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ORIGINAL ARTICLE

Design, synthesis, molecular modeling and biological evaluation of novel diaryl heterocyclic analogs as potential selective cyclooxygenase-2 (COX-2) inhibitors

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KEYWORDS

Selective COX-2 inhibitors;
Docking;
Synthesis;
Anti-inflammatory

Abstract New series of 3,4-diaryl-2-thioxoimidazolidin-4-ones and 3-alkylthio-4,5-diaryl-4*H*-1,2,4-triazoles were designed, synthesized and evaluated for their activity as anti-inflammatory agents. Compounds **20**, **21**, **23** and **34** are highly selective inhibitors of COX-2 enzyme at a concentration of 100 mM relative to celecoxib, the standard reference. (±)-3-(4-Phenoxy-phenyl)-5-phenyl-2-thioxoimidazolidin-4-ones **23** exhibited the most active anti-inflammatory agent.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) constitute one of the most widely used classes of drugs. NSAIDs **1**–**11** are commonly used as anti-inflammatory, analgesic, antirheumatic and antipyretic agents (Rodriguez et al., 1998); (Chart 1). The common mechanism of action for NSAIDs is inhibition of the synthesis of PGs by inhibiting the key regulatory cyclooxygenase "COX" enzyme. In 1989, it was determined that there were at least 2 isoforms of cyclooxygenase: COX-1, or prostaglandin H_1 synthase, and COX-2, prostaglandin H_2 synthase. COX-1 is expressed in most tissues, regulates physiological processes such as gastric cytoprotection, kidney function, and platelet aggregation, and is stimulated by growth factors and hormones. It has been called the "house keeping" enzyme (Sperling, 1995; Kulkarni and Jain, 2005; Singh, 1998; Graumlich, 2001; Lee, 2011; Noble et al., 2000).

However, the unfavorable side effect of NSAID drugs mainly is the whole GI tract damage including a wide spectrum

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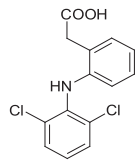
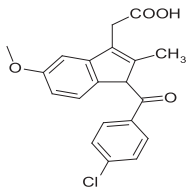
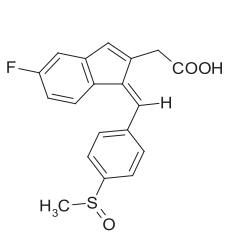


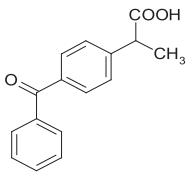
Diclofenac (**1**)



Indomethacin (**2**)



Sulindac(**3**)



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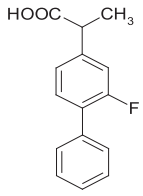

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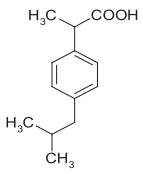
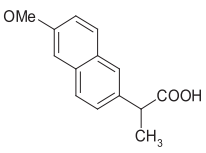


Ibuprofen (**6**)



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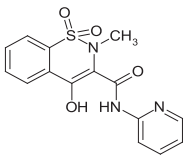
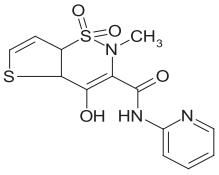
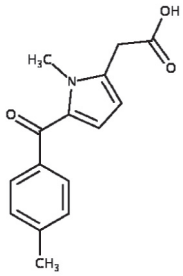


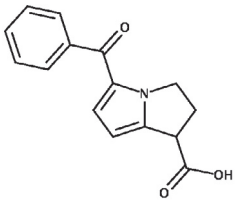
piroxicam (**8**)



Tenoxicam (**9**)



Tolmetin (**10**)



Ketorolac (**11**)

Chart 1 Classic NSAIDs.

of lesions. About 1–2% of NSAID users experienced a serious GI complication during treatment due to the inhibition of COX-1 (Griffin et al., 1991; Hollander, 1994; Laine, 1996; Scheiman, 1996).

