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Discovery of potential anti-inflammatory drugs: diaryl-1,2,4-triazoles bearing *N*-hydroxyurea moiety as dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase†

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A series of hybrids from diaryl-1,2,4-triazole and hydroxamic acid or *N*-hydroxyurea were synthesized and evaluated as novel anti-inflammatory agents. The biological data showed that (i) all the compounds showed dual COX-2/5-LOX inhibitory activities *in vitro*, and **15e** showed optimal inhibitory activities (COX-2: IC_{50} = 0.15 μM, 5-LOX: IC_{50} = 0.85 μM), (ii) **15e** selectively inhibited COX-2 relative to COX-1 with selectivity index (SI = 0.012) comparable to celecoxib (SI = 0.015), (iii) **15e** exhibited potent anti-inflammatory activity (inhibition: 54.1%) which was comparable to the reference drug celecoxib (inhibition: 46.7%) in a xylene-induced ear edema assay, and (iv) **15e** displayed promising analgesic activity in acetic acid-induced writhing response and hot-plate assay. Finally, a molecular modeling study revealed the binding interactions of **15e** with COX-2 and 5-LOX. Our findings suggest that **15e** may be a promising anti-inflammatory agent for further evaluation.

1. Introduction

Inflammation is an extremely complex biological process which implicates a great number of mediators originating from the over-activation of multiple cascades. Eicosanoids including prostaglandins (PGs) and leukotrienes (LTs) synthesized from arachidonic acid (AA) play an important role in many inflammatory and allergic disorders, *e.g.* rheumatoid arthritis (RA), osteoarthritis (OA), asthma and psoriasis.[1] Following its release from membrane-bound phospholipids, AA undergoes further biotransformation *via* cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) pathways. The COX pathway has long been used as a target for anti-inflammatory drugs. Both traditional non-steroidal anti-inflammatory drugs (NSAIDs) (ibuprofen, indomethacin and naproxen) and selective COX-2 inhibitors coxibs (celecoxib, rofecoxib and valdecoxib) block AA metabolism by inhibiting the COX.[2] However, the prolonged use of traditional NSAIDs are associated with several serious side effects such as kidney failure, ulcers and prolonged bleeding after an injury or surgery.[3,4] Furthermore, the withdrawal of rofecoxib and valdecoxib from the market due to an increased risk of cardiovascular complications (*e.g.*, myocardial infarction and stroke) aroused great concern about coxibs' safety problem.[5–7] In spite of advances in the last decades, safe and effective anti-inflammatory drug design remains a great challenge.

It is generally agreed that drugs acting on an individual target usually exert unwanted therapeutic effects and even severe toxicity, whereas multi-target therapeutics which regulate multiple nodes of the disease network simultaneously exhibit a synergistic effect and provide optimal clinical use.[8] 5-LOX is another key enzyme in AA metabolism and responsible for the conversion of AA into LTs which are potent mediators of inflammation and allergic reactions and may play a role in cardiovascular diseases.[9] It has also been well established that inhibition of the COX enzymes may shunt AA metabolism to the 5-LOX pathway,[10] but when both COX-2 and 5-LOX were blocked the production of inflammatory mediators could be completely shut off.[11] Thus, dual inhibition of COX-2 and 5-LOX constitutes a rational concept for the design of more efficacious anti-inflammatory agents with an improved safety profile.[12] Besides, both COX-2 and 5-LOX are implicated

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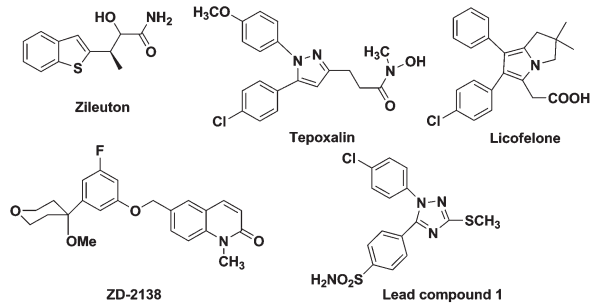


Fig. 1 Structures of zileuton, representative dual COX-2/5-LOX inhibitors and lead compound **1**.

in a number of therapeutic areas of interest including cancers[13–15] and age-related degenerative conditions, particularly Alzheimer's and Parkinson's diseases.[16,17]

Various structures of dual COX/5-LOX inhibitors have been developed, and the representatives are tepoxalin, licofelone and ZD-2138 (Fig. 1).[18–20] Tepoxalin has been approved for the treatment of OA in dogs in the United States and the European Union.[20,21] Licofelone is a promising dual COX/5-LOX inhibitor. The results of Phase III clinical trials suggested that licofelone is effective in the treatment of OA with reduced gastrointestinal (GI) toxicity and thromboembolic risk compared to traditional NSAIDs and COX-2 selective inhibitors.[22–25] Besides the above-mentioned dual inhibitors, natural products are also an important source of dual inhibitors,[26–29] among them, flavocoxid (Limbrel®) has been launched to market as an FDA-regulated medical food for the clinical dietary management of the metabolic aspects of OA in the United States.[30]

Recently, we have reported that 1,5-diaryl-1,2,4-triazole derivatives were identified as selective COX-2 inhibitors, among which compound **1** exhibited potent and selective COX-2 inhibitory activity (IC_{50} = 0.37 μM, SI = 0.018), being equipotent to celecoxib.[31] Meanwhile we have noticed a report about hydroxamic acid or *N*-hydroxyurea structures, which have provided one of the most successful strategies to develop iron-chelating 5-LOX inhibitors—zileuton (Fig. 1).[32] As a part of our continuing effort to find new NSAIDs candidates, we became interested in exploring dual COX-2/5-LOX inhibitors with novel structural characteristics, better anti-inflammatory effects and lower cardiovascular risk. Accordingly, we designed a series of novel hybrids in which the selective inhibitory COX-2 moiety diaryl-1,2,4-triazole of compound **1** was incorporated with hydroxamic acid or *N*-hydroxyurea group. Herein we report the synthesis, biological evaluation and docking studies of these diaryl-1,2,4-triazoles bearing the hydroxamic acid or hydroxyurea moiety as dual COX/5-LOX inhibitors (Fig. 2).

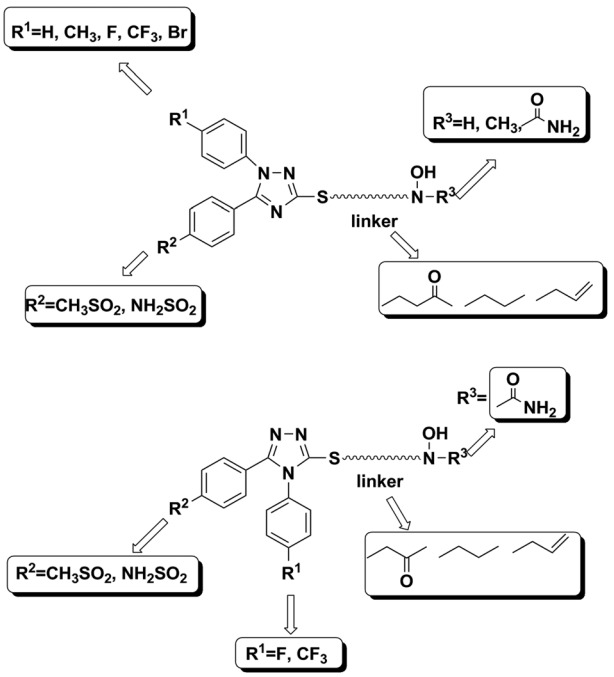


Fig. 2 Diaryl-1,2,4-triazoles bearing the hydroxamic acid or *N*-hydroxyurea moiety.

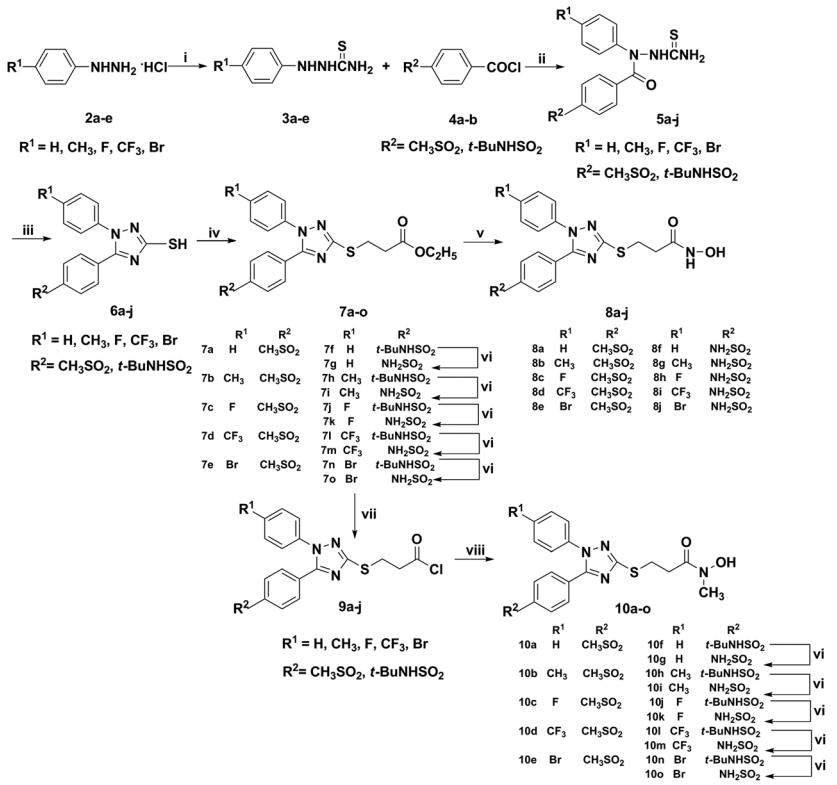
2. Results and discussion

2.1. Chemistry

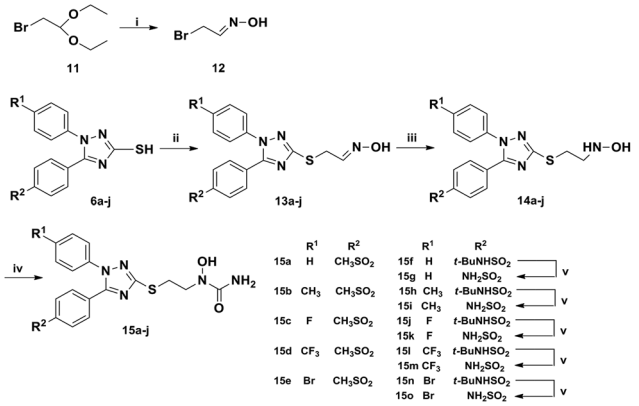
The synthetic route of target compounds **8a–j** and **10a–o** is shown in Scheme 1. Using commercially available *para*-position substituted phenylhydrazine hydrochloride as initial material, the critical intermediates **6a–j** were synthesized as described in our previous report.[31] The thiols bearing 1,2,4-triazole were readily converted into the corresponding esters **7a–o** by treatment with ethyl 3-bromopropionate in good yields. The target hydroxamic acid derivatives **8a–j** could be directly prepared by mixing **7a–o** with hydroxylamine methanol solution in the presence of KOH at room temperature. Nevertheless, it was difficult to obtain *N*-methyl hydroxamic acid derivatives **10a–o** by the above-mentioned procedures, presumably owing to the steric hindrance of methyl. Thus, the esters **7a–o** were converted to more active acyl chloride **9a–j**, then by reaction with *N*-methyl hydroxylamine hydrochloride in the presence of Et_3N to give the desired *N*-methylhydroxamic acid compounds **10a–e**. The target compounds bearing *p*-NH_2SO_2 moiety at N-1 phenyl ring were obtained by deprotection of *tert*-butyl in CF_3COOH–$PhOCH_3$ solution.

