0	0.88 ± 0.23# (34.8)	1.28 ± 0.28# (35.0)	1.74 ± 0.33# (34.8)	2.27 ± 0.62§ (28.6)	4.4 ± 1.3#
	7.5	1.07 ± 0.32 (20.7)	1.46 ± 0.24# (25.9)	1.92 ± 0.29§ (28.1)	2.55 ± 0.34 (19.8)	4.8 ± 1.4§

§ $p < 0.05$; # $p < 0.01$ *vs.* model; inhibition values are in (); & the results are expressed as percentage of inhibition following 30, 15, 7.5 mg/kg oral dose of compound **6c**.

drug celecoxib (64.9%). In contrast, those compounds with a methoxyl group as an electron donor exhibited a weaker potency, while in our previous work [24] the methyl group as an electron donor was investigated and the results had shown that compounds with a methyl group at the *para*-position of the N-1 phenyl ring had provided equipotent inhibitory activity compared to the reference drug celecoxib. Further studies are needed to address if the lipophilicity of the compound is beneficial to the inhibitory potency.

COX-1/COX-2 inhibition assay in vitro

Based on the results of preliminary pharmacological tests *in vivo*, the best compound **6c** has interesting pharmacological properties and was selected to be tested in inhibition assays against COX-1 and COX-2 enzymes, using celecoxib as a reference drug [26]. As summarized in Table 2, compound **6c** exhibited good COX-2 inhibitory activity and selectivity (63.5% inhibition at 1 μM; COX-1 $IC_{50} = 20.0$ μM; COX-2 $IC_{50} = 0.37$ μM; SI = 0.018), which is equipotent to celecoxib (59.4% inhibition at 1 μM; COX-1 $IC_{50} = 16.3$ μM; COX-2 $IC_{50} = 0.26$ μM; SI = 0.015) [27]. The results suggest that compound **6c** is a selective COX-2 inhibitor.

Carrageenan-induced paw edema and acetic acid-induced vascular permeability assays [28, 29]

Anti-inflammatory activity evaluations *in vivo* for **6c** were carried out, and the results are illustrated in Tables 3 and 4. In the rat carrageenan-induced paw edema assay, compound **6c** exhibited a slightly less potent anti-inflammatory activity (34.8% and 35%) than the reference drug celecoxib (40.4% and 41.1%) at 1 h and 2 h after administration, respectively, for a 15 mg/kg oral dose. In addition, it exhibited the ability to decrease the permeability of celiac blood capillary and to block the release of mediators of inflammation in the acetic acid-induced vascular permeability assay. These data indicate that compound **6c** has certain potentials equivalent to the reference drug celecoxib in the acute inflammation model.

Table 4. The effect of compound **6c** on acid-induced vascular permeability in mice.

Compound	Dose (mg/kg)[&]	A_{590} (×10)
Model	–	2.47 ± 0.52
Celecoxib	30	1.36 ± 0.38[#]
	60	1.21 ± 0.36[#]
6c	30	1.78 ± 0.50[#]
	15	1.93 ± 0.60[§]

$n = 10$, $\bar{x} \pm$ S; § $p < 0.05$, # $p < 0.01$ *vs.* Model; & the results are expressed as mean ± SEM ($n = 10$) following 60, 30, 15 mg/kg oral dose of compound **6c**.

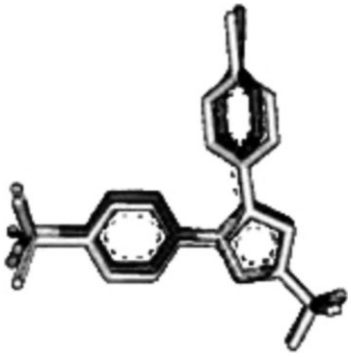


Figure 3. Conformational superposition of SC-558 from the crystal structure (gray) and that from the Gold result (light gray).