A new generation of selective cyclooxygenase-2 (COX-2) inhibitors allowed the desired synthesis of cytoprotective prostaglandins, in conjunction with a simultaneous inhibition of pro-inflammatory prostaglandin synthesis, thereby reducing dyspepsia and ulceration. COX-2 selective inhibitors are also known to suppress synthesis of prostacyclin, a potent vasodilator and gastro-protective. They are validated as anti-inflammatory therapeutics for the treatment of rheumatoid arthritis with less gastrointestinal and renal toxicity (Crofford et al., 2000; Gauthier et al., 2006; Navidpour et al., 2006; Chrischilles and Wallace, 1993; DuBois et al., 1988).

The pharmacophore structural features of the selective COX-2 inhibitors are possessing a central heterocyclic five membered ring system bearing two vicinal aryl moieties, such as pyrazole (celcoxib, **12**), 2(5*H*)furanone (refecoxib, **13**), and isoxazole (valdecoxib, **14**) (Chart 2) (Penning et al., 1997; Li et al., 1999; Talley et al., 2000a,b; Li et al., 2003). Also, the substituted sulfonyl group is considered one of the pharmacophoric moieties responsible for the selective recognition of the key amino acid residues at COX-2 active site pocket.

On the basis of these considerations, and in view of the reported COX-2 inhibitory activity of certain 2-thioimidazolidin-4-one derivatives (Gauthier et al., 2006), a series of (±)-2-thioxoimidazolidin-4-ones of the general structure (**A**) bearing two aryl moieties at 3- and 5-positions of the imidazolidine ring and carrying different substituents on the 3-aryl residue was synthesized. COX-2 inhibitory potency of several 1,2,4-triazole derivatives was evaluated (Maxwell et al., 1984; Gosowami et al., 1984; Amir and Shikla, 2004). Accordingly, certain 4,5-diaryl-4*H*-1,2,4-triazoles of the general structure (**B**) and (**C**) possessing C_3-thio and alkylthio substituents and carrying a methylsulfonyl moiety in the 4-position of one aryl moiety were synthesized. In addition, and in order to enhance COX-2 selectivity, the benzylthio derivatives (**D**) were synthesized. The proposed compounds have a characteristic molecular pattern and bulk volume to fulfill the pharmacophoric requirements for better recognition at the COX-2 binding active site. The newly synthesized analogs were evaluated for their COX selectivity and their anti-inflammatory activity.

2. Chemistry

To spotlight on the significance of the functional groups that will be used as a key to identify the difference in recognition in both COX-1 and COX-2 active sites and to achieve proper selective COX-2 inhibitors, Schemes 1 and 2 were used to prepare different series of (±)-3-(Substituted phenyl)-5-phenyl-2-thioxo-imidazolidin-4-ones **20**–**23**, 1-(4-methylsulfonyl phenyl)-4-substituted phenyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **29**–**32**, and 3-(4-methyl or chloro-benzylthio)-4-