The strategy to synthesize *N*-hydroxyurea derivatives **15a–j** is shown in Scheme 2. Commercially available bromoacetaldehyde diethyl acetal **11** was converted directly to oxime **12**,[33] which was treated with intermediates **6a–j** to yield the requisite oximes **13a–j** in high overall yields. Reduction of the oximes to the hydroxylamines **14a–j** was carried out by treatment of **13a–j** with a 3-fold excess of $NaBH_3CN$ in the presence of 4 N HCl. The conversion of **14a–j** to the aimed compounds *N*-hydroxyureas **15a–j** was performed *via* the addition of KOCN/HOAc in methanol.

The target 3,4,5-substituted-1,2,4-triazole derivatives **21a–d** and **22a–d** were prepared using the synthetic route illustrated in Scheme 3. Starting with appropriate benzoic acids **16a–b**,



Scheme 1 Synthesis of hydroxamic acid derivatives of 1,3,5-substituted-1,2,4-triazoles. *Reagents and conditions*: (i) KSCN, con. HCl, anhydrous EtOH, reflux, 5 h; (ii) Et_3N, acetone, reflux, 2 h; (iii) a. 10% NaOH, MeOH, reflux, 2 h; b. 10% HCl; (iv) ethyl 3-bromopropionate, K_2CO_3, acetone, reflux, 3 h; (v) a. 2 mol L^{-1} NH_2OH/MeOH, KOH, r.t., 1 h; b. glacial acetic acid; (vi) TFA/methylanisole, 0 °C–r.t., 24 h; (vii) a. 10% NaOH, MeOH, reflux, 2 h; b. 10% HCl; c. oxalyl dichloride, dry DCM, r.t., 3 h; (viii) *N*-methylhydroxylamine hydrochloride, Et_3N, dry DCM, r.t., 2 h.



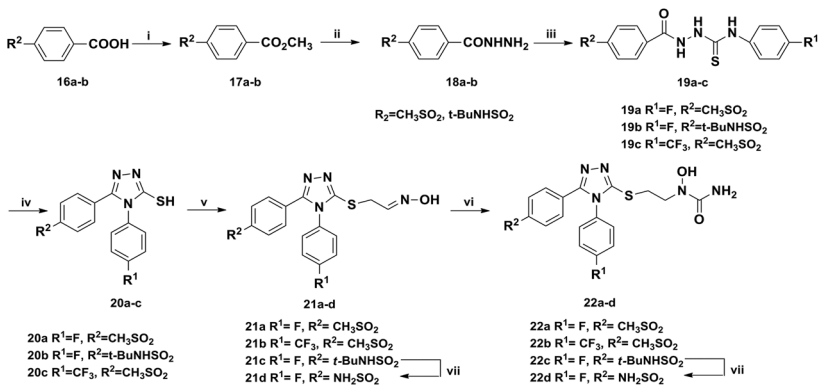
Scheme 2 Synthesis of *N*-hydroxyurea derivatives of 1,3,5-substituted-1,2,4-triazoles. *Reagents and conditions*: (i) hydroxylamine hydrochloride, 1 N HCl, 30 °C, 24 h; (ii) **12**, K_2CO_3, acetone, reflux, 3 h; (iii) sodium cyanoborohydride, 4 N HCl, 30 °C, 4 h; (iv) potassium cyanate, glacial acetic acid, MeOH, 30 °C, 4 h; (v) TFA/methylanisole, 0 °C–r.t., 24 h.

benzoic acid hydrazides **18a–b** formed by the reaction of methyl esters **17a–b** with *para*-position substituted phenyl isothiocyanate. **19a–c** were cyclized into 4,5-diaryl-4*H*-l,2,4-triazole-3-thiols (**20a–c**) in aqueous NaOH/MeOH solution.[34] The thiols **20a–c** were further treated with acetaldehyde oxime **12** to form target oximes **21a–d** and *N*-hydroxyureas **22a–d**.

2.2. *In vitro* COX-2 and 5-LOX inhibitory activities assay

All the target compounds were initially evaluated *in vitro* to determine their COX-2 and 5-LOX inhibitory activities by measuring PGE_2 and LTB_4 levels from macrophages and leukocytes, respectively. Celecoxib (COX-2 inhibitor), and zileuton (5-LOX inhibitor) were reference drugs. As summarized in Table 1, all of the compounds exhibited modest to potent COX-2/5-LOX inhibitory activities. Among them, **8c**, **8d**, **8e**, **10e**, **10k**, **10m** and **15e** showed potent COX-2 inhibitory activity (IC_{50} = 0.13–0.19 μM) comparable to that of the reference drug celecoxib (IC_{50} = 0.14 μM), meanwhile, **8e**, **10e**, **10k**, **10m**, **15c**, **15d**, **15e**, **15k**, **15m**, **15o**, **21a**, **21b**, **21d**, **22b** and **22d** exhibited equipotent inhibitory activity of 5-LOX (IC_{50} = 0.74–0.89 μM) to that of the reference drug zileuton (IC_{50} = 0.82 μM). We guessed that the moieties of target compounds in charge of 5-LOX inhibition are hydroxamic acid or *N*-hydroxyurea, so the compounds with the same pharmacophore showed small statistical difference in 5-LOX assay.

It is notable that introduction of electron-withdrawing group (F, CF_3 and Br) at the *para*-position of the C-5 phenyl ring provided potent dual COX-2/5-LOX inhibitory activity, indicating that lipophilicity is critical to the dual COX-2/5-LOX potency. In the hydroxamic acid substituted series, especially **8e** (COX-2: 0.14 μM; 5-LOX: 0.87 μM), **10e** (COX-2: 0.17 μM; 5-LOX: 0.86 μM), **10k** (COX-2: 0.17 μM; 5-LOX: 0.80 μM) and



Scheme 3 Synthesis of 3,4,5-substituted-1,2,4-triazole derivatives. *Reagents and conditions*: (i) iodomethane, K_2CO_3, DMF, 80 °C, 1 h; (ii) H_2NNH_2, H_2O, EtOH, reflux, 6 h; (iii) *p*-fluorophenyl isothiocyanate or *p*-trifluorophenyl isothiocyanate, anhydrous EtOH, reflux, 5 h; (iv) a. 10% NaOH, MeOH, reflux, 2 h; b. 10% HCl. (v) **12**, K_2CO_3, acetone, reflux, 3 h; (vi) a. sodium cyanoborohydride, 4 N HCl, 30 °C, 4 h; b. potassium cyanate, glacial acetic acid, MeOH, 30 °C, 4 h; (vii) TFA/methylanisole, 0 °C–r.t., 24 h.

Table 1 Structures and inhibitory activities against COX-2 and 5-LOX of target compounds

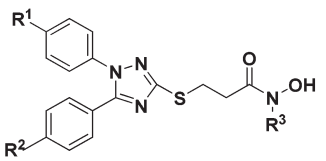
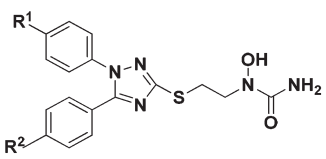
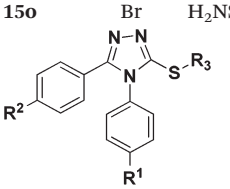
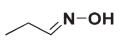
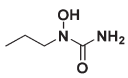
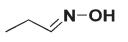
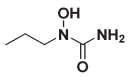
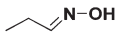
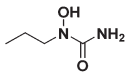
Compds	R^1	R^2	R^3	COX-2 IC_{50} [a] (μM)	5-LOX IC_{50} [a] (μM)
 **8a-j, 10a-e, 10g,10i,10k,10m,10o**					
8a	H	CH_3SO_2	H	1.24 ± 0.11	0.92 ± 0.03
8b	CH_3	CH_3SO_2	H	0.66 ± 0.07	1.05 ± 0.10
8c	F	CH_3SO_2	H	0.15 ± 0.01	0.93 ± 0.21
8d	CF_3	CH_3SO_2	H	0.13 ± 0.03	0.89 ± 0.11
8e	Br	CH_3SO_2	H	0.14 ± 0.02	0.87 ± 0.12
8f	H	H_2NSO_2	H	1.71 ± 0.20	1.08 ± 0.08
8g	CH_3	H_2NSO_2	H	1.05 ± 0.22	1.24 ± 0.17
8h	F	H_2NSO_2	H	0.72 ± 0.16	0.98 ± 0.05
8i	CF_3	H_2NSO_2	H	0.84 ± 0.05	0.97 ± 0.09
8j	Br	H_2NSO_2	H	0.93 ± 0.07	1.11 ± 0.12
10a	H	CH_3SO_2	CH_3	0.94 ± 0.14	0.98 ± 0.04
10b	CH_3	CH_3SO_2	CH_3	0.56 ± 0.08	1.02 ± 0.23
10c	F	CH_3SO_2	CH_3	0.25 ± 0.05	0.92 ± 0.17
10d	CF_3	CH_3SO_2	CH_3	0.21 ± 0.01	0.91 ± 0.05
10e	Br	CH_3SO_2	CH_3	0.17 ± 0.06	0.86 ± 0.03
10g	H	H_2NSO_2	CH_3	0.92 ± 0.13	0.93 ± 0.15
10i	CH_3	H_2NSO_2	CH_3	0.51 ± 0.04	0.98 ± 0.07
10k	F	H_2NSO_2	CH_3	0.17 ± 0.09	0.80 ± 0.07
10m	CF_3	H_2NSO_2	CH_3	0.18 ± 0.05	0.74 ± 0.12
10o	Br	H_2NSO_2	CH_3	0.44 ± 0.03	0.91 ± 0.18
 **15a-e, 15g, 15i,15k,15m,15o**					
15a	H	CH_3SO_2	—	0.97 ± 0.16	0.91 ± 0.13
15b	CH_3	CH_3SO_2	—	0.53 ± 0.11	0.90 ± 0.08
15c	F	CH_3SO_2	—	0.28 ± 0.03	0.89 ± 0.17
15d	CF_3	CH_3SO_2	—	0.26 ± 0.04	0.87 ± 0.05
15e	Br	CH_3SO_2	—	0.15 ± 0.07	0.85 ± 0.03
15g	H	H_2NSO_2	—	0.87 ± 0.12	1.03 ± 0.14
15i	CH_3	H_2NSO_2	—	0.69 ± 0.06	0.90 ± 0.15
15k	F	H_2NSO_2	—	0.26 ± 0.05	0.88 ± 0.12

Table 1 *(Contd.)*

Compds	R^1	R^2	R^3	COX-2 IC_{50} [a] (μM)	5-LOX IC_{50} [a] (μM)
15m	CF_3	H_2NSO_2	—	0.22 ± 0.09	0.80 ± 0.06
15o	Br	H_2NSO_2	—	0.32 ± 0.14	0.87 ± 0.09
 **21a,21b,21d,22a,22b,22d**					
21a	F	CH_3SO_2		0.85 ± 0.11	0.87 ± 0.08
22a	F	CH_3SO_2		0.97 ± 0.24	0.95 ± 0.15
21d	F	H_2NSO_2		1.02 ± 0.31	0.83 ± 0.06
22d	F	H_2NSO_2		1.15 ± 0.15	0.85 ± 0.04
21b	CF_3	CH_3SO_2		0.89 ± 0.02	0.78 ± 0.09
22b	CF_3	CH_3SO_2		0.96 ± 0.08	0.84 ± 0.13
Celecoxib				0.14 ± 0.05	—
Zileuton				—	0.82 ± 0.07

[a] The test compound concentration required to produce 50% inhibition of COX-2 or 5-LOX *in vitro*. The result (IC_{50}, μM) is the mean of four determinations.