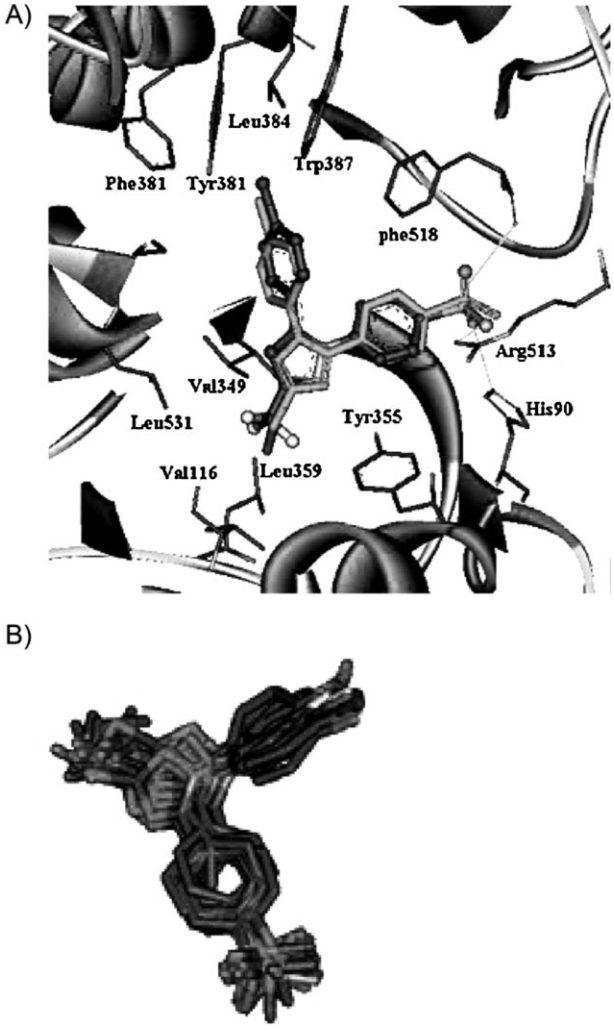


Figure 4. (A) Binding mode of compound **6c** (dark gray) and SC-558 (gray) in the COX-2 active site. (B) Likely binding conformations of 1,5-diaryl-1,2,4-triazole derivatives.

Docking study

The superposition between the GOLD-predicted conformation of the known selective COX-2 inhibitor SC-558 and that of the X-ray crystallographic complex is shown in Fig. 3. The root mean square deviation (RMSD) between these two conformations is 0.84 Å, indicating that the parameter set for the GOLD program is reasonable to reproduce the X-ray structure.

A docking study was carried out to investigate the binding interactions of compound **6c** within the COX-2 binding sites. As illustrated in Fig. 4, compound **6c** binds in the active site of COX-2 in a similar mode to that of known selective COX-2 inhibitor SC-558 [30]. The chlorophenyl ring is oriented in a hydrophobic pocket at the apex of the COX-2 binding site formed by Trp^{387}, Leu^{384}, Tyr^{381}, and Phe^{381}. The methylthio group is bound in the vicinity pocket formed by Val^{349}, Leu^{531}, Val^{116}, Leu^{359}, and Tyr^{355}. The sulfamoyl group forms hydrogen bonds with the Arg^{513}, Phe^{518}, and His^{90}, which are responsible for COX-2 selectivity.

Conclusions

In search for novel selective COX-2 inhibitors, 1,5-diaryl-1,2,4-triazole derivatives were synthesized and evaluated. The results showed that the target compounds displayed a broad range of anti-inflammatory activities in the xylene-induced ear edema assay *in vivo*. Among them, eight compounds **5b**, **6b**, **6c**, **7c**, **8b**, **8d**, **9c**, and **9d** have potent anti-inflammatory activity ($p < 0.01$), while compounds **6b**, **6c**, and **9c** exhibit marked potency. In the COX inhibition assay *in vitro*, compound **6c** was identified as a potent and selective inhibitor of COX-2 (COX-2 $IC_{50} = 0.37$ μM; SI = 0.018), being equipotent to celecoxib (COX-2 $IC_{50} = 0.26$ μM; SI = 0.015). In a rat carrageenan-induced paw edema assay, **6c** exhibited moderate anti-inflammatory activity (35% inhibition of inflammation) at 2 h after administration of 15 mg/kg oral dose. A docking study also revealed that compound **6c** binds in the active site of COX-2 in a similar mode to that of the known selective COX-2 inhibitor SC-558. These results may provide some useful information for the development of 1,5-diaryl-1,2,4-triazole derivatives as selective COX-2 inhibitors. As an interesting substance, compound **6c** is currently under further investigation.

Experimental

Chemistry

Reagents were purchased from Shanghai Chemical Reagent Company (China) and were used without further purification. Column chromatography (CC): silica gel 60 (200–300 mesh). Thin-layer chromatography (TLC): silica gel 60 F254 plates (250 μm; Qingdao Ocean Chemical Company, China). M. p.: RDCSY-I melting point apparatus; uncorrected. IR spectra: Shimadzu FTIR-8400S spectrometer (in cm^{-1}; Shimadzu, Japan). ^{1}H-NMR spectra: Bruker ACF-300Q apparatus at 300 MHz (Bruker, USA), in $CDCl_3$ unless otherwise indicated; δ in ppm rel. to Me_4Si, J in Hz. Mass spectrometry (MS): Hewlett-Packard 1100 LC/MSD spectrometer (Hewlett-Packard, USA); in *m/z*. Elemental analysis: Elementar Vario EL III instrument (Elementar, Germany).