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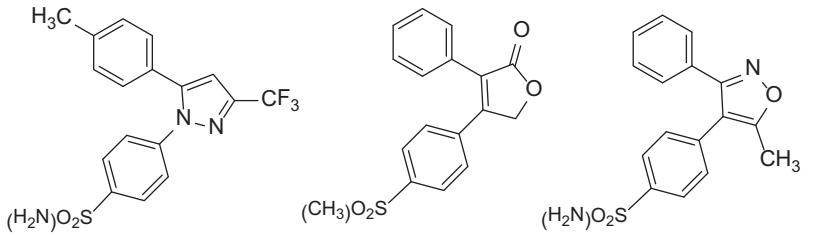
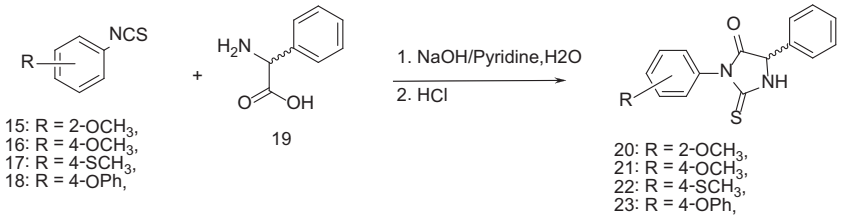
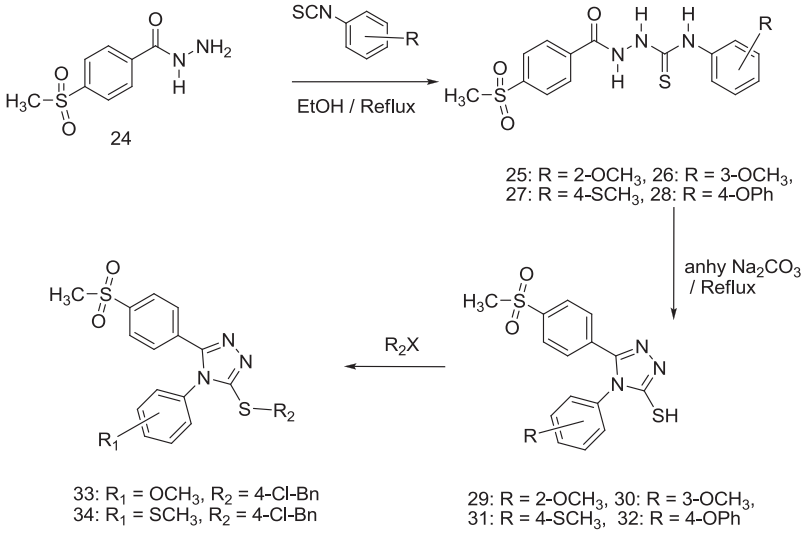


Chart 2 Representative examples of selective COX-2 inhibitors.



Scheme 1



Scheme 2

substituted phenyl-5-(4-methylsulfonyl)-4*H*-1,2,4-triazoles **33** and **34**.

(±)-3-(Substituted phenyl)-5-phenyl-2-thioxoimidazolidin-4-ones **20**–**23** were prepared following the reported procedures (Gauthier et al., 2006). The isothiocyanate derivatives **15**–**18** were reacted with a solution of (±)-α-phenylglycine **19** in 1:1 water/pyridine mixture at alkaline pH, followed by acidification with 1N hydrochloric acid. The reaction yielded the desired final compounds **20**–**23**, Table 1. The crude products were purified by applying preparative thin layer chromatography using $CHCl_3$:CH_3OH (7:3, v/v).

In the present work methyl 4-methyl sulfonyl benzoate ester was easily prepared following the classical esterification method (El-Emam and Ibrahim, 1991), by heating 4-methyl sulfonyl benzoic acid with pure methanol in the presence of sulfuric acid as a dehydrating agent to yield the desired ester in 98% yield. The target hydrazide **24** was prepared in high yield by heating the methyl ester with hydrazine hydrate in ethanol (El-Emam and Ibrahim, 1991). The acid hydrazide **24** was allowed to react with the appropriate isothiocyanate in ethanol to obtain the corresponding thiosemicarbazides **25**–**28**. Compounds **25**–**28** were refluxed in saturated aqueous sodium carbonate solution to afford the corresponding target compounds **29**–**32**.

Alkylation of 5-(4-methylsulfonyl phenyl)-4-substituted phenyl-2,4-dihydro-3*H*-1,2,4-triazol-3-thiones **30** and **31** was achieved through utilizing anhydrous potassium carbonate in ethanol, followed by treatment with methyl iodide or *p*-chlorobenzyl chloride to afford the corresponding alkylated compounds **33** and **34**.

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Table 1 Percentage inhibitory activity against COX-1, COX-2 and Percentage increase in right paw weight carrageenan-induced edema of the tested compounds.