10 m (COX-2: 0.18 μM; 5-LOX: 0.74 μM) are much more effective dual COX-2/5-LOX inhibitors *in vitro*. While in the *N*-hydroxyurea substituted series, compounds with *p*-CH_3SO_2 moiety at N-1 phenyl ring and F, CF_3 and Br group at *para*-position of C-5 phenyl ring provided more potent COX-2/5-LOX inhibition, such as **15c** (COX-2: 0.28 μM; 5-LOX: 0.89 μM), **15d** (COX-2: 0.26 μM; 5-LOX: 0.87 μM), **15e** (COX-2: 0.15 μM; 5-LOX: 0.85 μM). It is also of interest that shifting the N-1 aromatic ring to N-4 position led to a dramatic decrease in COX-2

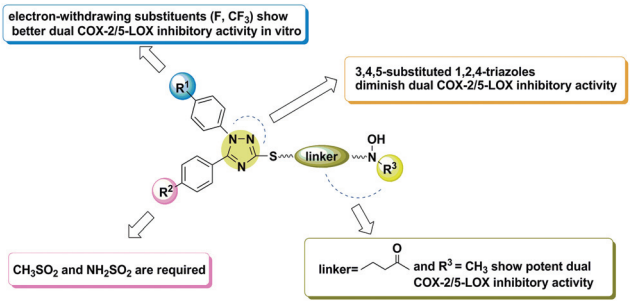


Fig. 3 The preliminary structure–activity relationships of diaryl-1,2,4-triazoles.

inhibitory activity (COX-2: 0.85–1.15 μM, celecoxib: 0.14 μM), such as 3,4,5-substituted-1,2,4-triazole derivatives **21a**, **21b**, **21d**, **22a**, **22b** and **22d**, presuming that the "Y-type" structure of 1,5-diaryl-1,2,4-triazoles is favorable for the compounds binding in the COX-2 and 5-LOX active site. The structure–activity relationships are summarized in Fig. 3.

2.3. Assay of COX-1/COX-2 inhibitory and selectivity index of 10e, 10m, 15e and 15m

Based on the favorable results of preliminary pharmacological tests, compounds **10e**, **10m**, **15e** and **15m** were evaluated in concentration–response studies to determine the COX-1/COX-2 inhibitory IC_{50}. The main problem with the COX-2 drugs is over-high selectivity to COX-2, leading to an imbalance between prostacyclin and thromboxane A_2 (TXA2), which may produce potential cardiovascular risks, which is the reason why rofecoxib was withdrawn from the market. Therefore, the design of COX-2 "preferential" inhibitors keeping a slight effect on COX-1 at therapeutic dosage could theoretically limit the imbalance of prostacyclin/TXA2, while the balance between prostacyclin and TXA2 is significant to avoid cardiovascular side-effects, such as celecoxib, which possess suitable selectivity for inhibition of COX-2/COX-1, is still available in clinical. So it is necessary to evaluate selectivity index (SI) of the compounds to predict the cardiovascular safety of them. As summarized in Table 2, **15e** exhibited potent and selective COX-2 inhibitory activity (COX-1 IC_{50} = 12.5 μM, COX-2 IC_{50} = 0.15 μM, SI = 0.012) which was comparable to that of the reference drug celecoxib (COX-1 IC_{50} = 7.7 μM; COX-2 IC_{50} = 0.12 μM; SI = 0.015).[35] While **15e** displayed equipotent 5-LOX inhibition (IC_{50} = 0.85 μM) to that of reference drug zileuton (IC_{50} = 0.82 μM, see Table 1). Thus, the favorable IC_{50} value of COX-1/COX-2 and 5-LOX as well as reasonable SI value implied that **15e** may have more potent anti-inflammatory activity and relative cardiovascular safety profile.

Table 2 *In vitro* COX-1 inhibitory activity and selectivity index for **10e**, **10m**, **15e** and **15m**

Compds	COX-1 IC_{50} [a] (μM)	Selectivity index [b] (SI)
10e	15.5 ± 1.84	0.011
10m	20.0 ± 3.61	0.009
15e	12.5 ± 0.79	0.012
15m	24.4 ± 5.03	0.009
Celecoxib	7.7[c]	0.015[c]

[a] The result (IC_{50}, μM) is the mean of four determinations. [b] *In vitro* COX-2 selectivity index (COX-2 IC_{50}/COX-1 IC_{50}). [c] See ref. 35.

Table 3 *In vivo* anti-inflammatory activity of **8d**, **10k**, **10m** and **15e** on xylene-induced ear edema in mice (n = 7, $\bar{x} \pm s$)

Groups	Swollen extent (weight, mg)	Inhibition (%)
Model	4.70 ± 1.27	—
Celecoxib	2.51 ± 1.44*	46.7
8d	4.62 ± 3.10	1.7
10k	3.72 ± 1.95	20.9
10m	3.68 ± 1.06	21.7
15e	2.16 ± 0.47**	54.1

*P < 0.05, **P < 0.01 *vs.* model.

2.4. Xylene-induced ear edema in mice assay

Compounds **8d**, **10k**, **10m**, and **15e** were selected for the study of their anti-inflammatory activity *in vivo* using xylene-induced ear edema in mice model and the results are listed in Table 3. Unfortunately, the hydroxamic acid substituted series with good dual COX-2/5-LOX inhibitory activity *in vitro* did not exhibit potent anti-inflammatory activity (inhibition: 1.7–21.7% at a dose of 30 mg kg^{-1}) *in vivo* as expected. It is presumed that the low bioavailability of hydroxamic acid substituted compounds affects their anti-inflammatory activity. Nevertheless, *N*-hydroxyurea substituted compound **15e** displayed the most potent anti-inflammatory activity (inhibition: 54.1% at a dose of 30 mg kg^{-1}), which is comparable to the reference drug celecoxib (inhibition: 46.7% at a dose of 30 mg kg^{-1}).

2.5. Albumen-induced rat paw edema and acetic acid-induced mouse vascular permeability assays

As an interesting new entity, **15e** was selected for further anti-inflammatory activity evaluation *in vivo* and the results are shown in Tables 4 and 5. In the albumen-induced rat paw edema assay, the high and medium dosage groups of **15e** exhibited significant anti-inflammatory activity (P < 0.01)

Table 4 The effect of **15e** on albumen-induced paw edema in rats (n = 10, $\bar{x} \pm s$)

Groups	Dose[a] (mg kg^{-1})	Swollen extent (×10, mL) 0.5 h	1 h	2 h	3 h
Model	—	1.40 ± 0.41	1.09 ± 0.44	0.67 ± 0.44	0.63 ± 0.41
Celecoxib	30	0.72 ± 0.38**	0.62 ± 0.29*	0.53 ± 0.34	0.49 ± 0.28
15e	60	0.69 ± 0.27**	0.65 ± 0.17*	0.63 ± 0.24	0.50 ± 0.24
	30	0.71 ± 0.34**	0.70 ± 0.32*	0.64 ± 0.30	0.56 ± 0.27
	15	0.85 ± 0.53*	0.82 ± 0.47	0.74 ± 0.48	0.65 ± 0.43

*P < 0.05, **P < 0.01 *vs.* model. [a] The results are expressed as percentage of inhibition following 60, 30, 15 mg kg^{-1} oral dose of **15e**.

Table 5 The effect of **15e** on acid-induced vascular permeability in mice (n = 10, $\bar{x} \pm s$)

Groups	Dose[a] (mg kg^{-1})	A_{590}
Model	—	0.375 ± 0.312
Celecoxib	30	0.142 ± 0.098*
15e	60	0.077 ± 0.069**
	30	0.098 ± 0.111*
	15	0.167 ± 0.191

*P < 0.05, **P < 0.01 *vs.* model. [a] The results are expressed as mean ± SEM (n = 10) following 60, 30, 15 mg kg^{-1} oral dose of **15e**.

comparable to that of the reference drug celecoxib at 30 min after compound administration, and lasted for 1 h (P < 0.05) in high dosage group. On the other hand, **15e** dose dependently decreases the permeability of celiac blood capillary in the acetic acid-induced mouse vascular permeability assay. These data indicated that **15e** had certain potency equivalent to that of the reference drug celecoxib in the acute inflammation model.

2.6. Analgesic activity evaluation

Both chemical (acetic acid-induced writhing response) and thermal method (hot-plate assay) in mice were employed to investigate the analgesic activity of **15e**. As illustrated in Fig. 4, **15e** significantly (P < 0.01) increased the pain threshold towards the thermal source in a dose-dependent manner after 90 min of administration, indicating that **15e** may contribute to analgesic activity. In the acetic acid-induced writhing response assay, all dosage groups of **15e** dramatically (P < 0.01) increased the writhe latency and reduced the number of abdominal constrictions and stretching of hind limbs induced by the injection of acetic acid (Fig. 5). These results demonstrated that **15e** exhibited potent analgesic activity comparable to that of the reference drug celecoxib and may involve pro-inflammatory mediator PGE_2 release.

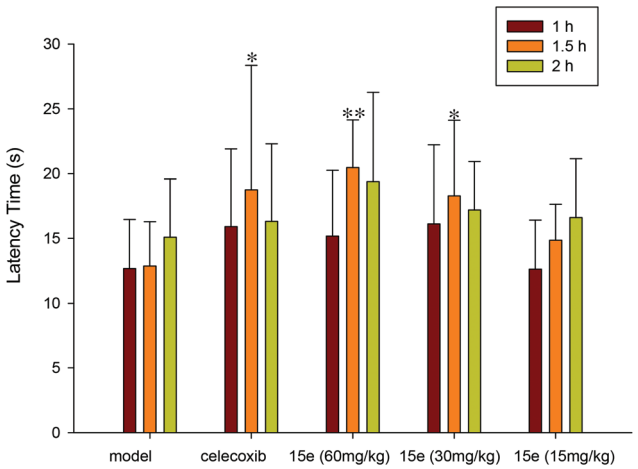


Fig. 4 The analgesic activity of **15e** on hot-plate assay in mice (n = 10, $\bar{x} \pm s$). *P < 0.05, **P < 0.01 *vs.* model.

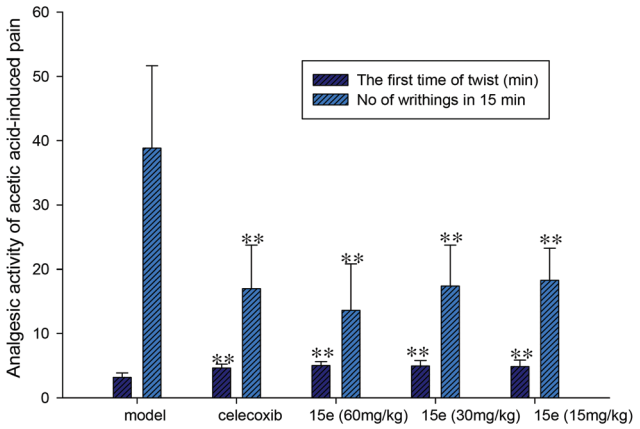


Fig. 5 The analgesic activity of **15e** on acetic acid-induced writhing response in mice (n = 10, $\bar{x} \pm s$). *P < 0.05, **P < 0.01 *vs.* model.

2.7. Molecular modeling (docking) studies

Molecular modeling (docking) studies were carried out to investigate the binding interactions of these derivatives with the COX-2 and 5-LOX active sites. For clarity, only **15e** was displayed as it exhibits balanced COX-2/5-LOX inhibitory activity. Fig. 6 shows the conformational superposition of **15e** and SC-558, the known selective COX-2 inhibitor. Both of the compounds exhibit a Y-type structure and the two phenyl rings stretch in the same direction indicating that it is preferential for **15e** binding in the active site of COX-2. As illustrated in Fig. 7, the N-1 bromophenyl ring is oriented towards the apex of the COX-2 active site composed of Phe^{381}, Leu^{384}, Tyr^{385}, Trp^{387} and Ser^{530}. The C-5 *p*-CH_3SO_2-phenyl moiety undergoes interactions with hydrophobic residues Tyr^{355}, Phe^{518}, Val^{523} and Ser^{353}. The SO_2CH_3 moiety is positioned in a relatively polar pocket, wherein the two oxygen atoms form hydrogen bonds with Arg^{513} and His^{90}, respectively, which are crucial for

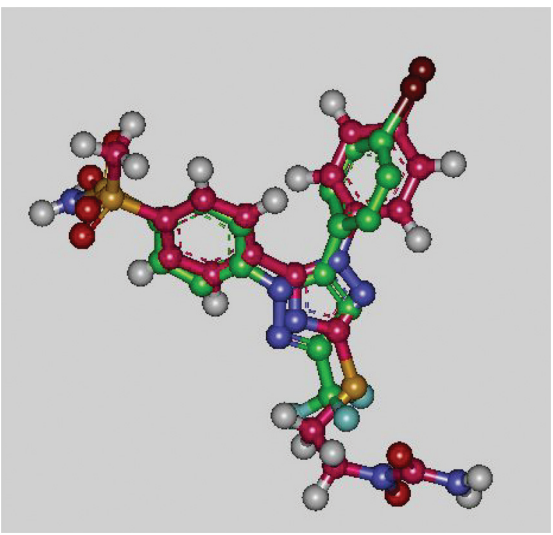


Fig. 6 Conformational superposition of **15e** (red) and selective COX-2 inhibitor SC-558 (green).