*General procedure for synthesis of compounds **5a–5d***

1-(4-Substituted)-5-(4-(methylsulfonyl)phenyl)-1*H*-1,2,4-triazole-3-thiols (1 mmol) and K_2CO_3 (1.1 mmol) were suspended in 10 mL acetone at 50°C for 30 min, then MeI (1.1 mmol) was added, and the mixture was allowed to reflux for 3 h. Then, the mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was purified by flash chromatography (ethyl acetate/petroleum ether, 1:2) to get the desired compounds.

*1-(4-Methoxyphenyl)-5-(4-(methylsulfonyl)phenyl)-3-(methylthio)-1H-1,2,4-triazole **5a***

Yield: 53%, white solid, m. p.: 178–180°C; IR (KBr): 2916, 1595, 1606, 1513, 1300, 1257, 837 cm^{-1}; ^{1}H-NMR (DMSO-d_6), (δ, ppm): 2.61 (3H, s), 3.26 (3H, s), 3.81 (3H, s), 7.05 (2H, d, $J = 6.80$ Hz), 7.39 (2H, d, $J = 6.81$ Hz), 7.69 (2H, d, $J = 8.43$ Hz), 7.95 (2H, d, $J = 8.45$ Hz); ESI-MS: 376 $[M + H]^+$. Anal. calcd. for $C_{17}H_{17}N_3O_3S_2$: C, 54.38; H, 4.56; N, 11.19. Found: C, 54.45; H, 4.82; N, 10.81.

*1-(4-Fluorophenyl)-5-(4-(methylsulfonyl)phenyl)-3-(methylthio)-1H-1,2,4-triazole **5b***

Yield: 60%, white solid; m. p.: 185–188°C; IR (KBr): 2916, 1600, 1511, 1222, 1310, 1144, 847 cm^{-1}; ^{1}H-NMR (DMSO-d_6), (δ, ppm): 2.62 (3H, s), 3.27 (3H, s), 7.37 (2H, m), 7.54 (2H, m), 7.69 (2H, d, $J = 8.60$ Hz), 7.96 (2H, d, $J = 8.62$ Hz); ESI-MS: 364 $[M + H]^+$. Anal. calcd. for $C_{16}H_{14}FN_3O_2S_2$: C, 52.88; H, 3.88; N, 11.56. Found: C, 53.24; H, 4.15; N, 11.23.

*1-(4-Chlorophenyl)-5-(4-(methylsulfonyl)phenyl)-3-(methylthio)-1H-1,2,4-triazole **5c***

Yield: 76%, white solid; m. p.: 154–156°C; IR (KBr): 2996, 1600, 1494, 1303, 1151, 1091, 839 cm^{-1}; ^{1}H-NMR (DMSO-d_6), (δ, ppm): 2.62 (3H, s), 3.26 (3H, s), 7.49 (2H, d, $J = 4.95$ Hz), 7.59 (2H, d, $J = 6.74$ Hz), 7.71 (2H, d, $J = 6.73$ Hz), 7.97 (2H, d, $J = 5.10$ Hz); ESI-MS: 380 $[M + H]^+$. Anal. calcd. for $C_{16}H_{14}ClN_3O_2S_2$: C, 50.59; H, 3.71; N, 11.06. Found: C, 50.18; H, 4.09; N, 10.69.

*1-(4-Bromophenyl)-5-(4-(methylsulfonyl)phenyl)-3-(methylthio)-1H-1,2,4-triazole **5d***

Yield: 79%, white solid; m. p.: 157–159°C; IR (KBr): 2923, 1639, 1490, 1302, 1152, 833, 535 cm^{-1}; ^{1}H-NMR (DMSO-d_6), (δ, ppm): 2.62 (3H, s), 3.26 (3H, s), 7.42 (2H, d, $J = 8.37$ Hz), 7.71 (4H, m), 7.98 (2H, d, $J = 8.02$ Hz); ESI-MS: 424 $[M + H]^+$. Anal. calcd. for $C_{16}H_{14}BrN_3O_2S_2$: C, 45.29; H, 3.33; N, 9.90. Found: C, 45.57; H, 3.11; N, 9.72.

*General procedure for synthesis of compounds **6a–6d***

A solution of the corresponding compounds **5a–d** (1 mmol), CF_3COOH (2 mL), and two drops of methylphenyl ether were stirred at room temperature for 24 h. The mixture was

evaporated *in vacuo* and the residue was purified by flash chromatography (ethyl acetate/petroleum ether, 2:3) to get the desired compounds.