Compd	R or R_1	R_2	% Inhibition		% Increase in right paw weight
			COX-1	COX-2	
Celecoxib	–	–	–	80.6	66.2 ± 12.6
20	2-OCH_3	–	0	49.3	Toxic
21	4-OCH_3	–	0	62.9	37.0 ± 5.5*
22	4-SCH_3	–	0	28.8	Toxic
23	4-OPh	–	0	59.6	16.6 ± 4.3**
29	2-OCH_3	–	7.0	3.9	74.3 ± 5.7
30	3-OCH_3	–	0	0	71.0 ± 4.2
31	4-SCH_3	–	0	0	60.0 ± 13.8
32	4-Oph	–	0	0	68.8 ± 11.7
33	3-OCH_3	4-Cl-Bn	0	0	50.8 ± 14.9
34	4-SCH_3	4-Cl-Bn	0	60.1	68.6 ± 6.9

For COX-1, COX-2 inhibition, all compounds were tested at a concentration of 100 μM, except the standard (celecoxib) which was used at 50.0 μM.
For carrageenan-induced paw edema data are expressed as mean ± standard error of mean (SEM), where ($n = 4$) represents the number of animals.
* $p < 0.05$ compared with control group using one-way ANOVA followed by Dunnett's post-hoc test.
** $p < 0.01$ compared with control group using one-way ANOVA followed by Dunnett's post-hoc test.

3. Results and discussion

3.1. In vitro COXs inhibitory effect and selectivity of tested compounds

The effect of the tested compounds on COX-1 and COX-2 activity was carried out using a commercially available COX inhibitor screening enzyme immuno-assay (EIA) kit Pradelles et al., 1985. The results of this assay (Table 1) showed that compounds **20**, **21**, **23** and **34** are highly selective inhibitors of COX-2 enzyme. Although these compounds were tested at a high concentration (100 μM), no inhibition of COX-1 activity was detected, supporting the conclusion that these compounds are highly selective against COX-2. Conversely, other compounds were inactive against either COX-1 or COX-2.

3.2. In vivo anti-inflammatory effect of tested compounds

Subplantar injection of carrageenan in the rat paw elicited an inflammatory response that was characterized by an increase in the right paw weight (Winter et al., 1962; Mielens et al., 1968). Pre-treatment of rats with compound **23** resulted in a significant decrease in the carrageenan-induced paw edema. The % increase in the right paw edema was significantly lower than that of control group (Table 1). The obtained results suggest that compound **23** is active anti-inflammatory *in vivo*. Conversely, pre-treatment of rats with other compounds did not affect the carrageenan-induced paw edema significantly. Moreover, compounds **20** and **22** were toxic to the animals at the tested dose (150 mg kg^{-1}) showing symptoms of CNS stimulation.

Considering the results of both *in vitro* and *in vivo* experiments revealed that, compound **23** is a highly selective COX-2 inhibitor with active anti-inflammatory effect (see Fig. 1).

3.3. Molecular modeling study

The binary complex of the cyclooxygenase-2 enzyme (1CX2) coupled with the selective COX-2 inhibitor; **SC-558** was used as a reference to modeling and docking study (Fig. 2). Studying the hydrogen bonding interaction of the pyrazole hetero-ring of **SC558** with the 1CX2 active site revealed that the N_1 of the pyrazole ring contributed preferable hydrogen bonds with the key pocket residue Tyr355. The sulfonyl oxygen and the terminal amino group conferred three H-bonds with the 'catalytic triad' residues of 1CX2 pocket Phe518, His90 and Arg513, respectively.

Comparative computational study was performed to the designed compounds **20**–**23** and **29**–**34** to examine their degree of selective recognition at the binding active site with the conserved amino acids of both COX-1 and COX-2 binding pockets. Compound **20** with the 2-methoxy substituted group showed hydrogen binding recognition with Leu352, which is considered one of the common shared conserved residues in both COX-1 and COX-2 binding pockets. However, compound **20** showed high degree of recognition with the key amino acid residues of COX-2 pocket namely Tyr355, Val523 and Ala527 and that is in agreement with the *in vitro* binding data (Fig. 3).