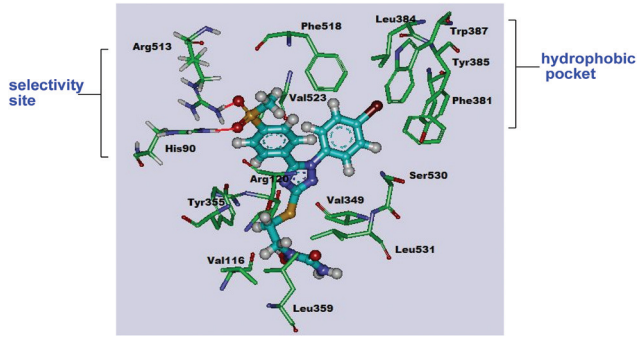


Fig. 7 Binding mode of **15e** in the active site of COX-2. Red lines represent hydrogen bonds.

COX-2 selectivity. On the other hand, the 5-LOX pharmacophore *N*-hydroxyurea moiety is oriented in the bottom of the hydrophobic pocket formed by Val^{116}, Val^{349}, Tyr^{355}, Leu^{359} and Leu^{531}. The binding mode is similar to that of SC-558.[36]

We employed the newly reported human 5-LOX (PDB code 3O8Y) for docking model.[37] As shown in Fig. 8, a U-shape conformation is found for **15e** in the 5-LOX catalytic domain. 1,2,4-Triazole moiety is positioned in the centre of the active site, and the C-5 *p*-CH_3SO_2-phenyl moiety is oriented deep into the bottom channel of the hydrophobic pocket formed by Tyr^{181}, Ala^{603}, Trp^{599}, His^{600}, Asn^{425}, and Phe^{421}, wherein Tyr^{181}, Ala^{603}, Trp^{599} and His^{600} are specific to the 5-LOX sequence. It is noteworthy that one of the oxygen atoms of the *p*-CH_3SO_2 substituent forms a hydrogen bond with Asn^{425}. The N-1 bromophenyl ring is oriented in a more elongated cleft and forms a hydrogen bond with Thr^{364}. The non-heme iron, which plays an important role in 5-LOX cascade, is coordinated by three conserved histidines (His^{367}, His^{372} and His^{550}) as well as Ile^{673}. The hydroxyl oxygen of *N*-hydroxylurea moiety and the sulphur atom of **15e** form hydrogen bonds with His^{372} and Ile^{673}, respectively. Furthermore, the sulphur atom is much closer to iron (distance 2.83 Å) than the ligands His^{367}, His^{372} and His^{550}. The results of our docking studies demonstrated that **15e** binds with both active sites of COX-2 and 5-LOX by hydrophobic interactions with relative amino acid residues, and stabilizes binding conformation by one or more hydrogen bonds. The results are in agreement with reported studies,[31] and may validate the potent dual inhibitory activity of **15e**.

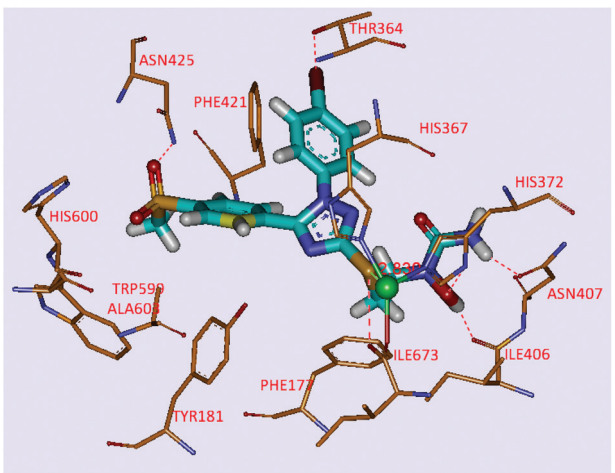


Fig. 8 Binding mode of **15e** in human 5-LOX active site. Green lines are distance between non-heme iron to ligands, and the red dashed lines are hydrogen bonds.

3. Conclusions

In summary, a series of novel hybrids from diaryl-1,2,4-triazole and hydroxamic acid or *N*-hydroxyurea were designed, synthesized and evaluated as dual COX-2/5-LOX inhibitors. It was found that (i) 1,3,5-substituted-1,2,4-triazole scaffold was critical for dual COX-2/5-LOX inhibition; (ii) the hydroxamic acid hybrid 1,3,5-substituted-1,2,4-triazoles, especially **10k** and **10m**, were potent dual COX-2/5-LOX inhibitors *in vitro*; (iii) in the *N*-hydroxyurea hybrid 1,3,5-substituted-1,2,4-triazoles, **15e** exhibited optimal dual COX-2/5-LOX inhibitory activities *in vitro* and potent oral anti-inflammatory and analgesic activities *in vivo*,[38] (iv) the molecular modeling study revealed that **15e** binds with both active sites of COX-2 and 5-LOX by hydrophobic interactions and stabilizes binding conformation by one or more hydrogen bonds. Collectively, **15e** might be a promising anti-inflammatory agent for further investigations.

4. Experimental section

4.1 Chemistry

Reagents and all solvents were purchased from Shanghai Chemical Reagent Company and used without further purification. All of the experiments were monitored by analytical thin-layer chromatography (TLC) performed on silica gel GF254 precoated plates. Column chromatography was carried out using silica gel (200–300 mesh). The purities of all of the compounds (>95%) used for biological screening were determined by high-performance liquid chromatography (HPLC) (Agilent, 1260 INFINITY) using a Gemini C18 column (4.6 mm × 150 mm, 5 μm) eluted with a gradient mixture of acetonitrile and water. Melting points were determined using MEL-TEMP II melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker-ACF spectrometer (300 or 500 MHz) in DMSO-d_6. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane (TMS) as an internal standard. Multiplicities are given as s (singlet), d (doublet), dd (double-doublet), t (triplet), q (quadruplet), m (multiplet) and br s (broad signal). Mass spectra were recorded on an Agilent LC/MSD TRAP SL spectrometer equipped with an electrospray ionisation (ESI) interface; Elemental analysis: *Elementar Vario EL III* instrument. IR (in cm^{-1}) spectra in KBr pellets on a Bruker FT-IR TENSOR 27 instrument.

General procedure for synthesis of compounds 7a–e, 7f, 7h, 7j, 7l, 7n. Corresponding 1,2,4-triazole-3-thiols **6a–j**

(4.8 mmol) and K_2CO_3 (6.8 mmol) were suspended in 10 mL acetone at 50 °C for 30 min, then ethyl 3-bromopropionate (6.8 mmol) was added, the mixture was allowed to reflux for 3 h, then was filtered and the filtrate was evaporated *in vacuo*. The residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1 : 2, v/v) as eluent to afford a white solid.

General procedure for synthesis of compounds 7g, 7i, 7k, 7m, 7o. A solution of the corresponding compounds **7f**, **7h**, **7j**, **7l**, **7n** (1 mmol), CF_3COOH (2 mL) and two drops of methylphenyl ether was stirred at room temperature for 24 h. The mixture was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1 : 3, v/v) as eluent to afford a white solid.

General procedure for synthesis of compounds 8a–j. Hydroxylamine hydrochloride (0.025 mol) was suspended in methanol (4.35 mL) at reflux for 1 h, and KOH (0.025 mol) was refluxed in methanol (8.15 mL) for 1 h. After being cooled to 40 °C, KOH methanol solution was poured into hydroxylamine hydrochloride suspension, then the precipitated KCl was filtered. The filtrate is 2 mol L^{-1} hydroxylamine methanol solution. Corresponding ethyl esters **7a–o** (0.76 mmol) were added (over 30 min) to the 2 mol L^{-1} hydroxylamine methanol solution (7 mL, 14 mmol) followed by addition of KOH to adjust to pH = 10. The mixture was stirred at room temperature for 1 h. The mixture was poured into stirring cold water, and the pH was adjusted to 7 by adding acetic acid. The aqueous solution was extracted with ethyl acetate (3 × 10 mL), and the combined extracts were dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (20 : 1, v/v) as eluent to afford a white solid.

N-*Hydroxy-3-(5-(4-(methylsulfonyl)phenyl)-1-phenyl-1*H-*1,2,4-triazol-3-ylthio)propanamide (**8a**).* The title compound was obtained as a white solid (0.12 g, 37.5%), mp 83–85 °C. Found: C, 52.01; H, 4.03; N, 13.07. Calc. for $C_{18}H_{18}N_4O_4S_2$: C, 51.66; H, 4.34; N, 13.39. IR (KBr/cm^{-1}): 3214, 3002, 2923, 1661, 1498, 1453, 1303, 1148, 958, 780, 694, 530. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 10.47 (s, 1H, -O*H*), 8.83 (s, 1H, -N*H*), 7.96 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8.5 Hz, 2H), 7.54 (m, 3H), 7.46 (m, 2H), 3.33 (t, J = 6.9 Hz, 2H, -CH_2-), 3.26 (s, 3H, SO_2CH_3), 2.52 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 417 $[M - H]^-$.

N-*Hydroxy-3-(5-(4-(methylsulfonyl)phenyl)-1-*p-*tolyl-1*H-*1,2,4-triazol-3-ylthio)propanamide (**8b**).* The title compound was obtained as a white solid (0.21 g, 39.2%), mp 88–90 °C. Found: C, 52.44; H, 4.94; N, 12.55. Calc. for $C_{19}H_{20}N_4O_4S_2$: C, 52.76; H, 4.66; N, 12.95. IR (KBr/cm^{-1}): 3452, 3003, 2924, 1646, 1513, 1304, 1150, 988, 779, 566. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: ppm 10.44 (s, 1H, -O*H*), 8.79 (s, 1H, -N*H*), 7.95 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 8.5 Hz, 2H), 7.33 (s, 4H), 3.33 (t, J = 7.0 Hz, 2H, -CH_2-), 3.25 (s, 3H, SO_2CH_3), 2.51 (t, J = 7.0 Hz, 2H, -CH_2-), 2.38 (s, 3H, phenyl-CH_3). MS (ESI, m/z): 431 $[M - H]^-$.

*3-(1-(4-Fluorophenyl)-5-(4-(methylsulfonyl)phenyl)-1*H-*1,2,4-triazol-3-ylthio)-*N-*hydroxypropanamide (**8c**).* The title compound was obtained as a white solid (0.14 g, 31%), mp 92–94 °C. Found: C, 49.71; H, 4.18; N, 12.48. Calc. for $C_{18}H_{17}FN_4O_4S_2$: C, 49.53; H, 3.93; N, 12.84. IR (KBr/cm^{-1}): 3423, 2963, 2923, 1657, 1510, 1304, 1149, 844, 779, 564. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: ppm 10.51 (s, 1H, -O*H*), 8.87 (s, 1H, -N*H*), 8.03 (d, J = 8.6 Hz, 2H), 7.75 (d, J = 8.7 Hz, 2H), 7.61 (d, J = 8.2 Hz, 2H), 7.44 (d, J = 8.2 Hz, 2H), 3.41 (t, J = 6.9 Hz, 2H, -CH_2-), 3.31 (s, 3H, SO_2CH_3), 2.56 (t, J = 7.0 Hz, 2H, -CH_2-). MS (ESI, m/z): 435 $[M - H]^-$.