4-(1-(4-Methoxyphenyl)-3-(methylthio)-1H-1,2,4-triazol-5-yl)benzenesulfonamide ***6a***

Yield: 72%, white solid; m. p.: 245–249°C; IR (KBr): 3446, 3317, 2980, 1607, 1513, 1348, 1164, 1018, 838 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 2.67 (3H, s), 3.87 (3H, s), 4.80 (2H, s), 6.95 (2H, d, J = 4.56 Hz), 7.24 (2H, d, J = 2.21 Hz), 7.65 (2H, d, J = 4.85 Hz), 7.86 (2H, d, J = 1.91 Hz); ESI-MS: 377 $[M + H]^+$. Anal. calcd. for $C_{16}H_{16}N_4O_3S_2$: C, 51.05; H, 4.28; N, 14.88. Found: C, 51.46; H, 4.30; N, 14.65.

4-(1-(4-Fluorophenyl)-3-(methylthio)-1H-1,2,4-triazol-5-yl)benzenesulfonamide ***6b***

Yield: 67%, white solid; m. p.: 233–236°C; IR (KBr): 3451, 3322, 3075, 1601, 1512, 1341, 1161, 1223, 840 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.40 (3H, s), 4.87 (2H, s), 7.20 (2H, m), 7.39 (2H, m), 7.69 (2H, d, J = 6.74 Hz), 7.95 (2H, d, J = 7.76 Hz); ESI-MS: 365 $[M + H]^+$. Anal. calcd. for $C_{15}H_{13}FN_4O_2S_2$: C, 49.44; H, 3.60; N, 15.37. Found: C, 49.21; H, 3.89; N, 15.72.

4-(1-(4-Chlorophenyl)-3-(methylthio)-1H-1,2,4-triazol-5-yl)benzenesulfonamide ***6c***

Yield: 73%, white solid; m. p.: 235–238°C; IR (KBr): 3463, 2921, 1644, 1497, 1335, 1160, 1102, 834 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 2.67 (3H, s), 4.87 (2H, s), 7.28 (2H, d, J = 8.57 Hz), 7.43 (2H, d, J = 8.64 Hz), 7.64 (2H, d, J = 8.30 Hz), 7.91 (2H, d, J = 8.36 Hz); ESI-MS: 379 $[M - H]^-$. Anal. calcd. for $C_{15}H_{13}ClN_4O_2S_2$: C, 47.30; H, 3.44; N, 14.71. Found: C, 47.03; H, 3.70; N, 14.98.

4-(1-(4-Bromophenyl)-3-(methylthio)-1H-1,2,4-triazol-5-yl)benzenesulfonamide ***6d***

Yield: 62%, white solid; m. p.: 241–244°C; IR (KBr): 3427, 1586, 1493, 1333, 1162, 830, 618 cm^{-1}; ^{1}H-NMR($CDCl_3$), (δ, ppm): 2.67 (3H, s), 4.84 (2H, s), 7.22 (2H, d, J = 8.79 Hz), 7.58 (2H, d, J = 8.78 Hz), 7.64 (2H, d, J = 8.70 Hz), 7.92 (2H, d, J = 8.69 Hz); ESI-MS: 425 $[M + H]^+$. Anal. calcd. for $C_{15}H_{13}BrN_4O_2S_2$: C, 42.36; H, 3.08; N, 13.17. Found: C, 42.57; H, 3.46; N, 12.83.

General procedure for synthesis of compounds ***7a–7d***

The corresponding compounds **5a–d** (1 mmol) were suspended in methanol (30 mL), heated to 50°C, and then quickly added to the solution of $NaIO_4$ (3 mmol) in 5 mL of water in one portion. The mixture was heated to reflux for 5 h and then was filtered. The filtrate was evaporated *in vacuo*. The residue was purified by flash chromatography (acetone/petroleum ether, 1:1) to get the desired compounds.

1-(4-Methoxyphenyl)-3-(methylsulfinyl)-5-(4-(methylsulfonyl)phenyl)-1H-1,2,4-triazole ***7a***

Yield: 61%, white solid; m. p.: 197–199°C; IR (KBr): 2922, 1607, 1515, 1310, 1148, 1071, 1025, 840 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.07 (3H, s), 3.14 (3H, s), 3.88 (3H, s), 6.99 (2H, d, J = 9.02 Hz), 7.28 (2H, d, J = 9.05 Hz), 7.76 (2H, d, J = 8.74 Hz), 7.94 (2H, d, J = 8.72 Hz); ESI-MS: 392 $[M + H]^+$. Anal. calcd. for $C_{17}H_{17}N_3O_4S_2$: C, 52.16; H, 4.28; N, 10.73. Found: C, 52.51; H, 4.05; N, 10.30.

