Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 2507–2517
Bioorganic & Medicinal Chemistry

Design, synthesis, and biological evaluation of substituted 3-alkylthio-4,5-diaryl-4*H*-1,2,4-triazoles as selective COX-2 inhibitors

Latifeh Navidpour,[a] Hamed Shafaroodi,[b] Khosrou Abdi,[a] Mohsen Amini,[a] Mohammad H. Ghahremani,[c] Ahmad Reza Dehpour[d] and Abbas Shafiee[a,*]

[a]*Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14174, Iran*
[b]*Department of Pharmacology, Tehran Medical Unit, Islamic Azad University, Tehran, Iran*
[c]*Department of Pharmacology and Toxicology and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14174, Iran*
[d]*Department of Pharmacology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran 14174, Iran*

Received 2 October 2005; revised 14 November 2005; accepted 15 November 2005
Available online 5 December 2005

Abstract—A new type of 4,5-diaryl-4*H*-1,2,4-triazole, possessing C-3 thio and alkylthio (SH, SMe or SEt) substituents, was designed and synthesized for evaluation as selective cyclooxygenase-2 (COX-2) inhibitors with in vivo anti-inflammatory activity. The compound, 3-ethylthio-5-(4-fluorophenyl)-4-(4-methylsulfonylphenyl)-4*H*-1,2,4-triazole (**10d**), exhibited a high in vitro selectivity (COX-1 IC_{50} = 20.5 nM; COX-2 IC_{50} = 1.8 nM; SI = 11.39) relative to the reference drug celecoxib (COX-1 IC_{50} = 3.7 nM; COX-2 IC_{50} = 2.2 nM; SI = 1.68) and also showed good anti-inflammatory activity compared to celecoxib in a carrageenan-induced rat paw edema assay.

1. Introduction

A new generation of anti-inflammatory drugs, celecoxib (Celebrex™)[1] and etoricoxib (Arcoxia®),[2] is being prescribed to treat acute or chronic inflammation by providing symptomatic pain relief.[3] A selective cyclooxygenase-2 (COX-2) inhibitor allows the desired synthesis of cytoprotective prostaglandins, in conjunction with a simultaneous inhibition of proinflammatory prostaglandin synthesis, thereby reducing dyspepsia and ulceration.[4] However, emerging evidence suggests that adverse reactions such as GI irritations or ulceration and renal liabilities are associated with prolonged use of COX-2 selective inhibitors. The adverse reactions have been attributed, at least in part, to COX-1 inhibition occurring with long-term exposure or at higher doses.[5] COX-2 selective inhibitors are also known to suppress synthesis of prostacyclin, a potent vasodilator, gastroprotectant, and platelet inhibitor, via inhibition of endothelial COX-2. COX-2 selective inhibitors do not inhibit production of thromboxane, a vasoconstrictor, and promoter of platelet aggregation, which is synthesized in platelets by COX-1.[6,7] Therefore, COX-2 inhibitors intrinsically lack anti-thrombotic activity, and some cardiovascular liabilities have been associated preclinically with them.[8] Thus, there is still a need for novel, selective, and potent COX-2 inhibitors with an improved profile compared to current COX-2 inhibitors.[3]

Diarylheterocycles, and other central ring pharmacophore templates, have been extensively studied as cyclooxygenase inhibitors. All these tricyclic molecules possess 1,2-diaryl substitution on a central four-, five-, or six-membered ring system such as cyclobutenone (**1**), pyrazole (**2**), 2-(5*H*)-furanone (**3**), isoxazole (**4**), or pyridine (**5**), respectively (Fig. 1).[1,2,9–11]

Structure–activity relationship (SAR) studies have shown that for optimum COX-2 selectivity and

Keywords: Cyclooxygenase-2 inhibitor; 1,2,4-Triazoles; Alkylthio; Celecoxib.
* Corresponding author. Tel.: +98 21 66406757; fax: +98 21 66461178; e-mail: ashafiee@ams.ac.ir

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.11.029

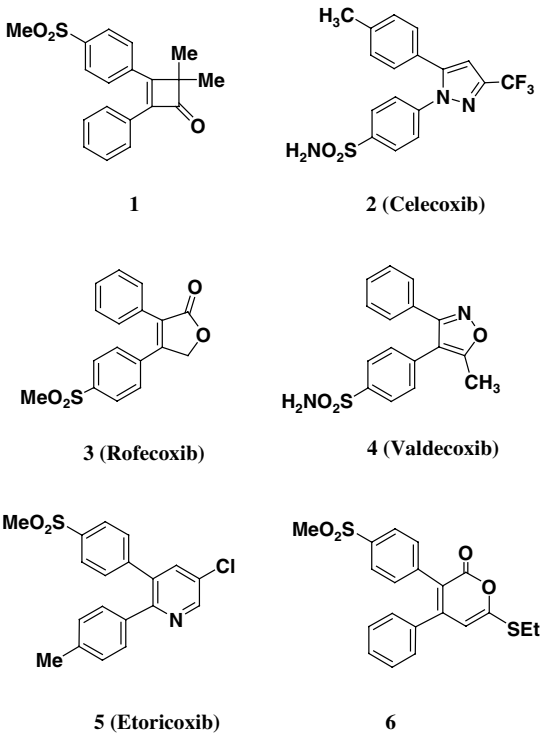
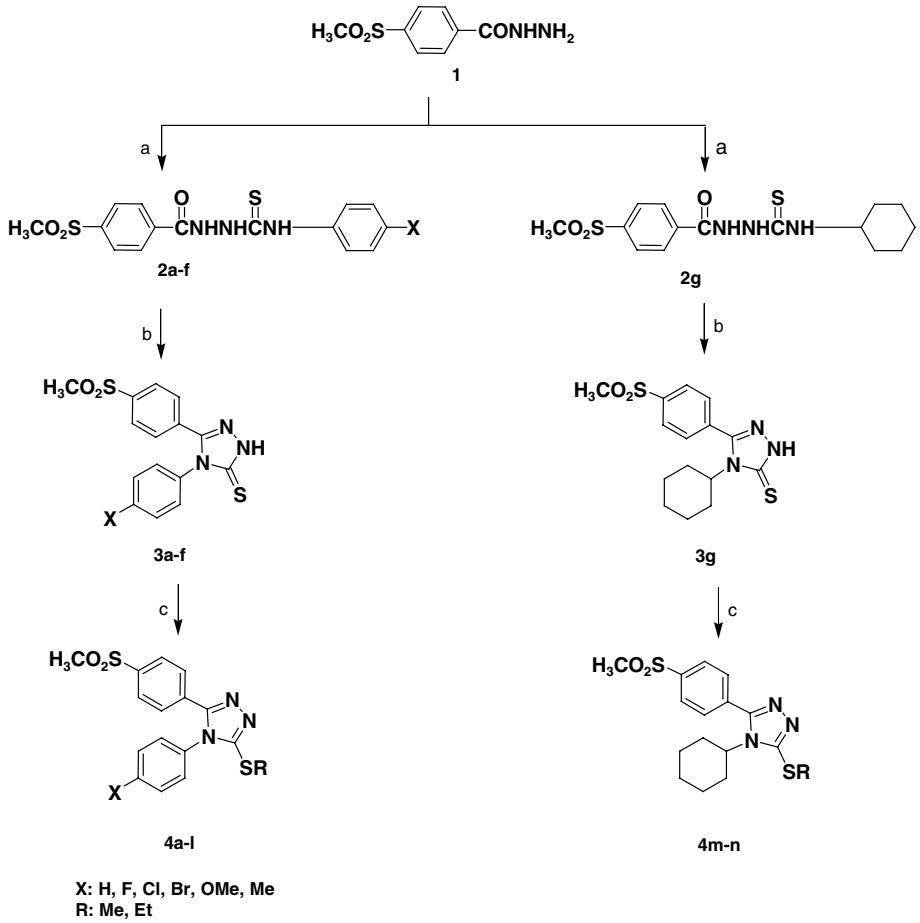


Figure 1. Representative examples of selective tricyclic COX-2 inhibitors.

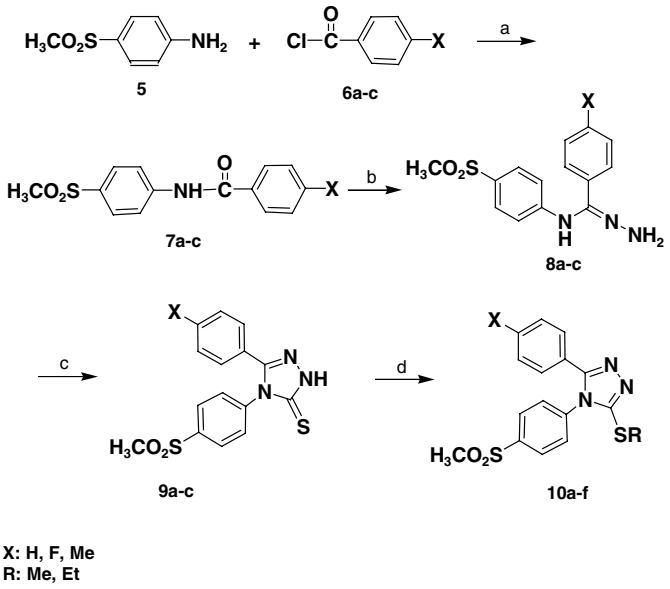
inhibitory potency, a SO_2Me or SO_2NH_2 substituent at the *para*-position of a phenyl ring, and the presence of a *p*-F substituent on a non-sulfonyl vicinal phenyl ring improve in vivo activity.[12] Recently, a novel class of 6-alkylthio-substituted six-membered lactone (pyrane-2-one) rings (**6**) has been designed and exhibited very good in vitro COX-2 inhibitory potency and selectivity.[13] Furthermore, differently substituted 1,2,4-triazole-3-thione derivatives exhibited anti-inflammatory activity.[14–18] Considering these results and as part of our ongoing program to design novel selective COX-2 inhibitors, we describe herein the design, synthesis, and biological evaluation of a novel diverse group of alkylthio-substituted-5-(4-methylsulfonylphenyl)-4-phenyl (or cyclohexyl) triazoles and alkylthio-substituted-4-(4-methylsulfonylphenyl)-5-phenyltriazoles as selective COX-2 inhibitors with anti-inflammatory activity.