N-*Hydroxy-3-(5-(4-(methylsulfonyl)phenyl)-1-(4-(trifluoromethyl)-phenyl)-1*H-*1,2,4-triazol-3-ylthio)propanamide (**8d**).* The title compound was obtained as a white solid (0.14 g, 32%), mp 84–86 °C. Found: C, 46.63; H, 3.76; N, 11.90. Calc. for $C_{19}H_{17}F_3N_4O_4S_2$: C, 46.91; H, 3.52; N, 11.52. IR (KBr/cm^{-1}): 3344, 2925, 1660, 1445, 1326, 1149, 988, 848, 777, 533. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: ppm 10.46 (s, 1H, -O*H*), 8.82 (s, 1H, -N*H*), 7.99 (d, J = 8.3 Hz, 2H), 7.91 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 3.37 (t, J = 6.9 Hz, 2H, -CH_2-), 3.27 (s, 3H, SO_2CH_3), 2.52 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 485 $[M - H]^-$.

*3-(1-(4-Bromophenyl)-5-(4-(methylsulfonyl)phenyl)-1*H-*1,2,4-triazol-3-ylthio)-*N-*hydroxypropanamide (**8e**).* The title compound was obtained as a white solid (0.14 g, 27%), mp 82–84 °C. Found: C, 43.69; H, 3.61; N, 11.60. Calc. for $C_{18}H_{17}BrN_4O_4S_2$: C, 43.47; H, 3.45; N, 11.26. IR (KBr/cm^{-1}): 3418, 3228, 3019, 2923, 1660, 1492, 1303, 1150, 987, 780, 534. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: ppm 10.46 (s, 1H, -O*H*), 8.81 (s, 1H, -N*H*), 7.98 (d, J = 8.5 Hz, 2H), 7.72 (m, 4H), 7.73 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.6 Hz, 2H), 3.35 (t, J = 6.9 Hz, 2H, -CH_2-), 3.26 (s, 3H, SO_2CH_3), 2.51 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 496 $[M - H]^-$.

N-*Hydroxy-3-(1-phenyl-5-(4-sulfamoylphenyl)-1*H-*1,2,4-triazol-3-ylthio)propanamide (**8f**).* The title compound was obtained as a white solid (0.04 g, 33.2%), mp 92–95 °C. Found: C, 48.42; H, 4.35; N, 16.58. Calc. for $C_{17}H_{17}N_5O_4S_2$: C, 48.68; H, 4.08; N, 16.70. IR (KBr/cm^{-1}): 3345, 3072, 2962, 2922, 1656, 1512, 1451, 1303, 1151, 822, 776, 538. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: ppm 10.45 (s, 1H, -O*H*), 8.80 (s, 1H, -N*H*), 7.82 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.48 (s, 2H, SO_2NH_2), 7.54 (m, 3H), 7.46 (m, 2H), 3.37 (t, J = 6.9 Hz, 2H, -CH_2-), 2.52 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 418 $[M - H]^-$.

N-*Hydroxy-3-(5-(4-sulfamoylphenyl)-1-*p-*tolyl-1*H-*1,2,4-triazol-3-ylthio)propanamide (**8g**).* The title compound was obtained as a white solid (0.11 g, 29.2%), mp 120–122 °C. Found: C, 50.09; H, 4.68; N, 16.47. Calc. for $C_{18}H_{19}N_5O_4S_2$: C, 49.87; H, 4.42; N, 16.16. IR (KBr/cm^{-1}): 3442, 2962, 2922, 2851, 1644, 1513, 1335, 1163, 822, 613. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 10.46 (s, 1H, -O*H*), 8.84 (s, 1H, -N*H*), 7.82 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.48 (s, 2H, SO_2NH_2), 7.32 (m, 4H), 3.48 (t, J = 6.9 Hz, 2H, -CH_2-), 2.50 (t, J = 6.9 Hz, 2H, -CH_2-), 2.38 (s, 3H, phenyl-CH_3). MS (ESI, m/z): 432 $[M - H]^-$.

*3-(1-(4-Fluorophenyl)-5-(4-sulfamoylphenyl)-1*H-*1,2,4-triazol-3-ylthio)-*N-*hydroxypropanamide (**8h**).* The title compound was obtained as a white solid (0.17 g, 31.3%), mp 84–86 °C. Found: C, 46.44; H, 3.85; N, 15.72. Calc. for $C_{17}H_{16}FN_5O_4S_2$: C, 46.67; H, 3.69; N, 16.01. IR (KBr/cm^{-1}): 3442, 2962, 2922, 2851, 1644, 1513, 1335, 1163, 822, 613. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 10.47 (s, 1H, -O*H*), 8.83 (s, 1H, -N*H*), 8.00 (d, J = 8.6 Hz,

2H), 7.77 (d, J = 8.7 Hz, 2H), 7.55 (d, J = 8.7 Hz, 2H), 7.48 (s, 2H, SO_2NH_2), 7.32 (d, J = 8.7 Hz, 2H), 3.42 (t, J = 6.9 Hz, 2H, -CH_2-), 2.67 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 436 $[M - H]^-$.

N-*Hydroxy-3-(5-(4-sulfamoylphenyl)-1-(4-(trifluoromethyl)phenyl)-1*H*-1,2,4-triazol-3-ylthio)propanamide (**8i**).* The title compound was obtained as a white solid (0.18 g, 26.5%), mp 84–86 °C. Found: C, 44.02; H, 3.69; N, 14.75. Calc. for $C_{18}H_{16}F_3N_5O_4S_2$: C, 44.35; H, 3.31; N, 14.37. IR (KBr/cm^{-1}): 3396, 1656, 1454, 1325, 1166, 1063, 847, 621, 543. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 10.49 (s, 1H, -O*H*), 8.80 (s, 1H, -N*H*), 7.91 (d, J = 8.5 Hz, 2H), 7.79 (d, J = 8.5 Hz, 2H), 7.61 (m, 4H), 7.35 (s, 2H, SO_2NH_2), 3.42 (t, J = 6.9 Hz, 2H, -CH_2-), 2.59 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 486 $[M - H]^-$.

*3-(1-(4-Bromophenyl)-5-(4-sulfamoylphenyl)-1*H*-1,2,4-triazol-3-ylthio)-*N*-hydroxypropanamide (**8j**).* The title compound was obtained as a white solid (0.17 g, 34.2%), mp 96–98 °C. Found: C, 41.35; H, 3.56; N, 14.43. Calc. for $C_{17}H_{16}BrN_5O_4S_2$: C, 40.97; H, 3.24; N, 14.05. IR (KBr/cm^{-1}): 3395, 2373, 2346, 1655, 1492, 1335, 1160, 987, 835, 620. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 10.45 (s, 1H, -O*H*), 8.80 (s, 1H, -N*H*), 7.84 (d, J = 8.5 Hz, 2H), 7.72 (d, J = 8.7 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H), 7.46 (s, 2H, SO_2NH_2), 7.40 (d, J = 8.7 Hz, 2H), 3.34 (t, J = 6.9 Hz, 2H, -CH_2-), 2.49 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 497 $[M - H]^-$.

General procedure for synthesis of compounds 9a–j. To a solution of the appropriate esters **7a–o** (0.01 mol) in 10 mL methanol, 5 mL 10% NaOH was added, and the mixture was heated under reflux for 1 h. The solution was cooled and acidified to pH = 2 by adding 10% HCl. The precipitated yellow solid was filtered, washed with water and dried to afford corresponding carboxylic acids. Oxalyl chloride (0.2 mL, 1.97 mmol) and one drop of DMF as catalyst were added to a solution of appropriate carboxylic acid (0.656 mmol) in dry CH_2Cl_2 (10 mL). The resulting mixture was stirred for 3 h at room temperature and the solvents were evaporated *in vacuo*. The residue was used immediately without further purification for the preparation of the target compounds **10a–o**.

General procedure for synthesis of compounds 10a–o. An appropriate solution of acyl chloride **9a–j** in 10 mL dry CH_2Cl_2 was added dropwise to the suspension of *N*-methylhydroxylamine hydrochloride (1.312 mmol) and Et_3N (1.312 mmol) in 5 mL dry CH_2Cl_2. The mixture was stirred for an additional 2 h at room temperature. Water (30 mL) was added to dilute, and the CH_2Cl_2 layer was further washed by 10% HCl and brine. The organic layer was collected, and dried over anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (60 : 1, v/v) as eluent to afford a white solid.

N-*Hydroxy*-N-*methyl-3-(5-(4-(methylsulfonyl)phenyl)-1-phenyl-1*H*-1,2,4-triazol-3-ylthio)propanamide (**10a**).* The title compound was obtained as a white solid (0.25 g, 39.5%), mp 165–167 °C. Found: C, 53.02; H, 4.58; N, 12.73. Calc. for $C_{19}H_{20}N_4O_4S_2$: C, 52.76; H, 4.66; N, 12.95. IR (KBr/cm^{-1}): 3440, 3172, 3063, 2947, 2925, 1636, 1501, 1460, 1319, 1265, 1149, 956, 781, 535. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 9.84 (s, 1H, -O*H*), 7.95 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8.5 Hz, 2H), 7.53 (m, 3H), 7.45 (m, 2H), 3.35 (t, J = 6.8 Hz, 2H, -CH_2-), 3.25 (s, 3H, SO_2CH_3), 3.10 (s, 3H, -NCH_3), 2.52 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 433 $[M + H]^+$.

N-*Hydroxy*-N-*methyl-3-(5-(4-(methylsulfonyl)phenyl)-1-*p*-tolyl-1*H*-1,2,4-triazol-3-ylthio)propanamide (**10b**).* The title compound was obtained as a white solid (0.2 g, 41.7%), mp 178–180 °C. Found: C, 53.65; H, 4.55; N, 12.81. Calc. for $C_{20}H_{22}N_4O_4S_2$: C, 53.79; H, 4.97; N, 12.55. IR (KBr/cm^{-1}): 3443, 3093, 2941, 2922, 2802, 1644, 1515, 1318, 1152, 819, 780, 532. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 9.66 (s, 1H, -O*H*), 7.96 (d, J = 8.5 Hz, 2H), 7.72 (d, J = 8.5 Hz, 2H), 7.33 (s, 4H), 7.45 (m, 2H), 3.32 (t, J = 6.8 Hz, 2H, -CH_2-), 3.25 (s, 3H, SO_2CH_3), 3.12 (s, 3H, -NCH_3), 2.89 (t, J = 6.9 Hz, 2H, -CH_2-), 2.38 (s, 3H, phenyl-CH_3). MS (ESI, m/z): 481 $[M + Cl]^-$.

*3-(1-(4-Fluorophenyl)-5-(4-(methylsulfonyl)phenyl)-1*H*-1,2,4-triazol-3-ylthio)-*N*-hydroxy-*N*-methylpropanamide (**10c**).* The title compound was obtained as a white solid (0.2 g, 34.7%), mp 169–171 °C. Found: C, 50.42; H, 4.41; N, 12.68. Calc. for $C_{19}H_{19}FN_4O_4S_2$: C, 50.65; H, 4.25; N, 12.44. IR (KBr/cm^{-1}): 3454, 2926, 1641, 1511, 1463, 1323, 1156, 850, 781, 567. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 9.85 (s, 1H, -O*H*), 7.98 (d, J = 8.5 Hz, 2H), 7.74 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.2 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 3.32 (t, J = 6.9 Hz, 2H, -CH_2-), 3.25 (s, 3H, SO_2CH_3), 3.10 (s, 3H, -NCH_3), 2.89 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 451 $[M + H]^+$.