1-(4-Fluorophenyl)-3-(methylsulfinyl)-5-(4-(methylsulfonyl)phenyl)-1H-1,2,4-triazole ***7b***

Yield: 49%, white solid; m. p.: 214–216°C; IR (KBr): 2924, 1633, 1512, 1307, 1149, 1083, 844 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.07 (3H, s), 3.13 (3H, s), 7.18 (2H, m), 7.36 (2H, m), 7.73 (2H, d, J = 8.52 Hz), 7.95 (2H, d, J = 8.51 Hz); ESI-MS: 380 $[M + H]^+$. Anal. calcd. for $C_{16}H_{14}FN_3O_3S_2$: C, 50.65; H, 3.72; N, 11.07. Found: C, 50.43; H, 3.99; N, 10.67.

1-(4-Chlorophenyl)-3-(methylsulfinyl)-5-(4-(methylsulfonyl)phenyl)-1H-1,2,4-triazole ***7c***

Yield: 53%, white solid; m. p. 192–195°C; IR (KBr): 2916, 1632, 1496, 1310, 1148, 1093, 1079, 838 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.08 (3H, s), 3.15 (3H, s), 7.33 (2H, d, J = 8.89 Hz), 7.49 (2H, d, J = 8.90 Hz), 7.75 (2H, d, J = 8.73 Hz), 7.98 (2H, d, J = 8.72 Hz); ESI-MS: 396 $[M + H]^+$. Anal. calcd. for $C_{16}H_{14}ClN_3O_3S_2$: C, 48.54; H, 3.56; N, 10.61. Found: C, 48.66; H, 3.90; N, 10.25.

1-(4-Bromophenyl)-3-(methylsulfinyl)-5-(4-(methylsulfonyl)phenyl)-1H-1,2,4-triazole ***7d***

Yield: 61%, white solid; m. p. 179–182°C; IR (KBr): 2924, 1628, 1490, 1309, 1146, 1079, 837, 534 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.08 (3H, s), 3.14 (3H, s), 7.26 (2H, d, J = 8.82 Hz), 7.64 (2H, d, J = 8.81 Hz), 7.74 (2H, d, J = 8.72 Hz), 7.98 (2H, d, J = 8.68 Hz); ESI-MS: 442 $[M + H]^+$. Anal. calcd. for $C_{16}H_{14}BrN_3O_3S_2$: C, 43.64; H, 3.20; N, 9.54. Found: C, 43.32; H, 3.53; N, 9.21.

General procedure for synthesis of compounds ***8a–8d***

The corresponding compounds **5a–d** and **5f–h** (1 mmol) were added slowly to the solution of glacial acetic acid (2 mL), 30% H_2O_2 (3 mmol), and one drop of concentrated H_2SO_4 at 0°C with stirring. The mixture was warmed to room temperature and heated to 40°C for 3 h. After cooling down, 1 mL of water was added and the mixture stirred over 5 min, then filtered, the filter cake was washed with water (3 × 1 mL), and then purified by flash chromatography (ethyl acetate/petroleum ether, 1:1) to get the desired compounds.

1-(4-Methoxyphenyl)-3-(methylsulfonyl)-5-(4-(methylsulfonyl)phenyl)-1H-1,2,4-triazole ***8a***

Yield: 62%, white solid; m. p.: 189–191°C; IR (KBr): 2924, 1608, 1516, 1259, 1323, 1151, 841 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.08 (3H, s), 3.40 (3H, s), 3.89 (3H, s), 7.00 (2H, d, J = 9.03 Hz), 7.30 (2H, d, J = 6.87 Hz), 7.77 (2H, d, J = 9.03 Hz), 7.95 (2H, d, J = 6.90 Hz); ESI-MS: 408 $[M + H]^+$. Anal. calcd. for $C_{17}H_{17}N_3O_5S_2$: C, 50.11; H, 4.21; N, 10.31. Found: C, 49.83; H, 4.64; N, 10.58.

1-(4-Fluorophenyl)-3-(methylsulfonyl)-5-(4-(methylsulfonyl)phenyl)-1H-1,2,4-triazole ***8b***

Yield: 61%, white solid; m. p.: 189–191°C; IR (KBr): 2931, 1604, 1512, 1333, 1150, 1282, 843 cm^{-1};^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.09 (3H, s), 3.41 (3H, s), 7.22 (2H, d, J = 8.61 Hz), 7.40 (2H, m), 7.75 (2H, d, J = 8.61 Hz), 7.98 (2H, d, J = 8.61 Hz); ESI-MS: 430 $[M + Cl]^-$.

Anal. calcd. for $C_{16}H_{14}FN_3O_4S_2$: C, 48.60; H, 3.57; N, 10.63. Found: C, 48.39; H, 3.19; N, 10.35.