Comparative binding study of compound **23** indicated that the 4-phenoxy substitution forced the stabilization at W-shaped conformation that allows the terminal phenoxy group to be directed toward wide edge of the hydrophobic binding cavity. This conformational organization enhances the overall interactive recognition with the key amino acid residues of COX-2, and as a result imidazole ring was hanged with three stable hydrogen bonds with Ala527, Leu352 and Val523, the key residues present mainly in COX-2 binding pocket. The three phenyl rings of the **23** were stabilized within the lipophilic cavity where the *van der wall* interaction and the hydrophobic

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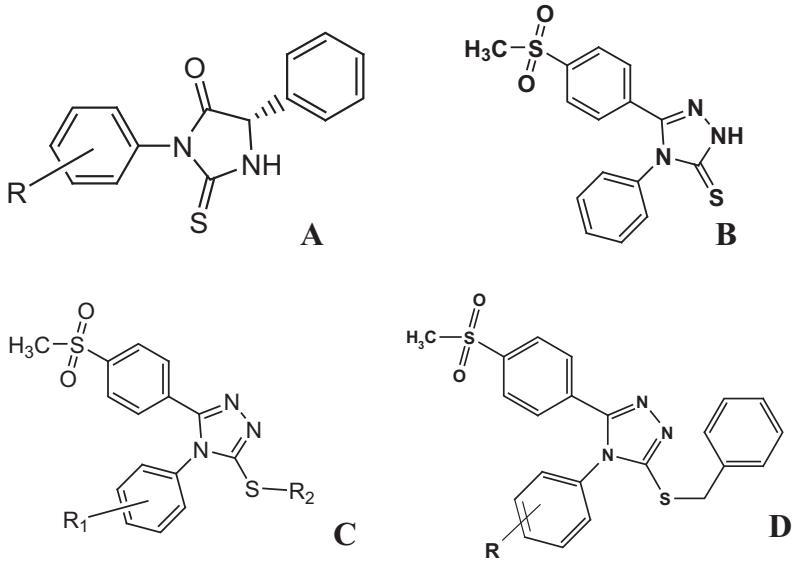

Figure 1 Newly synthesized tricyclic analogs.