N-*Hydroxy*-N-*methyl-3-(5-(4-(methylsulfonyl)phenyl)-1-(4-(trifluoromethyl)phenyl)-1*H*-1,2,4-triazol-3-ylthio)propanamide (**10d**).* The title compound was obtained as a white solid (0.18 g, 38.6%), mp 186–188 °C. Found: C, 47.82; H, 4.04; N, 11.45. Calc. for $C_{20}H_{19}F_3N_4O_4S_2$: C, 47.99; H, 3.83; N, 11.19. IR (KBr/cm^{-1}): 3442, 3091, 2923, 2852, 2814, 1643, 1453, 1315, 1150, 990, 850, 777, 531. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 9.84 (s, 1H, -O*H*), 7.99 (d, J = 8.4 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H), 7.73 (d, J = 8.3 Hz, 2H), 7.68 (d, J = 8.3 Hz, 2H), 3.32 (t, J = 6.7 Hz, 2H, -CH_2-), 3.26 (s, 3H, SO_2CH_3), 3.12 (s, 3H, -NCH_3), 2.91 (t, J = 6.7 Hz, 2H, -CH_2-). MS (ESI, m/z): 501 $[M + H]^+$.

*3-(1-(4-Bromophenyl)-5-(4-(methylsulfonyl)phenyl)-1*H*-1,2,4-triazol-3-ylthio)-*N*-hydroxy-*N*-methylpropanamide (**10e**).* The title compound was obtained as a white solid (0.11 g, 32.5%), mp 180–182 °C. Found: C, 44.87; H, 3.48; N, 11.24. Calc. for $C_{19}H_{19}BrN_4O_4S_2$: C, 44.62; H, 3.74; N, 10.96. IR (KBr/cm^{-1}): 3453, 3092, 2961, 2921, 2851, 2790, 1643, 1494, 1317, 1150, 824, 781, 531. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 9.88 (s, 1H, -O*H*), 7.98 (d, J = 8.4 Hz, 2H), 7.70 (m, 4H), 7.40 (d, J = 8.3 Hz, 2H), 3.32 (t, J = 7.0 Hz, 2H, -CH_2-), 3.26 (s, 3H, SO_2CH_3), 3.09 (s, 3H, -NCH_3), 2.88 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 513 $[M + H]^+$.

General procedure for synthesis of compounds 10g, 10i, 10k, 10m, 10o. A solution of the corresponding compounds **10f**, **10 h**, **10j**, **10 l**, **10n** (1 mmol), CF_3COOH (2 mL) and two drops of methylphenyl ether was stirred at room temperature for 24 h. The mixture was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (40 : 1, v/v) as eluent to afford a white solid.

N-*Hydroxy*-N-*methyl-3-(1-phenyl-5-(4-sulfamoylphenyl)-1*H-*1,2,4-triazol-3-ylthio)propanamide (**10g**).* The title compound was obtained as a white solid (0.04 g, 38.4%), mp 70–72 °C. Found: C, 49.69; H, 4.58; N, 15.84. Calc. for $C_{18}H_{19}N_5O_4S_2$: C, 49.87; H, 4.42; N, 16.16. IR (KBr/cm^{-1}): 3396, 2924, 2853, 1628, 1499, 1453, 1336, 1161, 769, 619. ^{1}H-NMR (DMSO-d$_6$, 500 MHz) δ: ppm 9.83 (s, 1H, -O*H*), 7.82 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.52 (m, 3H), 7.44 (m, 4H), 3.32 (t, J = 6.9 Hz, 2H, -CH_2-), 3.10 (s, 3H, -NCH_3), 2.89 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 434 [M + H]$^+$.

N-*Hydroxy*-N-*methyl-3-(5-(4-sulfamoylphenyl)-1*-p-*tolyl-1*H-*1,2,4-triazol-3-ylthio)propanamide (**10i**).* The title compound was obtained as a white solid (0.19 g, 47.8%), mp 47–49 °C. Found: C, 51.28; H, 4.95; N, 15.49. Calc. for $C_{19}H_{21}N_5O_4S_2$: C, 50.99; H, 4.73; N, 15.65. IR (KBr/cm^{-1}): 3430, 2925, 2854, 1680, 1639, 1541, 1206, 801, 732, 614. ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ: ppm 9.84 (s, 1H, -O*H*), 7.90 (m, 4H), 7.64 (m, 4H), 7.48 (s, 2H, SO_2NH_2), 3.34 (t, J = 6.7 Hz, 2H, -CH_2-), 3.10 (s, 3H, -NCH_3), 2.89 (t, J = 6.7 Hz, 2H, -CH_2-). MS (ESI, m/z): 448 [M + H]$^+$.

*3-(1-(4-Fluorophenyl)-5-(4-sulfamoylphenyl)-1*H-*1,2,4-triazol-3-ylthio)*-N-*hydroxy*-N-*methylpropanamide (**10k**).* The title compound was obtained as a white solid (0.1 g, 41.2%), mp 90–92 °C. Found: C, 47.56; H, 4.41; N, 15.33. Calc. for $C_{18}H_{18}FN_5O_4S_2$: C, 47.88; H, 4.02; N, 15.51. IR (KBr/cm^{-1}): 3384, 3081, 2931, 2374, 2323, 2248, 1628, 1510, 1337, 1160, 843, 614. ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ: ppm 9.85 (s, 1H, -O*H*), 7.83 (d, J = 8.6 Hz, 2H), 7.66 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.2 Hz, 2H), 7.40 (s, 2H, SO_2NH_2), 3.34 (t, J = 6.7 Hz, 2H, -CH_2-), 3.10 (s, 3H, -NCH_3), 2.89 (t, J = 6.7 Hz, 2H, -CH_2-). MS (ESI, m/z): 450 [M − H]$^-$.

N-*Hydroxy*-N-*methyl-3-(5-(4-sulfamoylphenyl)-1-(4-(trifluoromethyl)phenyl)-1*H-*1,2,4-triazol-3-ylthio)propanamide (**10m**).* The title compound was obtained as a white solid (0.15 g, 49.3%), mp 111–113 °C. Found: C, 45.77; H, 3.20; N, 14.15. Calc. for $C_{19}H_{18}F_3N_5O_4S_2$: C, 45.50; H, 3.62; N, 13.96. IR (KBr/cm^{-1}): 3445, 2377, 2350, 2310, 1617, 1520, 1446, 1325, 1167, 847, 622. ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ: ppm 9.84 (s, 1H, -O*H*), 7.90 (m, 4H), 7.64 (m, 4H), 7.48 (s, 2H, SO_2NH_2), 3.34 (t, J = 6.7 Hz, 2H, -CH_2-), 3.10 (s, 3H, -NCH_3), 2.89 (t, J = 6.7 Hz, 2H, -CH_2-), 2.41 (s, 3H, phenyl-CH_3). MS (ESI, m/z): 500 [M − H]$^-$.

*3-(1-(4-Bromophenyl)-5-(4-sulfamoylphenyl)-1*H-*1,2,4-triazol-3-ylthio)*-N-*hydroxy*-N-*methylpropanamide (**10o**).* The title compound was obtained as a white solid (0.1 g, 32.9%), mp 133–135 °C. Found: C, 42.56; H, 3.33; N, 13.49. Calc. for $C_{18}H_{18}BrN_5O_4S_2$: C, 42.19; H, 3.54; N, 13.67. IR (KBr/cm^{-1}): 3339, 3200, 2977, 2858, 1729, 1621, 1493, 1164, 766, 544. ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ: ppm 9.84 (s, 1H, -O*H*), 7.85 (d, J = 8.6 Hz, 2H), 7.72 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 8.6 Hz, 2H), 7.48 (s, 2H, SO_2NH_2), 7.40 (d, J = 8.8 Hz, 2H), 3.32 (t, J = 6.9 Hz, 2H, -CH_2-), 3.10 (s, 3H, -NCH_3), 2.89 (t, J = 6.8 Hz, 2H, -CH_2-). MS (ESI, m/z): 514 [M + H]$^+$.

Procedure for synthesis of bromoacetaldehyde oxime 12. Bromoacetaldehyde diethyl acetal **11** (12.2 mmol) was added to the solution of hydroxylamine hydrochloride (58.4 mmol) in 16 mL H_2O and 8 mL 1 N HCl. The biphasic solution was stirred at 30 °C until it became homogenous, at which time the reaction mixture was diluted and extracted with EtOAc (3 × 15 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and evaporated to give the oxime **12** as a volatile liquid, which was used without further purification.

General procedure for synthesis of compounds 13a–j. Corresponding 1,2,4-triazole-3-thiols **6a–j** (0.982 mmol) and K_2CO_3 (1.37 mmol) were suspended in 10 mL acetone at 50 °C for 30 min, then bromoacetaldehyde oxime **12** (1.37 mmol) was added. The mixture was allowed to reflux for 3 h, then was filtered and the filtrate was evaporated *in vacuo*. The residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether (1 : 2, v/v) as eluent to afford a white solid.

General procedure for synthesis of compounds 15a–j. $NaBH_3CN$ (4.65 mmol) and a trace of methyl orange were added to a solution of appropriate oximes **13a–j** (1.55 mmol) in 10 mL methanol/THF (1 : 1, v/v). The solution was stirred at 30 °C for 5 min prior to the addition of 4 N HCl that was added dropwise until the color remained orange red. The mixture was maintained at 30 °C for an additional 4 h with stirring. After removal of the organic solvents, water was added to dilute, the mixture was adjusted to pH = 9 using 10% NaOH, and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with brine and dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to provide the hydroxylamines **14a–j** as yellowish oils which were used immediately without further purification for the preparation of *N*-hydroxyureas **15a–j**.

To a stirred solution of hydroxylamines **14a–j** (0.26 mmol) in methanol and acetic acid (0.77 mmol) was added a solution of KOCN (0.77 mmol) in water (2 mL). The mixture was stirred for 4 h at 30 °C and diluted with 20 mL water, then extracted with EtOAc (3 × 10 mL) and the combined organic layers were washed with brine and dried over anhydrous Na_2SO_4 and concentrated to yield a yellowish oil. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (50 : 1, v/v) as eluent to afford a white solid.

*1-Hydroxy-1-(2-(5-(4-(methylsulfonyl)phenyl)-1-phenyl-1*H-*1,2,4-triazol-3-ylthio)ethyl)urea (**15a**).* The title compound was obtained as a white solid (0.07 g, 59.6%), mp 73–75 °C. Found: C, 49.75; H, 4.38; N, 16.44. Calc. for $C_{18}H_{19}N_5O_4S_2$: C, 49.87; H, 4.42; N, 16.16. IR (KBr/cm^{-1}): 3457, 2922, 2377, 2351, 2320, 1643, 1453, 1302, 1147, 780, 694, 528. ^{1}H-NMR (DMSO-d$_6$, 500 MHz) δ: ppm 9.42 (s, 1H, O*H*), 7.94 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8.5 Hz, 2H), 7.53 (m, 3H), 7.45 (m, 2H), 6.34 (s, 2H, NH_2), 3.72 (t, J = 6.8 Hz, 2H, -CH_2-), 3.33 (t, J = 6.9 Hz, 2H, -CH_2-), 3.25 (s, 3H, SO_2CH_3). MS (ESI, m/z): 434 [M + H]$^+$.

*1-Hydroxy-1-(2-(5-(4-(methylsulfonyl)phenyl)-1-*p-*tolyl-1*H-*1,2,4-triazol-3-ylthio)ethyl)urea (**15b**).* The title compound was obtained as a white solid (0.06 g, 62.7%), mp 94–96 °C. Found: C, 50.76; H, 4.44; N, 15.38. Calc. for $C_{19}H_{21}N_5O_4S_2$: C, 50.99; H, 4.73; N, 15.65. IR (KBr/cm^{-1}): 3382, 2922, 2351, 2310, 1653, 1513, 1303, 1150, 778, 566. ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ: ppm 9.41 (s, 1H, O*H*), 7.94 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8.5 Hz, 2H), 7.32 (s, 4H), 6.28 (s, 2H, NH_2), 3.74 (t, J = 6.8 Hz, 2H,

-CH_2-), 3.34 (t, J = 6.9 Hz, 2H, -CH_2-), 3.24 (s, 3H, SO_2CH_3), 2.37 (s, 3H, phenyl-CH_3). MS (ESI, m/z): 448 $[M + H]^+$.