1-(4-Chlorophenyl)-3-(methylsulfonyl)-5-(4-(methylsulfonyl)phenyl)-1H-1,2,4-triazole ***8c***

Yield: 60%, white solid; m. p.: 209–211°C; IR (KBr): 2916, 1632, 1495, 1308, 1144, 1084, 839 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.10 (3H, s), 3.40 (3H, s), 7.35 (2H, d, $J = 9.03$ Hz), 7.51 (2H, d, $J = 9.03$ Hz), 7.75 (2H, d, $J = 8.58$ Hz), 7.99 (2H, d, $J = 8.61$Hz); ESI-MS: 446 $[M + Cl]^-$. Anal. calcd. for $C_{16}H_{14}ClN_3O_4S_2$: C, 46.66; H, 3.43; N, 10.20. Found: C, 46.53; H, 3.80; N, 9.78.

1-(4-Bromophenyl)-3-(methylsulfonyl)-5-(4-(methylsulfonyl)phenyl)-1H-1,2,4-triazole ***8d***

Yield: 66%, white solid; m. p.: 208–210°C; IR (KBr): 2923, 1639, 1491, 1310, 1148, 836, 550 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.09 (3H, s), 3.39 (3H, s), 7.29 (2H, d, $J = 8.70$ Hz), 7.66 (2H, d, $J = 8.77$ Hz), 7.75 (2H, d, $J = 8.41$ Hz), 7.99 (2H, d, $J = 8.37$ Hz); ESI-MS: 456 $[M + H]^+$. Anal. calcd. for $C_{16}H_{14}BrN_3O_4S_2$: C, 42.11; H, 3.09; N, 9.21. Found: C, 42.56; H, 3.43; N, 9.57.

General procedure for synthesis of compounds ***9b–9d***

The preparation of compounds **9b–d** was according to the general procedure for the synthesis of compounds **6b–d**.

4-(1-(4-Fluorophenyl)-3-(methylsulfonyl)-1H-1,2,4-triazol-5-yl)benzenesulfonamide ***9b***

Yield: 68%, white solid; m. p. 236–240°C; IR (KBr): 3450, 2927, 1601, 1509, 1342, 1143, 1235, 842 cm^{-1}; ^{1}H-NMR ($CDCl_3$ + DMSO), (δ, ppm): 3.33 (3H, s), 6.92 (2H, s), 7.17 (2H, m), 7.37 (2H, m), 7.57 (2H, d, $J = 8.51$ Hz), 7.87 (2H, d, $J = 8.52$ Hz); ESI-MS: 397 $[M + H]^+$. Anal. calcd. for $C_{15}H_{13}FN_4O_4S_2$: C, 45.45; H, 3.31; N, 14.13. Found: C, 45.88; H, 3.05; N, 14.58.

4-(1-(4-Chlorophenyl)-3-(methylsulfonyl)-1H-1,2,4-triazol-5-yl)benzenesulfonamide ***9c***

Yield: 57%, white solid; m. p.: 254–256°C; IR (KBr): 3389, 3283, 2918, 1559, 1495, 1323, 1144, 1091, 838 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.40 (3H, s), 4.89 (2H, s), 7.34 (2H, d, $J = 9.03$ Hz), 7.50 (2H, d, $J = 9.02$ Hz), 7.70 (2H, d, $J = 8.58$ Hz), 7.96 (2H, d, $J = 8.58$ Hz); ESI-MS: 413 $[M + H]^+$. Anal. calcd. for $C_{15}H_{13}ClN_4O_4S_2$: C, 43.64; H, 3.17; N, 13.57. Found: C, 43.20; H, 3.58; N, 13.33.

4-(1-(4-Bromophenyl)-3-(methylsulfonyl)-1H-1,2,4-triazol-5-yl)benzenesulfonamide ***9d***

Yield: 66%, white solid; m. p.: 255–257°C; IR (KBr): 3387, 2917, 1605, 1491, 1323, 1142, 835, 621 cm^{-1}; ^{1}H-NMR ($CDCl_3$), (δ, ppm): 3.39 (3H, s), 4.88 (2H, s), 7.28 (2H, d, $J = 8.80$ Hz), 7.66 (2H, d, $J = 8.81$ Hz), 7.70 (2H, d, $J = 8.66$ Hz), 7.96 (2H, d, $J = 8.63$ Hz); ESI-MS: 457 $[M + H]^+$. Anal. calcd. for $C_{15}H_{13}BrN_4O_4S_2$: C, 39.40; H, 2.87; N, 12.25. Found: C, 39.75; H, 2.56; N, 11.90.

Pharmacology

Xylene-induced ear edema in mice

Swiss albino mice (18–22 g) were purchased from the Animal Centre of Henan Province (Zhengzhou, China). Mice were divided into twenty-one groups ($n = 5$). Compounds **5a–5d**, **6a–6d**, **7a–7d**, **8a–8d**, and **9b-9d** (24–30 mg/kg), celecoxib (250 mg/kg), and 0.5% CMC-Na solution were administered. One hour later, xylene (0.01 mL) was applied to the anterior and posterior surfaces of the right ear lobe and the left ear was regarded as control. After 30 min, the mice were killed by neck dislocation and both ears were removed. Circular sections were taken and weighed, using a cork borer with a diameter of 7 mm. The thickness of the ear was determined using a micrometer caliper. The percentage of ear edema was calculated based on the left ear without xylene.