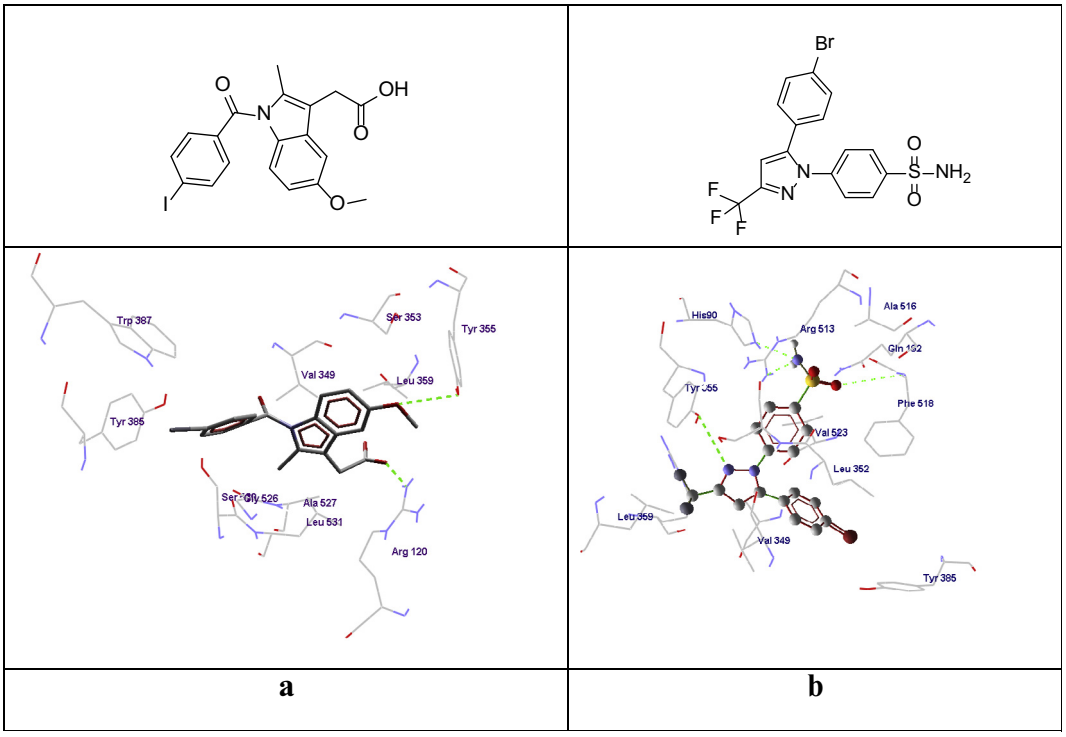

Figure 2 (a) Crystal structure of the non-selective COX-1 inhibitor 1MM (1PGF) showing the putative hydrogen bonding at the binding active site. (b) Crystal structure of the selective COX-2 inhibitor showing the putative hydrogen bonding at the 1CX2 active site. With its docked ligand; SC-558.

interaction were established due to the presence of Tyr348, Tyr385 and Tyr355. The phenoxy oxygen performed electrostatic interaction with the amino acid Ser353, the one of the conserved residues at the selective binding pocket (Fig. 4). Compound **23** showed proper recognition that goes properly with its biological effect in both *in vitro* and *in vivo* screenings.

The triazole analogs including compounds **29**–**34** showed no selectivity toward COX-1. This group of compounds is characterized by the presence of terminal sulfonyl moiety that was considered crucial in the compound's recognition with three conserved amino acid residues namely His90, Arg513 and Phe518. Modeling study of the binding mode of compound **29** indicated that, methyl-sulfonyl function performed conformational recognition with Ile517, Gln192, His90, while the terminal 2-methoxy group accomplished the binding with Ser530 (Fig. 4).

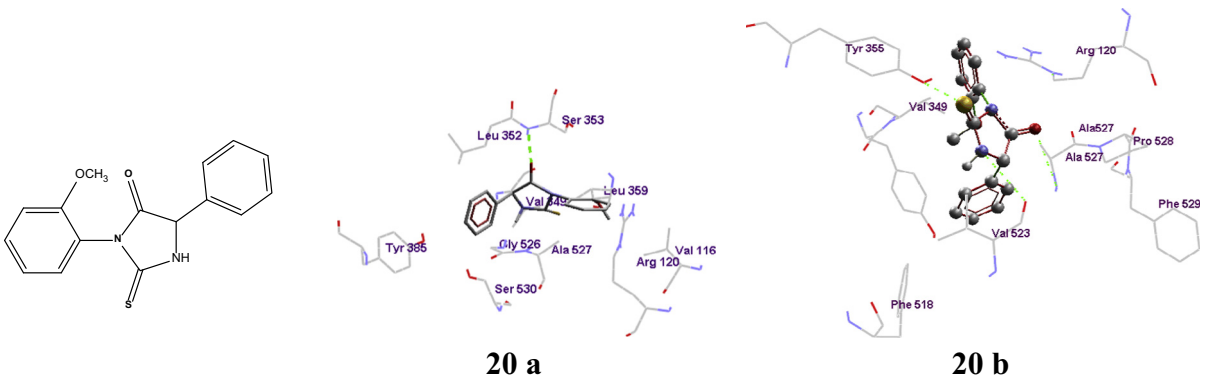

Figure 3 Comparative binding recognition of compound **20** at the two binding pockets of (a) COX-1 and (b) COX-2.