*1-(2-(1-(4-Fluorophenyl)-5-(4-(methylsulfonyl)phenyl)-1*H*-1,2,4-triazol-3-ylthio)ethyl)-1-hydroxyurea (**15c**).* The title compound was obtained as a white solid (0.1 g, 48.7%), mp 73–75 °C. Found: C, 47.50; H, 4.33; N, 15.36. Calc. for $C_{18}H_{18}FN_5O_4S_2$: C, 47.88; H, 4.02; N, 15.51. IR (KBr/cm^{-1}): 3446, 2923, 1653, 1510, 1463, 1305, 1150, 845, 779, 566. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 9.41 (s, 1H, O*H*), 7.96 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 8.5 Hz, 2H), 7.54 (m, 2H), 7.37 (m, 2H), 6.33 (s, 2H, NH_2), 3.73 (t, J = 6.8 Hz, 2H, -CH_2-), 3.34 (t, J = 6.9 Hz, 2H, -CH_2-), 3.25 (s, 3H, SO_2CH_3). MS (ESI, m/z): 452 $[M + H]^+$.

*1-Hydroxy-1-(2-(5-(4-(methylsulfonyl)phenyl)-1-(4-(trifluoromethyl)phenyl)-1*H*-1,2,4-triazol-3-ylthio)ethyl)urea (**15d**).* The title compound was obtained as a white solid (0.07 g, 37.7%), mp 136–98 °C. Found: C, 45.81; H, 3.24; N, 13.65. Calc. for $C_{19}H_{18}F_3N_5O_4S_2$: C, 45.50; H, 3.62; N, 13.96. IR (KBr/cm^{-1}): 3473, 2925, 1652, 1446, 1326, 1150, 1062, 849, 777, 533. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: ppm 9.42 (s, 1H, O*H*), 7.99 (d, J = 8.5 Hz, 2H), 7.89 (d, J = 8.5 Hz, 2H), 7.73 (d, J = 8.5 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H), 6.35 (s, 2H, NH_2), 3.73 (t, J = 6.8 Hz, 2H, -CH_2-), 3.34 (t, J = 6.7 Hz, 2H, -CH_2-), 3.26 (s, 3H, SO_2CH_3). MS (ESI, m/z): 502 $[M + H]^+$.

*1-(2-(1-(4-Bromophenyl)-5-(4-(methylsulfonyl)phenyl)-1*H*-1,2,4-triazol-3-ylthio)ethyl)-1-hydroxyurea (**15e**).* The title compound was obtained as a white solid (0.1 g, 41.7%), mp 135–137 °C. Found: C, 42.46; H, 3.25; N, 13.42. Calc. for $C_{18}H_{18}BrN_5O_4S_2$: C, 42.19; H, 3.54; N, 13.67. IR (KBr/cm^{-1}): 3461, 2927, 2854, 2346, 2310, 1644, 1493, 1311, 1149, 779, 534. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: ppm 9.42 (s, 1H, O*H*), 7.98 (d, J = 8.5 Hz, 2H), 7.71 (m, 4H), 7.42 (d, J = 8.5 Hz, 2H), 6.33 (s, 2H, NH_2), 3.72 (t, J = 6.8 Hz, 2H, -CH_2-), 3.32 (t, J = 6.7 Hz, 2H, -CH_2-), 3.25 (s, 3H, SO_2CH_3). MS (ESI, m/z): 513 $[M + H]^+$.

*4-(3-(2-(1-Hydroxyureido)ethylthio)-1-phenyl-1*H*-1,2,4-triazol-5-yl)benzenesulfonamide (**15g**).* The title compound was obtained as a white solid (0.04 g, 46.4%), mp 169–171 °C. Found: C, 46.74; H, 4.58; N, 19.66. Calc. for $C_{17}H_{18}N_6O_4S_2$: C, 46.99; H, 4.18; N, 19.34. IR (KBr/cm^{-1}): 3448, 2964, 2926, 1644, 1575, 1452, 1340, 1150, 801, 617. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 9.42 (s, 1H, O*H*), 7.82 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 7.51 (m, 3H), 7.45 (br, 2H), 7.42 (s, 2H, SO_2NH_2), 6.33 (s, 2H, NH_2), 3.72 (t, J = 6.8 Hz, 2H, -CH_2-), 3.33 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 435 $[M + H]^+$.

*4-(3-(2-(1-Hydroxyureido)ethylthio)-1-*p*-tolyl-1*H*-1,2,4-triazol-5-yl)benzenesulfonamide (**15i**).* The title compound was obtained as a white solid (0.1 g, 52.6%), mp 137–139 °C. Found: C, 48.11; H, 4.82; N, 18.96. Calc. for $C_{18}H_{20}N_6O_4S_2$: C, 48.20; H, 4.49; N, 18.74. IR (KBr/cm^{-1}): 3368, 2925, 1655, 1513, 1451, 1335, 1161, 823, 614. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 9.42 (s, 1H, O*H*), 7.82 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 8.4 Hz, 2H), 7.45 (s, 2H, SO_2NH_2), 7.30 (m, 4H), 6.34 (s, 2H, NH_2), 3.71 (t, J = 6.9 Hz, 2H, -CH_2-), 3.39 (t, J = 7.0 Hz, 2H, -CH_2-), 2.37 (s, 3H, phenyl-CH_3). MS (ESI, m/z): 449 $[M + H]^+$.

*4-(1-(4-Fluorophenyl)-3-(2-(1-hydroxyureido)ethylthio)-1*H*-1,2,4-triazol-5-yl)benzenesulfonamide (**15k**).* The title compound was obtained as a white solid (0.57 g, 49.6%), mp 166–168 °C. Found: C, 45.51; H, 3.95; N, 18.42. Calc. for $C_{17}H_{17}FN_6O_4S_2$: C, 45.12; H, 3.79; N, 18.57. IR (KBr/cm^{-1}): 3460, 2933, 2857, 2355, 2029, 1624, 1577, 1337, 1163, 855, 624. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 9.43 (s, 1H, O*H*), 7.81 (d, J = 8.6 Hz, 2H), 7.57 (m, 4H), 7.45 (s, 2H, SO_2NH_2), 7.43 (d, J = 8.6 Hz, 2H), 6.37 (s, 2H, NH_2), 3.71 (t, J = 6.6 Hz, 2H, -CH_2-), 3.35 (t, J = 6.2 Hz, 2H, -CH_2-). MS (ESI, m/z): 453 $[M + H]^+$.

*4-(3-(2-(1-Hydroxyureido)ethylthio)-1-(4-(trifluoromethyl)phenyl)-1*H*-1,2,4-triazol-5-yl)benzenesulfonamide (**15m**).* The title compound was obtained as a white solid (0.03 g, 44.6%), mp 157–159 °C. Found: C, 43.36; H, 3.74; N, 16.89. Calc. for $C_{18}H_{17}F_3N_6O_4S_2$: C, 43.02; H, 3.41; N, 16.72. IR (KBr/cm^{-1}): 3457, 2930, 2854, 2352, 2027, 1634, 1576, 1335, 1160, 852, 621. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: ppm 9.44 (s, 1H, O*H*), 7.88 (m, 4H), 7.66 (m, 4H), 7.49 (s, 2H, SO_2NH_2), 6.38 (s, 2H, NH_2), 3.73 (t, J = 6.8 Hz, 2H, -CH_2-), 3.35 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 503 $[M + H]^+$.

*4-(1-(4-Bromophenyl)-3-(2-(1-hydroxyureido)ethylthio)-1*H*-1,2,4-triazol-5-yl)benzenesulfonamide (**15o**).* The title compound was obtained as a white solid (0.1 g, 54.2%), mp 155–157 °C. Found: C, 39.98; H, 3.05; N, 16.61. Calc. for $C_{17}H_{17}BrN_6O_4S_2$: C, 39.77; H, 3.34; N, 16.37. IR (KBr/cm^{-1}): 3430, 2926, 2853, 2377, 2347, 2310, 1643, 1493, 1339, 1159, 832, 620. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: ppm 9.41 (s, 1H, O*H*), 7.81 (d, J = 8.7 Hz, 2H), 7.62 (d, J = 8.7 Hz, 2H), 7.53 (m, 2H), 7.41 (s, 2H, SO_2NH_2), 7.36 (m, 2H), 6.32 (s, 2H, NH_2), 3.72 (t, J = 6.9 Hz, 2H, -CH_2-), 3.32 (t, J = 6.9 Hz, 2H, -CH_2-). MS (ESI, m/z): 514 $[M + H]^+$.

General procedure for synthesis of compounds 17a–b. To a solution of the appropriate benzoic acids **16a–b** (5 mmol) in 10 mL DMF, K_2CO_3 (5.5 mmol) and CH_3I (5.5 mmol) were added, and the mixture was maintained at 80 °C for 1 h. The mixture was poured into ice water and the precipitate was filtered to give a white solid.

General procedure for synthesis of compounds 18a–b. A mixture of the appropriate methyl benzoates **17a–b** (3.27 mmol), hydrazine hydrate (16.3 mmol), and 10 mL of 95% ethanol was heated under reflux for 6 h. Yellow needle crystals were precipitated as the mixture cooled to room temperature. The precipitate was collected to give yellow needle crystals.

General procedure for synthesis of compounds 19a–c. Equimolar quantities of the appropriate benzoic acid hydrazide **18a–b** (1.3 mmol) and *para*-position substituted phenyl isothiocyanate (1.3 mmol) in 10 mL of absolute ethanol were heated under reflux for 5 h. The precipitate was collected to give a white solid.

General procedure for synthesis of compounds 20a–c. To a solution of the appropriate compounds **19a–c** (1 mmol) in 5 mL methanol, 5 mL 10% NaOH was added and the mixture was heated under reflux for 2 h. The solution was cooled and acidified to pH = 2 by adding 10% HCl. The precipitated solid was filtered and washed with water to give a white solid.

General procedure for synthesis of compounds 21a–d. As described in the procedure for synthesis of compounds **13a–j**.

*2-(4-(4-Fluorophenyl)-5-(4-(methylsulfonyl)phenyl)-4*H*-1,2,4-triazol-3-ylthio)acetaldehyde oxime (**21a**).* The title compound was obtained as a white solid (0.06 g, 43.4%), mp 85–87 °C. Found: C, 50.51; H, 3.66; N, 13.40. Calc. for $C_{17}H_{15}FN_4O_3S_2$: C, 50.23; H, 3.72; N, 13.78. IR (KBr/cm^{-1}): 3450, 3074, 2922, 2850, 2358, 1639, 1510, 1310, 1152, 960, 845, 779, 606. ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ: ppm 11.34 (s, 0.429H, *anti* O*H*), 10.94 (s, 0.563H, *syn* O*H*), 7.93 (d, J = 8.5 Hz, 2H), 7.61 (m, 4H), 7.47 (d, J = 8.5 Hz, 2H), 7.42 (t, J = 5.8 Hz, 0.588H, *syn* –C*H*═N), 6.93 (t, J = 5.3 Hz, 0.394H, *anti* –C*H*═N), 3.95 (d, J = 5.3 Hz, 1H, *anti* SCH_2–), 3.91 (d, J = 5.8 Hz, 1H, *syn* SCH_2–), 3.24 (s, 3H, SO_2CH_3). MS (ESI, m/z): 407 [M + H]$^+$.