In-vitro cyclooxygenase (COX) inhibition assays

6-Keto-$PGF_{1\alpha}$ production was measured in order to determine COX-1 activity. Bovine aortic endothelial cells were obtained from the thoracic aorta of newborn calves. The cells were maintained at 37°C and 5% CO_2 in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 60 mg/L penicillin, and 100 mg/L streptomycin. Cells were plated into 24-well plates at a density of 1.0×10^6 cells/well in 1 mL of DMEM. After 24 h of incubation, the cells were treated with compound **6c** (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} mol/L), DMSO, and the reference drug celecoxib (10^{-6} mol/L). These solutions were incubated for 20 min at 37°C and 5% CO_2, and then treated with arachidonic acid (10 μmol/L). After 20 min of incubation, the culture supernatants were collected and stored at −70°C until required for the 6-keto-$PGF_{1\alpha}$ determination. 6-Keto-$PGF_{1\alpha}$ was measured by using a radioimmuno assay kit according to the manufacturer's instructions.

PGE_2 production was measured in the culture medium in order to determine COX-2 activity. Peritoneal macrophages were gained by lavaging the peritoneal cavity of mice, which were killed by cervical dislocation for the PGE_2 induction assay, cells were plated into 24-well plates at a density of 1.0×10^6 cells/well in 1 mL of RPMI 1640 supplemented with 5% heat-inactivated fetal bovine serum, 2 mM glutamine, 60 mg/L penicillin, and 100 mg/L streptomycin. After 2 h of incubation, the cells were washed three times with fresh RPMI 1640 and the culture media were replaced with fresh RPMI 1640 containing LPS (1 μg/mL) and compound **6c** (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} mol/L), LPS (1 μg/mL) and celecoxib (10^{-6} mol/L), LPS (1 μg/mL) and DMSO, respectively. These solutions were incubated for 6 h at 37°C and 5% CO_2 and were treated with arachidonic acid (10 μmol/L). After 20 min of incubation, the culture supernatants were collected and stored at −70°C until required for PGE_2 determination. PGE_2 was measured by using a radioimmuno assay kit according to the manufacturer's instructions.

Anti-inflammatory activity

The tests of anti-inflammatory activity were adapted from carrageenan-induced paw edema and acetic acid-induced vascular permeability assays with some modifications, respectively.

Male Sprague-Dawley rats (130–170 g) were purchased from the Animal Centre of Henan Province (Zhengzhou, China). Rats were divided into five groups ($n = 10$) and dosed orally with compound **6c** (7.5, 15, and 30 mg/kg), celecoxib (15 mg/kg), and CMC-Na solution (0.5%). After one hour, 0.1 mL of a 1% suspension of carrageenan was injected into the plantar surface of the right hind foot of each rat. The injected (right) and non-injected (left) foot volumes were measured with a plethysmometer at 1, 2, 3, and 4 h after carrageenan injection. The mean

degree of swelling for each group was calculated and the results were expressed as percent inhibition of swelling.

The mice were divided into groups as described above and dosed orally with compound **6c** (15, 30, and 60 mg/kg), celecoxib (30 mg/kg), and CMC-Na solution (0.5%). After one hour, 0.5% Evan's blue dye solution in saline was injected (0.1 mL/10 g) through the tail vein immediately followed by the injection of 0.2 mL 0.6% solution of acetic acid intraperitoneally. 30 min later, the mice were killed by neck dislocation and the viscera were irrigated with normal saline. This was then collected and centrifuged at 3000 rpm for 15 min. The supernatants were increased in volume to 10 mL with normal saline, the absorption of which was measured at 590 nm.

Docking study

The crystal structure of murine COX-2 in complex with SC-558 was received from the RCSB Protein Data Bank (PDB; entry code 1cx2) and hydrogens were added. The docking experiment on COX-2 was carried out by superimposing the energy-minimized ligand on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The potential of the 3-D structures of COX-2 and COX-1 was assigned according to the Amber 4.0 force field with Kollmanall-atom charges encoded in Sybyl 6. The initial structures of nineteen 1,5-diaryl-1,2,4-triazoles were generated by the molecular modeling software Sybyl 6. The geometries of these compounds were subsequently optimized using the Tripos force field with Gasteiger-Huckel charges. The method of Powell available in the Maximin module encoded in Sybyl 6 was used for energy minimization using an 8-Å nonbond cutoff and an energy convergence gradient value of 0.005 kcal/(mol Å).