2. Chemistry

The synthetic reactions used for the synthesis of 3-alkylthio-5-(4-methylsulfonylphenyl)-4-phenyl (or cyclohexyl) triazoles (**4a–n**) and 3-alkylthio-4-(4-methylsulfonylphenyl)-5-phenyltriazoles (**10a–f**) are outlined in Schemes 1 and 2.



Scheme 1. Reagents and conditions: (a) R-N=C=S, EtOH, rt, overnight; (b) Na_2CO_3 aq reflux, overnight; (c) RI, KOH, rt, overnight.



Scheme 2. Reagents and conditions: (a) Et_3N, THF, 0 °C to rt, 24 h; (b) PCl_5, benzene, reflux, 3 h; then NH_2NH_2 (dry), THF, rt, overnight; (c) 1,1'-thiocarbonyldiimidazole, THF, rt, 18 h; (d) RI, KOH, rt, overnight.

Starting from 4-methylsulfonylbenzoic acid hydrazide **1**, 3-alkylthio-5-(4-methylsulfonylphenyl)-4-aryl (or cyclohexyl)-4*H*-1,2,4-triazole (**4a**–**n**) were prepared. The reaction of corresponding hydrazide **1** with respective aryl (or cyclohexyl) isothiocyanates yielded **2a**–**g**, which were cyclized in saturated sodium carbonate solution to **3a**–**g**. Subsequent alkylation of **3a**–**g** using alkyl iodide in basic media afforded the title compounds **4a**–**n** (see Scheme 1).[19]

The target 3-alkylthio-4-(4-methylsulfonylphenyl)-5-phenyl-4*H*-1,2,4-triazoles (**10a**–**f**) were prepared starting with the condensation of 4-methylsulfonylaniline **6** with respective benzoyl chloride **6** in the presence of Et_3N to produce *N*-(4-methylsulfonylphenyl)-4-substituted benzamides (**7a**–**c**). Subsequent reaction of **7a**–**c** with PCl_5, followed by reaction with dry hydrazine, yielded *N*-(4-methylsulfonylphenyl)aryl carbohydrazonamides (**8a**–**c**) that undergoes a ring closure using 1,1'-thiocarbonyldiimidazole and subsequent alkylation to afford **10a**–**f** (see Scheme 2).[20,21] 3-Alkylthio-1,2,4-triazoles (**11b**–**c**) were prepared according to the literature.[22]

3. Results and discussion

The effect of thio and alkylthio substituents on the central five-membered triazole ring on COX-2 selectivity and potency was determined by colorimetric COX (ovine) inhibitor screening assay (Tables 1–3).[23–25] As an initial in vitro screening, compounds were assayed against COX-2 at 5 and 10 nM, and COX-1 at 10 and 50 nM (Tables 1 and 3). For the more potent and selective COX-2 inhibitors (relative to reference compound, celecoxib), the IC_{50} values were then determined (Table 2).

In vitro enzyme inhibition studies for **3a**–**f** and **4a**–**l** showed weak to good COX-2 inhibitory activity. In general, for these compounds, COX-2 selectivity and potency were dependent upon steric properties of C-3 thio or alkylthio substituent on the central triazole ring and electronic properties of the C-4 phenyl ring substituent. For bulkier C-4-substituted compounds **3c**–**f** and **4e**–**l**, the size of the C-3 substituent had a significant effect on COX-2 inhibitory potency (SH > SMe > SEt) and COX-2 selectivity. Decreasing the size led to an increase in COX-2 potency and selectivity (see Table 1).

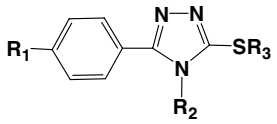
In addition, among the different substituents on C-4 of the phenyl ring, the best substituent was C-4 methyl. Thus, the most potent and selective COX-2 inhibitor was **3f**, (COX-2 IC_{50} = 0.6 nM; COX-1 IC_{50} = 3.1 nM; S.I. = 5.17) relative to reference drug celecoxib (COX-2 IC_{50} = 2.2 nM; COX-1 IC_{50} = 3.7 nM; S.I. = 1.68). This phenomenon is not particularly surprising or unexpected since it is also found in celecoxib at the 5-position of the central pyrazole ring.

In the cyclohexyl subgroup (**3g**, **4m**, and **4n**) also decreasing the size of the thio group significantly increased COX-2 selectivity with **3g**, showing a potent inhibition of COX-2 and good selectivity index (COX-2 IC_{50} = 0.6 nM; COX-1 IC_{50} = 2.4 nM; S.I. = 4.0) relative to reference compound celecoxib (COX-2 IC_{50} = 2.2 nM; COX-1 IC_{50} = 3.7 nM; S.I. = 1.68).

3-Alkylthio-4-(4-methylsulfonylphenyl)-5-phenyl-4*H*-1,2,4-triazoles (**10b**–**e**) exhibited weak COX-1 inhibition (3.8–20.5 nM range) with good COX-2 inhibition in the 1.6–2.5 nM range. In this series, **10d** was the most potent and selective COX-2 inhibitor (COX-2 IC_{50} = 1.8 nM; COX-1 IC_{50} = 20.5 nM; S.I. = 11.39) relative to reference compound celecoxib (COX-2 IC_{50} = 2.2 nM; COX-1 IC_{50} = 3.7 nM; S.I. = 1.68).

Further studies for 3-alkylthio-1,2,4-triazole subgroup (**11a**–**c**) showed good COX-1 inhibition with moderate

Table 1. In vitro inhibition of COX-1 and COX-2 by **3a–g**, **4a–n**, and **10a–f**



Compound	R_1	R_2	R_3	Inhibition of COX-2 at 5 nm[a]	Inhibition of COX-2 at 10 nm[a]	Inhibition of COX-1 at 10 nm[a]	Inhibition of COX-1 at 50 nm[a]	Ratio COX-2/COX-1 at 10 nm
3a	4-CH_3SO_2	Phenyl	H	0.74	1.18	0.82	0.87	1.44
3b	4-CH_3SO_2	4-F-phenyl	H	0.77	1.11	0.81	1.06	1.36
3c	4-CH_3SO_2	4-Cl-phenyl	H	0.99	1.09	0.99	1.15	1.09
3d	4-CH_3SO_2	4-Br-phenyl	H	0.91	0.99	0.87	0.90	1.13
3e	4-CH_3SO_2	4-OCH_3-phenyl	H	1.12	1.20	0.86	0.93	1.40
3f	4-CH_3SO_2	4-CH_3-phenyl	H	0.81	1.15	0.78	0.96	1.58
3g	4-CH_3SO_2	Cyclohexyl	H	0.90	1.05	0.94	0.98	1.12
4a	4-CH_3SO_2	Phenyl	CH_3	0.82	0.88	0.95	0.99	0.92
4b	4-CH_3SO_2	Phenyl	CH_2CH_3	0.86	0.98	0.83	0.87	1.17
4c	4-CH_3SO_2	4-F-phenyl	CH_3	0.79	0.85	0.83	0.97	1.03
4d	4-CH_3SO_2	4-F-phenyl	CH_2CH_3	0.91	1.00	0.98	0.97	1.02
4e	4-CH_3SO_2	4-Cl-phenyl	CH_3	0.85	0.93	0.91	0.98	1.02
4f	4-CH_3SO_2	4-Cl-phenyl	CH_2CH_3	0.48	0.65	0.92	1.13	0.71
4g	4-CH_3SO_2	4-Br-phenyl	CH_3	0.99	0.99	0.93	0.97	1.06
4h	4-CH_3SO_2	4-Br-phenyl	CH_2CH_3	0.54	0.64	0.97	0.91	0.66
4i	4-CH_3SO_2	4-OCH_3-phenyl	CH_3	1.00	1.02	0.80	1.00	1.27
4j	4-CH_3SO_2	4-OCH_3-phenyl	CH_2CH_3	1.47	0.83	0.95	0.97	0.87
4k	4-CH_3SO_2	4-CH_3-phenyl	CH_3	1.24	1.37	0.96	1.05	1.43
4l	4-CH_3SO_2	4-CH_3-phenyl	CH_2CH_3	0.43	0.65	0.96	0.89	0.68
4m	4-CH_3SO_2	Cyclohexyl	CH_3	1.27	1.38	0.96	1.08	1.44
4n	4-CH_3SO_2	Cyclohexyl	CH_2CH_3	1.11	1.35	0.79	1.14	1.71
10a	H	4-CH_3SO_2-phenyl	CH_3	1.11	1.17	1.04	1.11	1.13
10b	H	4-CH_3SO_2-phenyl	CH_2CH_3	1.14	1.17	0.98	1.11	1.19
10c	F	4-CH_3SO_2-phenyl	CH_3	0.93	1.10	0.85	1.06	1.30
10d	F	4-CH_3SO_2-phenyl	CH_2CH_3	0.98	1.01	0.62	0.86	1.64
10e	CH_3	4-CH_3SO_2-phenyl	CH_3	1.37	1.24	0.88	0.87	1.40
10f	CH_3	4-CH_3SO_2-phenyl	CH_2CH_3	1.17	0.91	0.86	0.98	1.06
Celecoxib				0.91	1.00	0.90	1.01	1.11

[a] Values are means of two determinations and deviation from the mean is <10% of the mean value.