*2-(5-(4-(Methylsulfonyl)phenyl)-4-(4-(trifluoromethyl)phenyl)-4*H*-1,2,4-triazol-3-ylthio)acetaldehyde oxime (**21b**).* The title compound was obtained as a white solid (0.08 g, 34.1%), mp 70–72 °C. Found: C, 47.08; H, 3.64; N, 12.55. Calc. for $C_{18}H_{15}F_3N_4O_3S_2$: C, 47.36; H, 3.31; N, 12.27. IR (KBr/cm^{-1}): 3422, 2923, 2852, 1722, 1608, 1439, 1325, 1153, 1066, 958, 779. ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ: ppm 11.36 (s, 0.42H, *anti* O*H*), 10.96 (s, 0.49H, *syn* O*H*), 8.00 (d, J = 8.3 Hz, 2H), 7.93 (d, J = 8.3 Hz, 2H), 7.63 (m, 4H), 7.43 (t, J = 5.8 Hz, 0.56H, *syn* –C*H*═N), 6.98 (t, J = 5.3 Hz, 0.46H, *anti* –C*H*═N), 3.95 (d, J = 5.1 Hz, 1H, *anti* SCH_2–), 3.92 (d, J = 5.7 Hz, 1H, *syn* SCH_2–), 3.24 (s, 3H, SO_2CH_3). MS (ESI, m/z): 457 [M + H]$^+$.

*4-(4-(4-Fluorophenyl)-5-(2-(hydroxyimino)ethylthio)-4*H*-1,2,4-triazol-3-yl)benzenesulfonamide (**21d**).* The title compound was obtained as a white solid (0.067 g, 76.6%), mp 119–121 °C. Found: C, 47.55; H, 3.06; N, 17.52. Calc. for $C_{16}H_{14}FN_5O_3S_2$: C, 47.17; H, 3.46; N, 17.19. IR (KBr/cm^{-1}): 3442, 2919, 2849, 2358, 1639, 1510, 1465, 1334, 1164, 843, 622. ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ: ppm 11.32 (s, 0.302H, *anti* O*H*), 10.92 (s, 0.386H, *syn* O*H*), 7.79 (d, J = 8.6 Hz, 2H), 7.55 (m, 4H), 7.45 (t, J = 8.6 Hz, 2H), 7.42 (s, 2H, SO_2NH_2), 7.39 (t, J = 5.9 Hz, 0.337H, *syn* –C*H*═N), 6.92 (t, J = 5.3 Hz, 0.313H, *anti* –C*H*═N), 3.94 (d, J = 5.3 Hz, 1H, *anti* SCH_2–), 3.89 (d, J = 5.9 Hz, 1H, *syn* SCH_2–). MS (ESI, m/z): 408 [M + H]$^+$.

General procedure for synthesis of compounds 22a–d. As described in the procedure for synthesis of compounds **15a–j**.

*1-(2-(4-(4-Fluorophenyl)-5-(4-(methylsulfonyl)phenyl)-4*H*-1,2,4-triazol-3-ylthio)ethyl)-1-hydroxyurea (**22a**).* The title compound was obtained as a white solid (0.73 g, 76.6%), mp 106–108 °C. Found: C, 47.59; H, 4.33; N, 15.78. Calc. for $C_{18}H_{18}FN_5O_4S_2$: C, 47.88; H, 4.02; N, 15.51. IR (KBr/cm^{-1}): 3462, 2963, 2923, 1653, 1510, 1441, 1261, 1090, 801, 510. ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ: ppm 9.47 (s, 1H, O*H*), 7.92 (d, J = 8.6 Hz, 2H), 7.58 (m, 4H), 7.42 (t, J = 8.8 Hz, 2H), 6.37 (s, 2H, NH_2), 3.69 (t, J = 6.8 Hz, 2H, -CH_2-), 3.36 (t, J = 6.9 Hz, 2H, -CH_2-), 3.23 (s, 3H, SO_2CH_3). MS (ESI, m/z): 452 [M + H]$^+$.

*1-Hydroxy-1-(2-(5-(4-(methylsulfonyl)phenyl)-4-(4-(trifluoromethyl)-phenyl)-4*H*-1,2,4-triazol-3-ylthio)ethyl)urea (**22b**).* The title compound was obtained as a white solid (0.067 g, 61.2%), mp 132–134 °C. Found: C, 45.82; H, 3.97; N, 13.70. Calc. for $C_{19}H_{18}F_3N_5O_4S_2$: C, 45.50; H, 3.62; N, 13.96. IR (KBr/cm^{-1}): 3473, 2925, 1652, 1446, 1326, 1150, 1062, 849, 777, 533. ^{1}H-NMR (DMSO-d$_6$, 500 MHz) δ: ppm 9.42 (s, 1H, O*H*), 7.99 (d, J = 8.5 Hz, 2H), 7.89 (d, J = 8.5 Hz, 2H), 7.73 (d, J = 8.5 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H), 6.35 (s, 2H, NH_2), 3.73 (t, J = 6.8 Hz, 2H, -CH_2-), 3.34 (t, J = 6.7 Hz, 2H, -CH_2-), 3.26 (s, 3H, SO_2CH_3). MS (ESI, m/z): 502 [M + H]$^+$.

*4-(4-(4-Fluorophenyl)-5-(2-(1-hydroxyureido)ethylthio)-4*H*-1,2,4-triazol-3-yl)benzenesulfonamide (**22d**).* The title compound was obtained as a white solid (0.06 g, 81.6%), mp 143–145 °C. Found: C, 45.48; H, 3.98; N, 18.34. Calc. for $C_{17}H_{17}FN_6O_4S_2$: C, 45.12; H, 3.79; N, 18.57. IR (KBr/cm^{-1}): 3485, 3336, 2377, 2350, 2319, 1634, 1511, 1347, 1158, 847, 621. ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ: ppm 9.47 (s, 1H, O*H*), 7.79 (d, J = 8.6 Hz, 2H), 7.55 (m, 4H), 7.43 (s, 2H, SO_2NH_2), 7.41 (d, J = 8.6 Hz, 2H), 6.35 (s, 2H, NH_2), 3.69 (t, J = 6.6 Hz, 2H, -CH_2-), 3.35 (t, J = 6.2 Hz, 2H, -CH_2-). MS (ESI, m/z): 453 [M + H]$^+$.

4.2. *In vitro* COX and 5-LOX inhibition assay

Stock solutions of test compounds were made by dissolving each compound in a minimum volume of DMSO. 6-keto-PGF$_{1\alpha}$ production was measured in order to determine COX-1 activity. Endotheliocyte was gained from the aorta pectoralis of neogenesis calf *in vitro*, the cells were maintained at 37 °C and 5% CO_2 in DMEM supplemented 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 60 mg L^{-1} penicillin and 100 mg L^{-1} streptomycin. Cells were plated into 24-well plates at a density of 1.0×10^6 cells per well in 1 mL of DMEM. After 24 h incubation, the cells were treated with test compounds (10^{-6} mol L^{-1}), DMSO and celecoxib (10^{-6} mol mL^{-1}). These solutions were incubated for 20 min at 37 °C and 5% CO_2, then were treated with arachidonic acid (10 μmol L^{-1}). After 20 min incubation, the culture supernatants were collected and stored at −70 °C until required for 6-keto-PGF$_{1\alpha}$ determination. 6-keto-PGF$_{1\alpha}$ was measured by using a radioimmunity assay kit according to the manufacturer's instructions.

The activity of the target compounds to inhibit COX-2 was determined by PGE_2 production assay. Macrophages were gained from the abdominal cavity of rats and plated into 24-well plates at a density of 1.0×10^6 cells per well in 1 mL of RPMI 1640 containing 5% FCS. After 24 h incubation at 37 °C and 5% CO_2, aspirin (1 mmol L^{-1}) was added to inactivate COX-1. Then the culture media were replaced with fresh media containing LPS (1 μg mL^{-1}) to induce COX-2. After 6 h incubation at 37 °C, the test compounds (10^{-6} mol L^{-1}), DMSO and celecoxib (10^{-6} mol L^{-1}) were added and incubated for 30 min at 37 °C and 5% CO_2. The cells were then treated with arachidonic acid (10 μmol L^{-1}) for 20 min, and the culture supernatants were collected for the determination of PGE_2 production using an enzyme immunoassay (EIA) kit (Cat-lot no. 414010, 96-well, Cayman Chemical Company) according to the manufacturer's instructions.

The 5-LOX inhibitor screening assay detects and measures the LTB_4 produced by stimulation of calcium ionophore A23187. Leukocytes were obtained from the abdominal cavity of Sprague-Dawley rats injected intraperitoneally with 20 mL kg^{-1} of a 0.2% (w/v) glycogen solution. The cell suspensions were collected with Hanks solution and plated into 24-well plates at a density of 1.0×10^6 cells per well. After 10 min

incubation at 37 °C, L-cysteine (10 mmol), indomethacin (1 mg L^{-1}), DMSO, celecoxib and test compounds were added in turn, and incubated at 37 °C for 30 min. LTB_4 production was initiated by adding calcium ionophore A23187 (5 μmol) and incubated for 30 min at 37 °C. After being clarified by centrifugation, the supernatant was plated into 96-well plates and incubated overnight at 4 °C. Chromogen was added and the plates were retained for 90 min. The 5-LOX activity was determined by using an EIA kit (Cat-lot no. 520111, 96-well, Cayman Chemical Company) according to the manufacturer's instructions. Percent inhibition was calculated according to the following formula:

$$\text{Inhibition } (\%) = 100 \times (\text{OD blank} - \text{OD comp})/\text{OD blank}.$$

The IC_{50} values were determined as the maximal inhibition (100%) by Bliss method.

4.3. Anti-inflammatory assay

Three models including xylene-induced ear edema in mice, albumen-induced paw edema in rats and acetic acid-induced mouse vascular permeability in mice were carried out to evaluate the anti-inflammatory activity of target compounds as described previously.[31]

4.4. Analgesic assay

Analgesic activity was determined using acetic acid-induced writhing response (chemical method) and hot-plate assay (thermal method) in mice.[39,40] Mice were divided into five groups (n = 10) and administrated orally with 0.5% CMC–Na solution, celecoxib (30 mg kg^{-1}) or compound **15e** (15, 30 and 60 mg kg^{-1}). 1 h post administration, acetic acid (0.7% v/v) was given intraperitoneally to all the groups at the dose of 0.1 mL per 10 g body weight. Analgesic activity was recorded by counting the first time of writhe and the number of writhes after the injection of acetic acid for a period of 15 min. A writhe is indicated by abdominal constriction and full extension of hind limb.

To perform hot-plate assay, animals were tested initially for baseline latency and the mice which showed hind paw-lick response within 6–8 s were selected for the study. To avoid tissue damage, animals were exposed to the hot plate for a maximum of 30 s. 1 h post administration of test and reference compounds, the animals were individually exposed to the hot plate maintained at 55 °C and latency was tested at 60, 90 and 120 min post-administration. The experimental protocols of animal studies were approved by the Animal Research Care Committee of China Pharmaceutic University.

4.5. Molecular modeling (docking) study

The crystal structure of murine COX-2 in complex with SC-558 was received from the RCSB Protein Data Bank (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 3D 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 crystal structure of 5-LOX was received from the RCSB Protein Data Bank (entry code 3O8Y). 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 $(mol\ Å)^{-1}$.

Docking studies were performed by the program of GOLD 3.0 running on a Discovery Studio 2.5 workstation. The X-ray coordinates of SC-558 bound to the active site of COX-2 were used to define 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. The procedures for definition of 5-LOX active site were carried out as described in a previous report.[31] The non-heme iron was added to the model in InsightII and it ligated to three histidines (His^{367}, His^{372}, His^{550}) as well as Ile^{674}.

Competing interest

The authors declare no competing financial interest.

Abbreviations

PGs	prostaglandins;
LTs	leukotrienes;
AA	arachidonic acid;
RA	rheumatoid arthritis;
OA	osteoarthritis;
COX	cyclooxygenase;
5-LOX	5-lipoxygenase;
NSAIDs	non-steroidal anti-inflammatory drugs;
TLC	thin-layer chromatography;
HPLC	high-performance liquid chromatography;
TMS	tetramethylsilane;
ESI	electrospray ionisation;
EIA	enzyme immunoassay;
MTT	methylthiazolyl tetrazolium;
PBS	phosphate-buffered solution;
ADP	adenosine diphosphate;
OH	hydroxyl radical;
NOS	nitric oxide synthase;
CAT	catalase;
PRP	platelet-rich plasma;
PPP	platelet-poor plasma.

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