Docking experiments were performed by the program of GOLD, version 3.0 [31]. This program from Cambridge Crystallographic Data Center, UK uses genetic algorithm for docking flexible ligands into protein binding sites to explore the full range of ligand conformational flexibility with partial flexibility of the protein. The results of the docking were demonstrated mainly by GOLD fitness score. The X-ray coordinates of SC-558 bound to the active site of COX-2 were used to define the active-site region with an active-site radius of 10 Å. The annealing parameters of van-der-Waals and hydrogen-bond interactions were considered within 4.0 and 2.5 Å, respectively, and other parameters were kept at the default setting.

For financial assistance, this study was supported by grants from the Key fund of Ministry of Education of China (No. 108069) and Jiangsu Provincial Natural Science Fund (No. BK2004109).

The authors have declared no conflict of interest.

References

[1] E. J. Topol, G. W. Falk, *Lancet* **2004**, *364*, 639–640.

[2] J. R. Vane, Y. S. Bakhle, R. M. Botting, *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 97–120.

[3] H. Herschman, *Biochim. Biophys. Acta* **1996**, *1299*, 125–140.

[4] D. Munroe, C. Y. Lau, *Chem. Biol.* **1995**, *2*, 343–350.

[5] J. R. Vane, *J. Physiol. Pharmacol.* **2000**, *51*, 573–586.

[6] D. L. Simmons, R. M. Botting, T. Hla, *Pharmacol. Rev.* **2004**, *56*, 387–437.

[7] L. Parente, M. Perretti, *Biochem. Pharmacol.* **2003**, *65*, 153–159.

[8] X. de Leval, F. Julemont, V. Benoit, M. Frederich, *et al.*, *Mini-Rev. Med. Chem.* **2004**, *4*, 597–601.

[9] D. O. Stichtenoth, J. C. Frölich, *Drugs* **2003**, *63*, 33–45.

[10] J. J. Talley, D. L. Brown, J. S. Carter, M. J. Graneto, *et al.*, *J. Med. Chem.* **2000**, *43*, 775–777.

[11] R. Hubbard, M. Kuss, S. Talwalker, *et al.*, *Ann. Emerg. Med.* **2000**, *36*, S69.

[12] K. Brune, B. Hinz, *Scand. J. Rheumatol.* **2004**, *33*, 1–6.

[13] www.fda.gov/Cder/Drug/Advisory/Cox2.Htm

[14] T. Grosser, S. Fries, G. A. Fitzgerald, *J. Clin. InVest.* **2006**, *116*, 4–15.

[15] L. A. Rodriguez, P. Patrignani, *Lancet* **2006**, *368*, 1745–1747.

[16] V. Strand, *Lancet* **2007**, *370*, 2138–2151.

[17] P. M. Gettigan, D. Henry, *JAMA* **2006**, *296*, 1633–1644.

[18] J. M. Dogne, C. T. Supuran, D. Pratico, *J. Med. Chem.* **2005**, *48*, 2251–2257.

[19] N. M. Gajraj, *Curr. Top. Med. Chem.* **2007**, *7*, 235–249.

[20] I. Moodley, *Cardiovasc. J. Afr.* **2008**, *19*, 102–107.

[21] F. H. Sarkar, S. Adsule, Y. W. Li, S. Padhye, *Mini-Rev. Med. Chem.* **2007**, *7*, 599–608.

[22] M. Nivsarkar, A. Banerjee, H. Padh, *Pharmacol. Rep.* **2008**, *60*, 692–698.

[23] G. Szilagyi, T. Somorai, T. Bozo, J. Lango, *et al.*, *Eur. J. Med. Chem.* **1990**, *25*, 95–101.

[24] J. Y. Xu, H. Q. Yao, Y. Zeng, *et al.*, *Chem. J. Chin. Univ.* **2005**, *26*, 2254–2258.

[25] K. Junping, N. Yun, N. Wang, L. Liang, *Bio. Pharm. Bull.* **2005**, *28*, 176–180.

[26] J. A. Mitchell, P. Akarasereenont, C. Thiemermann, *et al.*, *Proc. Natl. Acad. Sci. USA* **1994**, *90*, 11693.

[27] C. M. Reddy, V. B. Bhat, G. Kiranmai, M. N. Reddy, *Biochem. Biophys. Res. Commun.* **2000**, *277*, 599–603.

[28] C. A. Winter, E. Risley, G. Nuss, *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544–547.

[29] B. A. Whittle, *Br. J. Pharmacol. Chemother.* **1964**, *22*, 246–253.

[30] R. G. Kurumbail, A. M. Stevens, J. K. Gierse, J. J. McDonald, *Nature* **1996**, *384*, 644–648.

[31] G. Jones, P. Willett, R. C. Glen, *J. Mol. Biol.* **1995**, *245*, 43–53.