Table 2. IC_{50} values and anti-inflammatory activities of some of **3**, **4**, and **10**

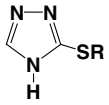
Compound	IC_{50} (nM)		Selectivity index (COX-1/COX-2)	AI activity	
	COX-1[a]	COX-2[a]		% inhibition at 3 h[b]	% inhibition at 5 h[b]
3a	3.5	1.6	2.19	59.1 ± 2.7	13.3 ± 6.2
3b	3.7	2.0	1.85	38.7 ± 2.8	28.8 ± 4.4
3f	3.1	0.6	5.17	25.8 ± 10.6	20.8 ± 7.9
3g	2.4	0.6	4.0	51.5 ± 4.6	36.7 ± 2.9
4c	3.7	2.3	1.61	70.4 ± 10.4	63.3 ± 3.3
4i	3.3	2.2	1.50	52.8 ± 6.2	51.7 ± 5.5
4k	4.7	2.1	2.24	64.0 ± 4.6	34.0 ± 6.6
4m	6.4	2.3	2.78	32.4 ± 4.6	32.4 ± 4.6
4n	3.7	1.9	1.95	44.0 ± 8.8	39.7 ± 7.8
10b	3.8	1.6	2.37	76.3 ± 8.2	71.1 ± 10.6
10c	12.1	2.0	6.05	53.8 ± 9.3	52.2 ± 7.7
10d	20.5	1.8	11.39	66.6 ± 5.8	43.4 ± 4.9
10e	4.1	2.5	1.64	66.1 ± 3.3	53.3 ± 3.3
Celecoxib	3.7	2.2	1.68	70.5 ± 4.7	50.0 ± 2.5

[a] Values are means of two determinations and deviation from the mean is <10% of the mean value.
[b] Inhibitory activity on carrageenan-induced rat paw edema. The results are expressed as means ± SEM (n = 4–6) following a 50 mg/kg oral dose of the test compound.

COX-2 inhibition relative to the reference drug celecoxib (Table 3). These compounds similar to classical NSAIDs inhibit both isoforms non-selectively.

The orientation of the highly potent and selective COX-2 inhibitors, 4-(4-methylphenyl)-5-(4-methylsulfonylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3f**) and

Table 3. In vitro inhibition of COX-1 and COX-2, and anti-inflammatory activity by **11a–c**



Compound	R	Inhibition of COX-2 at 5 nm[a]	Inhibition of COX-2 at 10 nm[a]	Inhibition of COX-1 at 10 nm[a]	Inhibition of COX-1 at 50 nm[a]	Ratio COX-2/COX-1 at 10 nm	AI activity	
							% inhibition at 3 h[b]	% inhibition at 5 h[b]
11a	H	0.80	0.81	0.91	0.92	0.89	57.0 ± 6.9	34.6 ± 11.8
11b	CH_3	0.72	0.76	0.85	0.89	0.89	46.5 ± 5.6	24.1 ± 15.2
11c	CH_2CH_3	0.76	0.80	0.93	0.95	0.86	59.3 ± 8.4	49.3 ± 9.5
Celecoxib		0.91	1.00	0.90	1.01	1.11	70.5 ± 4.7	50.0 ± 2.5

[a] Values are means of two determinations and deviation from the mean is <10% of the mean value.
[b] Inhibitory activity on carrageenan-induced rat paw edema. The results are expressed as means ± SEM (n = 4–6) following a 50 mg/kg oral dose of the test compound.

3-ethylthio-5-(4-fluorophenyl)-4-(4-methylsulfonylphenyl)-4*H*-1,2,4-triazole (**10d**), in the COX-2 active site was examined by a docking experiment.[26] This study showed that **3f** binds in the center of the primary binding site of COX-2 with the SO_2Me moiety interacting with the secondary pocket amino acid residues Phe[518], Gln[192], Arg[513], Leu[352], Ser[353], and Val[523]. One of the O-atoms of the SO_2Me substituent forms hydrogen bonds with the amine hydrogen (guanidine group) of Arg[513] (2.5 Å) and His[90] (2.7 Å), and the other oxygen forms hydrogen bond with Phe[518] (2.9 Å) as shown in Figure 2. Interestingly, the C-3 C=*S* sulfur atom is oriented toward the hydrophobic region formed by Ala[527], Leu[359], Leu[531], Met[113], Val[116], and Val[349]. This C=*S* sulfur atom is positioned about 4.5 Å from the NH_2 (guanidino) group of the polar amino acid Arg[120] and 5.9 Å from the OH group of Tyr[355]. These interactions may disrupt the salt bridge between His[90], Arg[120], Tyr[355], and Glu[524] at the mouth of the COX-2 active site. A similar docking study of **10d** showed that it binds in the primary binding site such that one of the O-atoms of the *p*-SO_2Me substituent forms hydrogen bonds with the amine hydrogen of Arg[513] (1.8 Å) and His[90] (2.5 Å), and the other oxygen forms hydrogen bond with Phe[518] (3.6 Å) as shown in Figure 3. The C-3 EtS-substituent

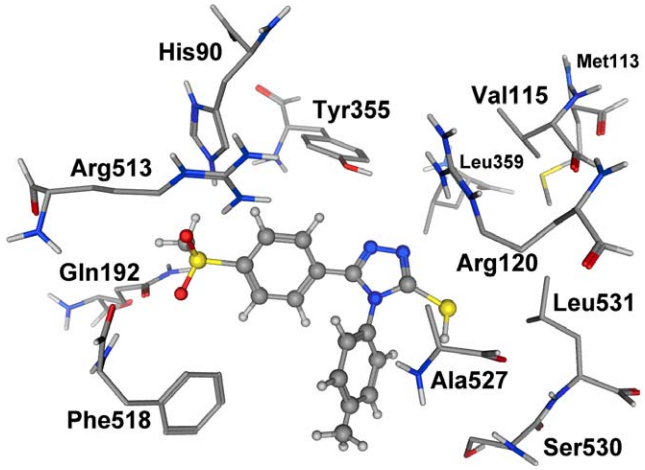


Figure 2. Docking of **3f** (ball and stick) in the active site of murine COX-2 (line and stick).

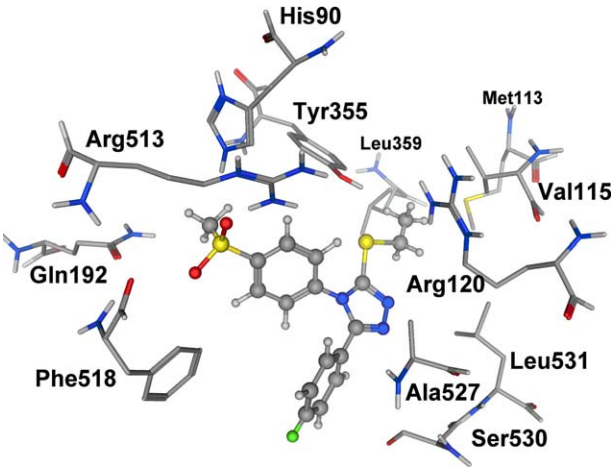


Figure 3. Docking of **10d** (ball and stick) in the active site of murine COX-2 (line and stick).

is located in a hydrophobic region formed by Ala[527], Leu[359], Leu[531], Met[113], Val[116], and Val[349], with the S-atom forming a weak hydrogen bond with the NH_2 (guanidino) group of the polar amino acid Arg[120] (3.3 Å) and with the OH group of Tyr[355] (2.8 Å).

These observations provide a good explanation for the potent and selective inhibitory activity of **3f** and **10d**.

In vivo pharmacological evaluation of **3**, **4**, and **10** was carried out to assess their potential anti-inflammatory activity.[27] Initial compound selection was based on the in vitro COX-1/COX-2 enzyme inhibition data obtained. Qualitative structure–activity relationship data, acquired using the anti-inflammatory rat paw edema assay, showed that this group of C-3 thio- and alkylthio-substituted 4,5-diaryl-4*H*-1,2,4-triazoles exhibits anti-inflammatory activity with moderate to good activity range (13–76% inhibition) (Table 2).

In the C-3 thio series (**3a**, **3b**, **3f**, and **3g**), 4-cyclohexyl-5-(4-methylsulfonylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**3g**) was the most active anti-inflammatory agent (51 and 37% reduction in inflammation at 3 and 5 h postdrug administration, respectively) for a

50 mg/kg oral dose as compared to celecoxib (70 and 50% reduction in inflammation at 3 and 5 h postdrug administration, respectively). In this series of compounds, **3b** and **3f** exhibit moderate anti-inflammatory activity. It is possible that these compounds are more susceptible to metabolic inactivation when administered by the oral route which may result in a lower amount of the drug localization at the inflammation site.

The C-3 alkylthio-substituted subgroup of **4** (**4c**, **4i**, **4k**, **4m**, and **4n**) reduced inflammation by 32–70% at different time intervals as shown in Table 2. 4-(4-Fluorophenyl)-5-(4-methylsulfonylphenyl)-3-methylthio-4*H*-1,2,4-triazole (**4c**) was the most potent anti-inflammatory agent in this series, producing a 70 and 63% reduction in inflammation at 3 and 5 h postdrug administration, respectively.

3-Alkylthio-4-(4-methylsulfonylphenyl)-5-phenyl-4*H*-1,2,4-triazoles (**10b–e**) exhibit good anti-inflammatory activity. In this series, **10b** was the most active anti-inflammatory agent (76 and 71% reduction in inflammation at 3 and 5 h postdrug administration, respectively).

The 3-thio and alkylthio-1,2,4-triazole group of compounds (**11a–c**) reduced inflammation by 24–59% at different time intervals as shown in Table 3.

4. Conclusions

A new class of 3-thio and alkylthio-4,5-diaryl-4*H*-1,2,4-triazoles was designed to develop further structure–activity relationship data. The results of this investigation show: (i) a C-3 SR substituent (**10d**) in this class of diarylheterocycles provides potent and selective inhibition of the COX-2 isozyme, (ii) molecular modeling studies indicate that the SO_2Me moiety inserts deep into the COX-2 secondary pocket and the C-3 *S*R sulfur atom forms a weak hydrogen bond with NH_2 atom of Arg^{120}, and (iii) these C-3 alkylthio compounds could serve as useful probes to study the function and catalytic activity of the COX-2 isozyme.

5. Experimental

Melting points were determined with a Reichert-Jung hot-stage microscope and are uncorrected. Infrared spectra were recorded on a Nicolet Magna 550-FT spectrometer. ^{1}H NMR (400 MHz) spectra were measured on a Varian Unity plus 400 spectrometer in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz. Spin multiples are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br (broad). Mass spectra were obtained on a Finnigan Mat TSQ-70 spectrometer. Elemental microanalyses were carried out with a Perkin-Elmer 240-C apparatus and were within ±0.4% of the theoretical values for C, H, and N. All solvents and reagents were purchased from the Fluka, Aldrich or Merck Chemical Company. Male Sprague–Dawley rats, used in the anti-inflammatory screens, were purchased from Pasteur Institute (Karaj, Iran), and experiments were carried out using protocols approved by the Ethics Committee of Tehran University of Medical Sciences.

5.1. General procedure for the preparation of 1-(4-methylsulfonylbenzoyl)-4-aryl (or cyclohexyl) thiosemicarbazides (2a–g)

To a solution of 4-methylsulfonylbenzoic acid hydrazide **1** (320 mg, 1.5 mmol) in ethanol (5 mL) was added respective isothiocyanate (1.5 mmol) and the mixture was stirred at room temperature for 24 h. The precipitate was filtered and crystallized from ethanol to give the title compounds **2a–g** as white solids.

5.1.1. 4-Phenyl-1-(4-methylsulfonylbenzoyl)thiosemicarbazide (2a). Yield, 86%; mp 201–203 °C; IR (KBr): 3307, 3261 (NH), 1681 (CO), 1293, 1146 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 10.52 (br s, 1H), 9.58 (br s, 1H), 8.22 (d, J = 8.5 Hz, 2H), 7.90 (d, J = 8.5 Hz, 2H), 7.75–7.12 (m, 5H), 3.11 (s, 3H); MS *m*/*z* (%) 349 (27), 331 (29), 313 (35), 298 (47), 242 (45), 202 (25), 173 (100), 129 (19), 75 (10), 49 (14). Anal. Calcd for $C_{15}H_{15}N_3O_3S_2$: C, 51.56; H, 4.33; N, 12.03. Found: C, 51.41; H, 4.45; N, 11.90.

5.1.2. 4-(4-Fluorophenyl)-1-(4-methylsulfonylbenzoyl)thiosemicarbazide (2b). Yield, 91%; mp 200–202 °C; IR (KBr): 3276, 3257 (NH), 1668 (CO), 1299, 1144 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 10.55 (br s, 1H), 9.56 (br s, 1H), 8.22 (d, J = 8.5 Hz, 2H), 7.90 (d, J = 8.5 Hz, 2H), 7.52 (dd, J = 5.1, 9.0 Hz, 2H), 7.02 (t, J = 8.4 Hz, 2H), 3.11 (s, 3H); MS *m*/*z* (%) 367 (5), 348 (100), 333 (75), 304 (16), 256 (33), 215 (15), 183 (11). Anal. Calcd for $C_{15}H_{14}FN_3O_3S_2$: C, 49.03; H, 3.84; N, 11.44. Found: C, 48.88; H, 3.95; N, 11.52.

5.1.3. 4-(4-Chlorophenyl)-1-(4-methylsulfonylbenzoyl)-thiosemicarbazide (2c). Yield, 87%; mp 196–198 °C; IR (KBr): 3310, 3239, 3165 (NH), 1663 (CO), 1298, 1145 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 10.72 (br s, 1H), 9.65 (br s, 1H), 8.20 (d, J = 8.5 Hz, 2H), 8.01 (d, J = 8.5 Hz, 2H), 7.59 (d, J = 8.7 Hz, 2H), 7.29 (d, J = 8.7 Hz, 2H), 3.11 (s, 1H); MS *m*/*z* (%) 383 (10), 365 (78), 349 (100), 336 (35), 256 (42), 214 (18), 183 (10), 169 (20). Anal. Calcd for $C_{15}H_{14}ClN_3O_3S_2$: C, 46.93; H, 3.68; N, 10.95. Found: C, 46.85; H, 3.82; N, 11.08.

5.1.4. 4-(4-Bromophenyl)-1-(4-methylsulfonylbenzoyl)-thiosemicarbazide (2d). Yield, 88%; mp 185–187 °C; IR (KBr): 3320, 3309 (NH), 1685 (CO), 1292, 1148 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 10.22 (br s, 1H), 9.35 (br s, 1H), 8.18 (d, J = 8.5 Hz, 2H), 8.05 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 8.7 Hz, 2H), 7.12 (d, J = 8.7 Hz, 2H), 3.12 (s, 1H). Anal. Calcd for $C_{15}H_{14}BrN_3O_3S_2$: C, 42.06; H, 3.29; N, 9.81. Found: C, 41.95; H, 3.39; N, 9.96.

5.1.5. 4-(4-Methoxyphenyl)-1-(4-methylsulfonylbenzoyl)thiosemicarbazide (2e). Yield, 95%; mp 195–197 °C; IR (KBr): 3328, 3247, 3177 (NH), 1664 (CO), 1297, 1144

(SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 10.72 (br s, 1H), 9.62 (br s, 1H), 8.20 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 3.80 (s, 3H), 3.11 (s, 3H); MS *m*/*z* (%) 379 (12), 361 (38), 328 (55), 313 (10), 256 (32), 239 (52), 213 (42), 183 (100), 165 (62), 150 (41), 122 (10). Anal. Calcd for $C_{16}H_{17}N_3O_4S_2$: C, 50.64; H, 4.52; N, 11.0. Found: C, 50.52; H, 4.60; N, 11.12.

5.1.6. 4-(4-Methylphenyl)-1-(4-methylsulfonylbenzoyl)-thiosemicarbazide (2f). Yield, 92%; mp 193–195 °C; IR (KBr): 3306, 3241 (NH), 1682 (CO), 1294, 1143 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 10.58 (br s, 1H), 9.65 (br s, 1H), 8.21 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.2 Hz, 2H), 7.13 (d, J = 8.2 Hz, 2H), 3.11 (s, 3H), 2.33 (s, 3H); MS *m*/*z* (%) 363 (5), 338 (20), 321 (60), 306 (52), 276 (78), 248 (48), 208 (32), 199 (42), 170 (100), 140 (45). Anal. Calcd for $C_{16}H_{17}N_3O_3S_2$: C, 52.87; H, 4.71; N, 11.56. Found: C, 52.99; H, 4.55; N, 11.44.

5.1.7. 4-Cyclohexyl-1-(4-methylsulfonylbenzoyl)thiosemicarbazide (2g). Yield, 65%; mp 200–202 °C; IR (KBr): 3419, 3320, 3244 (NH), 1692 (CO), 1293, 1149 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 10.55 (br s, 1H), 9.85 (br s, 1H), 8.18 (d, J = 8.5 Hz, 2H), 8.02 (d, J = 8.5 Hz, 2H), 4.25 (m, 1H), 3.11 (s, 3H), 2.60–1.20 (m, 10H). Anal. Calcd for $C_{15}H_{21}N_3O_3S_2$: C, 50.68; H, 5.95; N, 11.82. Found: C, 50.82; H, 6.05; N, 11.69.

5.2. General procedure for the preparation of 5-(4-methylsulfonylphenyl)-4-aryl (or cyclohexyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-thiones (3a–g)

A stirring mixture of compounds **2a**–**g** (1.5 mmol) and saturated aqueous sodium carbonate solution (15 mL) was refluxed overnight. After cooling, the solution was acidified with hydrochloric acid and the precipitate was filtered and rinsed with water, and then crystallized from methanol to give the title compounds **3a**–**g**.

5.2.1. 5-(4-Methylsulfonylphenyl)-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3a). Yield, 97%; mp 290–292 °C; IR (KBr): 3381 (NH), 1317, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 13.98 (br s, 1H), 7.85 (d, J = 8.2 Hz, 2H), 7.78–7.18 (m, 7H), 3.05 (s, 3H). Anal. Calcd for $C_{15}H_{13}N_3O_2S_2$: C, 54.36; H, 3.95; N, 12.68. Found: C, 54.22; H, 4.09; N, 12.61.

5.2.2. 4-(4-Fluorophenyl)-5-(4-methylsulfonylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3b). Yield, 94%; mp 296–298 °C; IR (KBr): 3323 (NH), 1327, 1153 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 14.05 (br s, 1H), 7.87 (d, J = 8.2 Hz, 2H), 7.82–7.15 (m, 6H), 3.08 (s, 3H). Anal. Calcd for $C_{15}H_{12}FN_3O_2S_2$: C, 51.56; H, 3.46; F, 5.44; N, 12.03. Found: C, 51.44; H, 5.29; N, 11.95.

5.2.3. 4-(4-Chlorophenyl)-5-(4-methylsulfonylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3c). Yield, 95%; mp 263–265 °C; IR (KBr): 3398 (NH), 1317, 1153 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 14.15 (br s, 1H), 7.89 (d, J = 8.2 Hz, 2H), 7.82–7.25 (m, 6H), 3.08 (s, 3H). Anal. Cacld for $C_{15}H_{12}ClN_3O_2S_2$: C, 49.24; H, 3.31; N, 11.49. Found: C, 49.12; H, 3.42; N, 11.66.

5.2.4. 4-(4-Bromophenyl)-5-(4-methylsulfonylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3d). Yield, 90%; mp 266–268 °C; IR (KBr): 3365 (NH), 1312, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 14.15 (br s, 1H), 7.89 (d, J = 8.2 Hz, 2H), 7.65 (d, J = 8.2 Hz, 2H), 7.51 (d, J = 8.2 Hz, 2H), 7.25 (d, J = 8.2 Hz, 2H), 3.07 (s, 3H). Anal. Calcd for $C_{15}H_{12}BrN_3O_2S_2$: C, 43.91; H, 2.95; N, 10.24. Found: C, 43.80; H, 3.06; N, 10.36.

5.2.5. 4-(4-Methoxyphenyl)-5-(4-methylsulfonylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3e). Yield, 85%; mp 296–298 °C; IR (KBr): 3328 (NH), 1328, 1171 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 14.25 (br s, 1H), 7.83 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H), 7.22 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 3.87 (s, 3H), 3.04 (s, 3H). Anal. Calcd for $C_{16}H_{15}N_3O_3S_2$: C, 53.17; H, 4.18; N, 11.63. Found: C, 53.10; H, 4.29; N, 11.80.

5.2.6. 4-(4-Methylphenyl)-5-(4-methylsulfonylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3f). Yield, 90%; mp 291–292 °C; IR (KBr): 3393 (NH), 1316, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 14.05 (br s, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.26 (d, J = 8.2 Hz, 2H), 7.10 (d, J = 8.2 Hz, 2H), 3.05 (s, 3H), 2.44 (s, 3H). Anal. Calcd for $C_{16}H_{15}N_3O_2S_2$: C, 55.63; H, 4.38; N, 12.16. Found: C, 55.85; H, 4.25; N, 12.26.

5.2.7. 4-Cyclohexyl-5-(4-methylsulfonylphenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3g). Yield, 76%; mp 301–303 °C; IR (KBr): 3434 (NH), 1315, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 13.75 (br s, 1H), 8.09 (d, J = 8.3 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 4.25 (m, 1H), 3.15 (s, 3H), 2.55–1.25 (m, 10H). Anal. Calcd for $C_{15}H_{19}N_3O_2S_2$: C, 53.39; H, 5.67; N, 12.45. Found: C, 53.48; H, 5.82; N, 12.53.

5.3. General procedure for the preparation of 3-alkylthio-5-(4-methylsulfonylphenyl)-4-aryl (or cyclohexyl)-4*H*-1,2,4-triazoles (4a–n)

To a stirring solution of compounds**3a**–**g** (0.5 mmol) and sodium hydroxide (0.5 mmol) in ethanol (5 mL) was added alkyl iodide (0.5 mL) and the mixture was stirred overnight. The volatiles were evaporated. Flash chromatography (MeOH/$CHCl_3$, 1:20) and crystallization from *n*-butanol gave the title compounds **4a**–**n**.

5.3.1. 5-(4-Methylsulfonylphenyl)-3-methylthio-4-phenyl-4*H*-1,2,4-triazole (4a). Yield, 83%; mp 215–216 °C; IR (KBr): 1311, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 7.59–7.51 (m, 3H), 7.22–7.29 (m, 2H), 3.04 (s, 3H), 2.75 (s, 3H); MS *m*/*z* 345 (28), 330 (58), 266 (5), 251 (100), 218 (9), 192 (18), 164 (22), 135 (12), 108 (27). Anal. Calcd for $C_{16}H_{15}N_3O_2S_2$: C, 55.63; H, 4.38; N, 12.16. Found: C, 55.78; H, 4.49; N, 12.03.

5.3.2. 3-Ethylthio-5-(4-methylsulfonylphenyl)-4-phenyl-4*H*-1,2,4-triazole (4b). Yield, 86%; mp 175–176 °C; IR (KBr): 1302, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (d, $J = 8.8$ Hz, 2H), 7.62 (d, $J = 8.8$ Hz, 2H), 7.59–7.51 (m, 3H), 7.29–7.22 (m, 2H), 3.37 (q, $J = 7.2$ Hz, 2H), 3.04 (s, 3H), 1.45 (t, $J = 7.2$ Hz, 3H); MS *m/z* (%) 359 (68), 330 (100), 296 (4), 279 (4), 256 (7). Anal. Calcd for $C_{17}H_{17}N_3O_2S_2$: C, 56.80; H, 4.77; N, 11.69. Found: C, 56.63; H, 4.59; N, 11.60.

5.3.3. 4-(4-Fluorophenyl)-5-(4-methylsulfonylphenyl)-3-methylthio-4*H*-1,2,4-triazole (4c). Yield, 90%; mp 201–203 °C; IR (KBr): 1307, 1157 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.87 (d, $J = 8.4$ Hz, 2H), 7.62 (d, $J = 8.4$ Hz, 2H), 7.15–7.31 (m, 4H), 3.05 (s, 3H), 2.76 (s, 3H); MS *m/z* (%) 363 (85), 283 (20), 281 (35), 249 (15), 236 (45), 207 (100), 197 (42), 176 (23), 145 (30), 135 (58), 109 (78), 91 (16). Anal. Calcd for $C_{16}H_{14}FN_3O_2S_2$: C, 52.88; H, 3.88; N, 11.56. Found: C, 52.69; H, 3.76; N, 11.62.

5.3.4. 3-Ethylthio-4-(4-fluorophenyl)-5-(4-methylsulfonylphenyl)-4*H*-1,2,4-triazole (4d). Yield, 84%; mp 191–192 °C; IR (KBr): 1307, 1153 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.88 (d, $J = 8.4$ Hz, 2H), 7.63 (d, $J = 8.4$ Hz, 2H), 7.21–7.29 (m, 4H), 3.33 (q, $J = 7.2$ Hz, 2H), 3.05 (s, 3H), 1.44 (t, $J = 7.2$ Hz, 3H); MS *m/z* (%) 377 (60), 349 (58), 348 (100), 276 (5), 250 (18), 197 (5), 78 (12), 52 (34), 45 (35). Anal. Calcd for $C_{17}H_{16}FN_3O_2S_2$: C, 54.09; H, 4.27; N, 11.13. Found: C, 54.22; H, 4.18; N, 11.05.

5.3.5. 4-(4-Chlorophenyl)-5-(4-methylsulfonylphenyl)-3-methylthio-4*H*-1,2,4-triazole (4e). Yield, 77%; mp 131–132 °C; IR (KBr): 1305, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.88 (d, $J = 8.8$ Hz, 2H), 7.62 (d, $J = 8.8$ Hz, 2H), 7.52 (d, $J = 8.8$ Hz, 2H), 7.20 (d, $J = 8.8$ Hz, 2H), 3.05 (s, 3H), 2.76 (s, 3H); MS *m/z* (%) 379 (100), 365 (65), 346 (100), 317 (40), 292 (88), 236 (68), 213 (48), 208 (12), 151 (28), 125 (90), 75 (12), 63 (13). Anal. Calcd for $C_{16}H_{14}ClN_3O_2S_2$: C, 50.59; H, 3.71; N, 11.06. Found: C, 50.48; H, 3.58; N, 10.95.

5.3.6. 4-(4-Chlorophenyl)-3-ethylthio-5-(4-methylsulfonylphenyl)-4*H*-1,2,4-triazole (4f). Yield, 82%; mp 175–176 °C; IR (KBr): 1317, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.87 (d, $J = 8.8$ Hz, 2H), 7.62 (d, $J = 8.8$ Hz, 2H), 7.52 (d, $J = 8.8$ Hz, 2H), 7.19 (d, $J = 8.8$ Hz, 2H), 3.33 (q, $J = 7.2$ Hz, 2H), 3.05 (s, 3H), 1.46 (t, $J = 7.2$ Hz, 3H); MS *m/z* (%) 394 (55), 366 (33), 364 (100), 330 (10), 292 (8), 250 (13), 213 (12), 139 (9), 63 (15). Anal. Caclcd for $C_{17}H_{16}ClN_3O_2S_2$: C, 51.83; H, 4.09; N, 10.67. Found: C, 51.90; H, 4.01; N, 10.88.

5.3.7. 4-(4-Bromophenyl)-5-(4-methylsulfonylphenyl)-3-methylthio-4*H*-1,2,4-triazole (4g). Yield, 80%; mp 146–147 °C; IR (KBr): 1304, 1149 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.89 (d, $J = 8.8$ Hz, 2H), 7.68 (d, $J = 8.8$ Hz, 2H), 7.62 (d, $J = 8.8$ Hz, 2H), 7.13 (d, $J = 8.8$ Hz, 2H), 3.05 (s, 3H), 2.76 (s, 3H); MS *m/z* (%) 425 (100), 423 (100), 390 (55), 360 (30), 338 (50), 311 (42), 259, 257 (25), 236 (45), 197, 195 (27), 171, 169 (75), 91 (13), 76 (14), 63 (12). Anal. Calcd for $C_{16}H_{14}BrN_3O_2S_2$: C, 45.29; H, 3.33; N, 9.90. Found: C, 45.45; H, 3.52; N, 9.81.

5.3.8. 4-(4-Bromophenyl)-3-ethylthio-5-(4-methylsulfonylphenyl)-4*H*-1,2,4-triazole (4h). Yield, 83%; mp 172–174 °C; IR (KBr): 1308, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.89 (d, $J = 8.4$ Hz, 2H), 7.67 (d, $J = 8.8$ Hz, 2H), 7.62 (d, $J = 8.4$ Hz, 2H), 7.12 (d, $J = 8.8$ Hz, 2H), 3.33 (q, $J = 7.2$ Hz, 2H), 3.05 (s, 3H), 1.46 (t, $J = 7.2$ Hz, 3H); MS *m/z* (%) 439 (52), 437 (52), 412 (81), 410 (81), 330 (11), 328 (11), 257 (17), 255 (17), 250 (72), 198 (16), 196 (16), 185 (100), 183 (100), 171 (25), 157 (13), 104 (33), 90 (32), 78 (20). Anal. Calcd for $C_{17}H_{16}BrN_3O_2S_2$: C, 46.58; H, 3.68; N, 9.59. Found: C, 46.42; H, 3.49; N, 9.73.

5.3.9. 4-(4-Methoxyphenyl)-5-(4-methylsulfonylphenyl)-3-methylthio-4*H*-1,2,4-triazole (4i). Yield, 76%; mp 176–177 °C; IR (KBr): 1301, 1151 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.86 (d, $J = 8.8$ Hz, 2H), 7.65 (d, $J = 8.8$ Hz, 2H), 7.16 (d, $J = 8.8$ Hz, 2H), 7.02 (d, $J = 8.8$ Hz, 2H), 3.89 (s, 3H), 3.05 (s, 3H), 2.75 (s, 3H); MS *m/z* (%) 375 (30), 376 (100), 342 (10), 312 (15), 288 (17), 209 (27), 165 (12), 147 (37), 121 (12), 102 (15), 89 (16), 63 (36). Anal. Calcd for $C_{17}H_{17}N_3O_3S_2$: C, 54.38; H, 4.56; N, 11.19. Found: C, 54.52; H, 4.77; N, 11.31.

5.3.10. 3-Ethylthio-4-(4-methoxyphenyl)-5-(4-methylsulfonylphenyl)-4*H*-1,2,4-triazole (4j). Yield, 80%; mp 150–152 °C; IR (KBr): 1308, 1149 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.86 (d, $J = 8.4$ Hz, 2H), 7.65 (d, $J = 8.4$ Hz, 2H), 7.15 (d, $J = 8.8$ Hz, 2H), 7.01 (d, $J = 8.8$ Hz, 2H), 3.89 (s, 3H), 3.31 (q, $J = 7.6$ Hz, 2H), 3.05 (s, 3H), 1.45 (t, $J = 7.6$ Hz, 3H); MS *m/z* (%) 389 (40), 373 (56), 360 (59), 346 (50), 344 (100), 288 (5), 250 (12), 209 (3), 135 (6), 119 (5), 65 (3). Anal. Calcd for $C_{18}H_{19}N_3O_3S_2$: C, 55.51; H, 4.92; N, 10.79. Found: C, 55.66; H, 5.12; N, 10.86.

5.3.11. 4-(4-Methylphenyl)-5-(4-methylsulfonylphenyl)-3-methylthio-4*H*-1,2,4-triazole (4k). Yield, 82%; mp 109–110 °C; IR (KBr): 1295, 1149 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (d, $J = 8.4$ Hz, 2H), 7.64 (d, $J = 8.4$ Hz, 2H), 7.33 (d, $J = 8.4$ Hz, 2H), 7.12 (d, $J = 8.4$ Hz, 2H), 3.05 (s, 3H), 2.75 (s, 3H), 2.46 (s, 3H); MS *m/z* (%) 359 (100), 345 (19), 326 (45), 297 (10), 279 (16), 273 (14), 235 (8), 131 (11), 105 (15). Anal. Calcd for $C_{17}H_{17}N_3O_2S_2$: C, 56.80; H, 4.77; N, 11.69. Found: C, 56.92; H, 4.89; N, 11.56.

5.3.12. 3-Ethylthio-4-(4-methylphenyl)-5-(4-methylsulfonylphenyl)-4*H*-1,2,4-triazole (4l). Yield, 76%; mp 147–148 °C; IR (KBr): 1298, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (d, $J = 8.8$ Hz, 2H), 7.64 (d, $J = 8.8$ Hz, 2H), 7.32 (d, $J = 8.4$ Hz, 2H), 7.11 (d, $J = 8.4$ Hz, 2H), 3.31 (q, $J = 7.2$ Hz, 2H), 3.04 (s, 3H), 2.46 (s, 3H), 1.45 (t, $J = 7.2$ Hz, 3H); MS *m/z* (%) 373 (26), 358 (68), 344 (45), 330 (100), 313 (4), 250 (16), 213 (5), 139 (10), 63 (5). Anal. Calcd for $C_{18}H_{19}N_3O_2S_2$: C, 57.88; H, 5.13; N, 11.25. Found: C, 57.69; H, 5.03; N, 11.16.

5.3.13. 4-Cyclohexyl-5-(4-methylsulfonylphenyl)-3-methylthio-4*H*-1,2,4-triazole (4m). Yield, 74%; mp 197–199 °C; IR (KBr) 1309, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.09 (d, $J = 8.4$ Hz, 2H), 7.72 (d, $J = 8.4$ Hz,

2H), 3.98 (m, 1H), 3.13 (s, 3H), 2.82 (s, 3H), 2.26–2.10 (m, 3H), 1.96–1.70 (m, 5H), 1.74–1.64 (m, 2H); MS *m/z* (%) 351(37), 336 (3), 269 (100), 242 (15), 223 (12), 207 (4), 190 (13), 182 (13), 161 (15), 83 (20), 65 (16). Anal. Calcd for $C_{16}H_{21}N_3O_2S_2$: C, 54.67; H, 6.02; N, 11.95. Found: C, 54.52; H, 6.18; N, 11.82.

5.3.14. 4-Cyclohexyl-3-ethylthio-5-(4-methylsulfonylphenyl)-4*H*-1,2,4-triazole (4n). Yield, 70%; mp 221–223 °C; IR (KBr): 1311, 1159 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.09 (d, $J = 8.4$ Hz, 2H), 7.73 (d, $J = 8.4$ Hz, 2H), 3.98 (m, 1H), 3.41 (q, $J = 7.6$ Hz, 2H), 3.13 (s, 3H), 2.23–2.15 (m, 7H), 1.49 (t, $J = 7.6$ Hz, 3H); MS *m/z* (%) 366 (M+1, 17), 365 (33), 336 (16), 284 (82), 283 (100), 256 (93), 255 (78), 251 (63), 203 (32), 182 (34),118 (14), 102 (17), 81 (15). Anal. Calcd for $C_{17}H_{23}N_3O_2S_2$: C, 55.86; H, 6.34; N, 11.50. Found: C, 55.69; H, 6.47; N, 11.66.

5.4. General procedure for the preparation of *N*-(4-methylsulfonylphenyl)-4-substituted benzamides (7a–c)

To a solution of 4-methylsulfonylaniline **5** (300 mg, 1.75 mmol) in THF (20 mL) was added dropwise 4-substituted benzoyl chloride **6a–c** (1.78 mmol) dissolved in THF (5 mL) under N_2 at 0 °C followed by addition of triethylamine (0.25 mL, 1.8 mmol). The reaction mixture was stirred overnight at 24 °C, filtered, and the precipitate was washed with THF. The filtrate was concentrated and crystallized from methanol to give the title compounds **7a–c**.

5.4.1. *N*-(4-Methylsulfonylphenyl)benzamide (7a). Compound **7a** was reported previously.[20]

5.4.2. *N*-(4-Methylsulfonylphenyl)-4-fluorobenzamide (7b). Yield, 93%; mp 218–219 °C; IR (KBr): 3375 (NH), 1680 (CO), 1291, 1143 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.18 (s, 1H), 8.08–7.90 (m, 4H), 7.90 (d, $J = 8.8$ Hz, 2H), 7.18 (t, $J = 8.4$ Hz, 2H), 3.06 (s, 3H). Anal. Calcd for $C_{14}H_{12}FNO_3S$: C, 57.33; H, 4.12; N, 4.78. Found: C, 57.50; H, 4.29; N, 4.90.

5.4.3. *N*-(4-Methylsulfonylphenyl)-4-methylbenzamide (7c). Yield, 96%; mp 258–259 °C; IR (KBr): 3365 (NH), 1674 (CO), 1294, 1142 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.81 (s, 1H), 7.96 (d, $J = 8.8$ Hz, 2H), 7.90 (d, $J = 8.8$ Hz, 2H), 7.79 (d, $J = 8.4$ Hz, 2H), 7.32 (d, $J = 8.4$ Hz, 2H), 3.06 (s, 3H), 2.45 (s, 3H). Anal. Calcd for $C_{15}H_{15}NO_3S$: C, 62.26; H, 5.23; N, 4.84. Found: C, 62.36; H, 5.36; N, 4.96.

5.5. General procedure for the preparation of *N*-(4-methylsulfonylphenyl)arylcarbohydrazonamides (8a–c)

N-(4-Methylsulfonylphenyl)-4-substituted benzamides **7a–c** (1.8 mmol) were dissolved in benzene (10 mL) under N_2, and phosphorus pentachloride (416 mg, 2 mmol) was added. The solution was heated at reflux for 3 h. It was concentrated to remove $POCl_3$, the residue was taken up in THF (15 mL) and added dropwise into a stirred THF (10 mL) solution of anhydrous hydrazine (0.6 mL) at 0 °C under N_2. The reaction mixture was stirred for 1 h at room temperature, poured into water (20 mL), and extracted with ethyl acetate. The organic phase was washed with brine, dried (Na_2SO_4) and evaporated. Crystallization of the residue gave the title compounds **8a–c**.

5.5.1. *N*-(4-Methylsulfonylphenyl)phenylcarbohydrazonamide (8a). Compound **8a** was reported previously.[20]

5.5.2. 4-Fluorophenyl-*N*-(4-methylsulfonylphenyl)carbohydrazonamide (8b). Yield, 83%; mp 199–201 °C; IR (KBr): 3416, 3309 (NH, NH_2), 1588 (C=N), 1286, 1132 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.78–7.35 (m, 4H), 7.05 (t, $J = 8.36$ Hz, 2H), 6.75 (d, $J = 8.6$ Hz, 2H), 5.95 (br s, 2H), 5.40 (br s, 1H), 2.99 (s, 3H). Anal. Calcd for $C_{14}H_{14}FN_3O_2S$: C, 54.71; H, 4.59; N, 13.67. Found: C, 54.88; H, 4.75; N, 13.49.

5.5.3. 4-Methylphenyl-*N*-(4-methylsulfonylphenyl)carbohydrazonamide (8c). Yield, 80%; mp 211–213 °C; IR (KBr): 3489 (NH), 3380, 3299 (NH_2), 1555 (C=N), 1298, 1132 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65 (d, $J = 8.6$ Hz, 2H), 7.40 (d, $J = 8.4$ Hz, 2H), 7.18 (d, $J = 8.4$ Hz, 2H), 6.75 (d, $J = 8.6$ Hz, 2H), 6.10 (br s, 2H), 5.52 (br s, 1H), 2.99 (s, 3H), 2.38 (s, 3H). Anal. Calcd for $C_{15}H_{17}N_3O_2S$: C, 59.38; H, 5.65; N, 13.85. Found: C, 59.56; H, 5.81; N, 13.66.

5.6. General procedure for the preparation of 4-(4-methylsulfonylphenyl-5-aryl-2,4-dihydro-3*H*-1,2,4-triazol-3-thiones (9a–c)

N-(4-Methylsulfonylphenyl)-4-substituted benzenecarbohydrazonamides **8a–c** (1.73 mmol) were dissolved in THF (100 mL) under N_2, and 1,1′-thiocarbonyldiimidazole (340 mg, 2 mmol) was added. The solution was stirred overnight at room temperature. The solvent was removed under reduced pressure. The residue was taken up in ethyl acetate and washed with 0.1 N HCl solution, water, and brine prior to drying (Na_2SO_4). The solvent was evaporated and the residue was crystallized from methanol to give the title compounds **9a–c**.

5.6.1. 4-(4-Methylsulfonylphenyl)-5-phenyl-2,4-dihydro-3*H*-1,2,4-triazol-3-thione (9a). Compound **9a** was reported previously.[20]

5.6.2. 5-(4-Fluorophenyl)-4-(4-methylsulfonylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-thione (9b). Yield, 48%; mp 227–229 °C; IR (KBr): 3396 (NH), 1367, 1114 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 13.90 (s, 1H), 8.07 (d, $J = 7.8$ Hz, 2H), 7.52–7.18 (m, 6H), 3.14 (s, 3H). Anal. Calcd for $C_{15}H_{12}FN_3O_2S_2$: C, 51.56; H, 3.46; N, 12.03. Found: C, 51.65; H, 3.59; N, 11.89.

5.6.3. 5-(4-Methylphenyl)-4-(4-methylsulfonylphenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-thione (9c). Yield, 55%; mp 239–241 °C; IR (KBr): 3412 (NH), 1319, 1149 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 13.68 (s, 1H), 8.02 (d, $J = 8$ Hz, 2H), 7.55 (d, $J = 8$ Hz, 2H), 7.28 (d, $J = 8.4$ Hz, 2H), 7.10 (d, $J = 8.4$ Hz, 2H), 3.13 (s, 3H), 2.44 (s, 3H). Anal. Calcd for $C_{16}H_{15}N_3O_2S_2$: C, 55.63; H, 4.38; N, 12.16. Found: C, 55.81; H, 4.45; N, 12.02.

5.7. General procedure for the preparation of 3-alkylthio-4-(4-methylsulfonylphenyl)-5-phenyl-4*H*-1,2,4-triazoles (10a–f)

To a stirring solution of compounds**9a**–**c** (1 mmol) and sodium hydroxide (1 mmol) in ethanol (5 mL) was added alkyl iodide (0.5 mL) and the mixture was stirred overnight. The volatiles were evaporated. Flash chromatography (MeOH/$CHCl_3$, 1:20) gave the title compounds **10a**–**f**.

5.7.1. 4-(4-Methylsulfonylphenyl)-3-methylthio-5-phenyl-4*H*-1,2,4-triazole (10a). Compound **10a** was reported previously.[20]

5.7.2. 3-Ethylthio-4-(4-methylsulfonylphenyl)-5-phenyl-4*H*-1,2,4-triazole (10b). Yield, 82%; mp 173–175 °C; IR (KBr): 1314, 1155 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.05 (d, $J = 8.4$ Hz, 2H), 7.43 (d, $J = 8.4$ Hz, 2H), 7.33 (s, 5H), 3.32 (q, $J = 7.2$ Hz, 2H), 3.12 (s, 3H), 1.49 (t, $J = 7.2$ Hz, 3H); MS *m*/*z* (%) 359 (78), 331 (100), 326 (10), 258 (22), 251 (14), 183 (32), 177 (65), 172 (73), 111 (9), 103 (10). Anal. Calcd for $C_{17}H_{17}N_3O_2S_2$: C, 56.80; H, 4.77; N, 11.69. Found: C, 56.92; H, 4.95; N, 11.56.

5.7.3. 5-(4-Fluorophenyl)-4-(4-methylsulfonylphenyl)-3-methylthio-4*H*-1,2,4-triazole (10c). Yield, 85%; mp 212–214 °C; IR (KBr): 1322, 1156 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.08 (d, $J = 8$ Hz, 2H), 7.42 (d, $J = 8$ Hz, 2H), 7.32 (dd, $J = 8.4$, 5.6 Hz, 2H), 7.01 (t, $J = 8.4$ Hz, 2H), 3.13 (s, 3H), 2.75 (s, 3H); MS *m*/*z* (%) 363 (100), 330 (10), 276 (12), 251 (12), 197 (22), 176 (47), 152 (15), 122 (17), 107 (92), 91 (25), 76 (27), 63 (20). Anal. Calcd for $C_{16}H_{14}FN_3O_2S_2$: C, 52.88; H, 3.88; N, 11.56. Found: C, 52.78; H, 3.72; N, 11.44.

5.7.4. 3-Ethylthio-5-(4-fluorophenyl)-4-(4-methylsulfonylphenyl)-4*H*-1,2,4-triazole (10d). Yield, 87%; mp 168–170 °C; IR (KBr): 1314, 1156 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.08 (d, $J = 8$ Hz, 2H), 7.44 (d, $J = 8$ Hz, 2H), 7.34 (dd, $J = 8.4$, 5.6 Hz, 2H), 7.02 (t, $J = 8.4$ Hz, 2H), 3.34 (q, $J = 7.2$ Hz, 2H), 3.15 (s, 3H), 1.46 (t, $J = 7.2$ Hz, 3H); MS *m*/*z* (%) 377 (64), 350 (80), 348 (100), 304 (23), 302 (95), 184 (22), 270 (12), 245 (17), 229 (20), 211 (45), 190 (92), 149 (62), 121 (37), 91 (57), 79 (38), 57 (45). Anal. Calcd for $C_{17}H_{16}FN_3O_2S_2$: C, 54.09; H, 4.27; N, 11.13. Found: C, 54.22; H, 4.10; N, 11.02.

5.7.5. 5-(4-Methylphenyl)-4-(4-methylsulfonylphenyl)-3-methylthio-4*H*-1,2,4-triazole (10e). Yield, 80%; mp 218–220 °C; IR (KBr): 1308, 1154 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.06 (d, $J = 8.8$ Hz, 2H), 7.44 (d, $J = 8.8$ Hz, 2H), 7.23 (d, $J = 8.4$ Hz, 2H), 7.11 (d, $J = 8.4$ Hz, 2H), 3.14 (s, 3H), 2.76 (s, 3H); MS *m*/*z* (%) 360 (66), 359 (100), 326 (28), 280 (17), 271 (26), 247 (35), 204 (24), 193 (39), 172 (64), 162 (37), 118 (35), 107 (69), 90 (43), 76 (31). Anal. Calcd for $C_{17}H_{17}N_3O_2S_2$: C, 56.80; H, 4.77; N, 11.69. Found: C, 56.69; H, 4.66; N, 11.55.

5.7.6. 3-Ethylthio-5-(4-methylphenyl)-4-(4-methylsulfonylphenyl)-4*H*-1,2,4-triazole (10f). Yield, 86%; mp 167–169 °C; IR (KBr): 1308, 1114 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.06 (d, $J = 8.8$ Hz, 2H), 7.43 (d, $J = 8.8$ Hz, 2H), 7.22 (d, $J = 8.4$ Hz, 2H), 7.11 (d, $J = 8.4$ Hz, 2H), 3.32 (q, $J = 7.2$ Hz, 2H), 3.14 (s, 3H), 1.45 (t, $J = 7.2$ Hz, 3H); MS *m*/*z* (%) 374 (26), 373 (94), 346 (92), 344 (100), 272 (22), 265 (15), 193 (58), 191 (90), 186 (92), 163 (19), 149 (17), 134 (18), 121 (37), 91 (73), 76 (48). Anal. Calcd for $C_{18}H_{19}N_3O_2S_2$: C, 57.88; H, 5.13; N, 11.25. Found: C, 57.69; H, 5.02; N, 11.15.

5.8. In vitro cyclooxygenase (COX) inhibition assay

The ability of the test compounds listed in Tables 1–3 to inhibit ovine COX-1 and COX-2 was determined using a colorimetric COX (ovine) inhibitor screening assay which utilizes the peroxidase component of cyclooxygenase. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm.[23–25]

5.9. Anti-inflammatory assay

The test compounds were evaluated using in vivo rat carrageenan-induced foot paw edema model reported previously.[27]

5.10. Molecular modeling (docking) studies

Docking studies were performed using MOE software version 2003.02 (CCG Inc.).[26] The coordinates of the X-ray crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme were obtained from the RCSB Protein Data Bank (1cx2). The ligand molecules were constructed using the Builder module and were energy optimized. The purpose of docking is to search for favorable binding configuration between the small flexible ligands and the rigid protein. Protein residues with atoms greater than 7.5 Å from the docking box were removed for efficiency. Searching is conducted within a specified 3D docking box using simulated annealing based on the Monte Carlo method and MMFF94 molecular mechanics force fields for 8000 iterations.

Acknowledgments

This work was supported by grants from the research council of Tehran University of Medical Sciences and Iranian National Science Foundation. We also thank Dr. H. Shadnia for helpful discussions.

References and notes

1. Penning, T. D.; Tally, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.
2. Riendeau, D.; Percival, M. D.; Brideau, C.; Dube, C. S.; Ethier, D.; Falgueyret, J. P.; Friesen, R. W.; Gordon, R.;

Greig, G.; Guay, J.; Girard, Y.; Prasit, P.; Zamboni, R.; Rodger, I. W.; Gresser, M.; Ford-Hutchinson, A. W.; Young, R. N.; Chan, C. C. *J. Pharmacol. Exp. Ther.* **2002**, *296*, 558.
3. Ranatunge, R. R.; Earl, R. A.; Garvey, D. S.; Janero, D. R.; Letts, L. G.; Martino, A. M.; Murty, M. G.; Richardson, S. K.; Schwalb, D. J.; Young, D. V.; Zemtseva, I. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6049.
4. Meade, E. A.; Smith, W. L.; DeWitt, D. L. *J. Biol. Chem.* **1993**, *268*, 6610.
5. Wolfe, M. M.; Lichtenstein, D. R.; Singh, G. N. *Engl. J. Med.* **1999**, *340*, 1888.
6. Catella-Lawson, F.; Crofford, L. J. *Am. J. Med.* **2001**, *110*, 28S.
7. Mukherjee, D.; Nissen, S. E.; Topol, E. J. *JAMA* **2001**, *286*, 954.
8. de Gaetano, G.; Donati, M. B.; Cerletti, C. *Trends Pharm. Sci.* **2003**, *24*, 245.
9. Friesen, R. W.; Dube, D.; Fortin, R.; Frenette, R.; Prescott, S.; Cromlish, W.; Greig, G. M.; Kargman, S.; Wong, E.; Chan, C. C.; Gordon, R.; Xu, L. *J. Bioorg. Med. Chem. Lett.* **1996**, *6*, 2677.
10. Prasit, P.; Wang, Z.; Brideau, C.; Chan, C.-C.; Charlson, S.; Cromlish, W.; Ethier, D.; Evans, J. F.; Ford-Hutchinson, A. W.; Gauthier, J. Y.; Gordon, R.; Guay, J.; Gresser, M.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Leger, S.; Mancini, J.; O'Neil, G. P.; Quellet, M.; Percival, M. D.; Perrier, H.; Riendeau, D.; Rodger, I.; Tagari, P.; Therien, M.; Vikers, P.; Wong, E.; Xu, L.-J.; Young, R. N.; Zamboni, R.; Boyce, S.; Rupniak, N.; Forrest, M.; Visco, D.; Patrick, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1773.
11. Talley, J. J.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. *J. Med. Chem.* **2000**, *43*, 775.
12. Talley, J. J. *Prog. Mad. Chem.* **1999**, *36*, 201.
13. Praveen Rao, P. N.; Amini, M.; Li, H.; Habeeb, A. G.; Knaus, E. E. *J. Med. Chem.* **2003**, *46*, 4872.
14. Tozkoparan, B.; Kupeli, E.; Yesilada, E.; Isik, S.; Ozalp, M.; Ertan, M. *Arzneimittelforschung* **2005**, *55*, 533.
15. Labanauskas, L.; Udrenaite, E.; Gaidelis, P.; Brukstus, A. *Farmaco* **2004**, *59*, 255.
16. Labanauskas, L.; Kalcas, V.; Udrenaite, E.; Gaidelis, P.; Brukstus, A.; Dauksas, U. *Pharmazie* **2001**, *56*, 617.
17. Sahin, G.; Palaska, E.; Kelican, P.; Demirdamar, R.; Altinok, G. *Arzneimittelforschung* **2001**, *51*, 478.
18. Achenone, S.; Bruno, O.; Ranise, A.; Bondavalli, F.; Filippelli, W.; Rossi, F.; Falcone, G. *Farmaco* **1998**, *53*, 590.
19. Shafiee, A.; Naimi, E.; Mansobi, P.; Foroumadi, A.; Shekari, M. *J. Heterocycl. Chem.* **1995**, *32*, 1235.
20. Navidpour, L.; Karimi, L.; Amini, M.; Vosooghi, M.; Shafiee, A. *J. Heterocycl. Chem.* **2004**, *41*, 2014.
21. Romine, L.; Martin, S. W.; Gribkoff, V. K.; Boissard, C. G.; Dworetzky, S. I.; Natale, J.; Li, J.; Li, Y.; Meanwell, N. A.; Starrett, J. E. *J. Med. Chem.* **2002**, *45*, 2942.
22. Stanovnik, B.; Mirtic, T.; Koren, B.; Tisler, M.; Belcic, B. *Vestnik Slovenskega Kemijskega Drustva* **1982**, *29*, 331.
23. Kulmacz, R. J.; Lands, W. E. M. *Prostaglandins* **1983**, *25*, 531.
24. Kulmacz, R. J. *Prostoglandins* **1987**, *34*, 225.
25. Rouzer, C. A.; Marnett, L. J. *Chem. Rev.* **2003**, *103*, 2239.
26. MOE: Molecular Operating Environment, Version 2003.02. In Bajorath, J., Crippen, G., Martin, E., Ripka, W., Spellmeyer, D., Eds.; The Chemical Computing Group, Montreal, Quebec, 2003.
27. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.