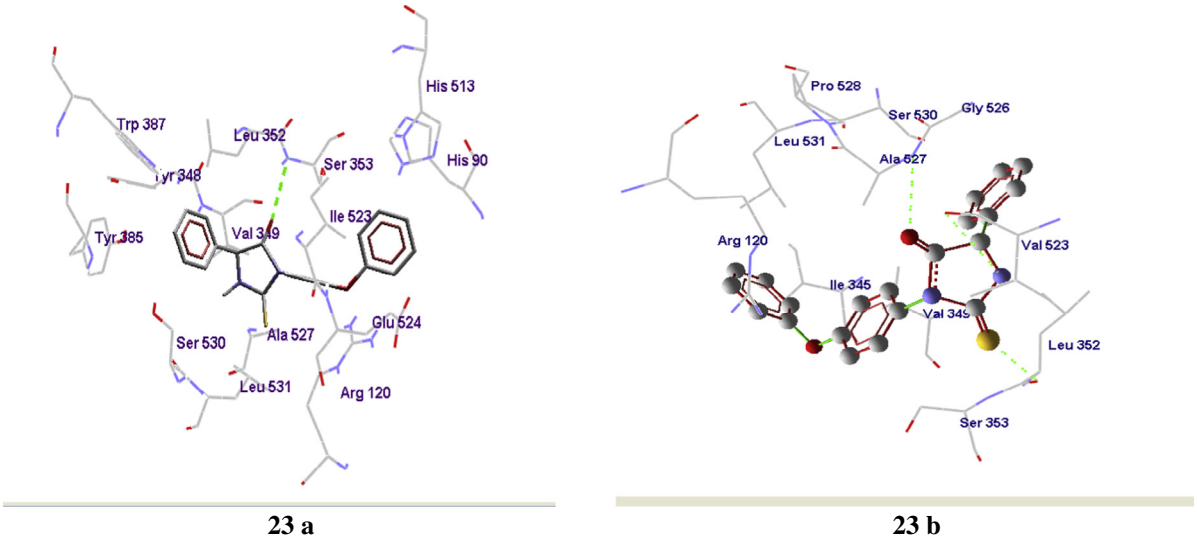

Figure 4 Comparative binding recognition of compound **23** at the two binding pockets of (a) COX-1 and (b) COX-2.

Compound **30** stabilized within the COX-2 binding pocket by the interaction with 3-methoxy group and the corresponding Tyr385 and Tyr355. The polar sulfonyl group also performed network of hydrogen bonding interaction with three conserved residues namely Arg513, Phe518 and His90. In compounds **31** and **32**, the methoxy substitution has been changed to a methylthio or a phenoxy group. This alteration led to a change in the binding style but maintained the minimum common feature required for recognition within the binding pocket, mainly the sulfonyl function group.

Compounds **33** and **34** substituted with the 4-chlorobenzyl group allowed the stabilization of the configuration by lipophilic interaction with the lipophilic pocket residues where the benzyl group oriented in a manner that allows the lipophilic lattice from the surrounding residues Phe205, Tyr385 and Tyr 348 (Fig. 5).

4. Experimental

Experimental synthesis has been done in the chemistry laboratory at pharmaceutical department; faculty of pharmacy; King Saud University; Female sector. All reagents and solvents were obtained from commercial suppliers and were used without further purification. Melting points (°C) were determined in open glass capillaries using Branstead 9001 electrothermal melting point apparatus and are uncorrected. Elemental analyses were recorded on a PERKIN-ELMER 2400 C,H,N elemental analyzer. NMR spectra were obtained on a Bruker AC 500 ultra shield NMR spectrometer (Fallanden, Switzerland) at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C, and the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) as internal standard; coupling constant (J) are expressed in Hz. Deuterio-chloroform ($CDCl_3$) and deuteriodimethyl sulfoxide (DMSO_d_6) were used as solvents. Mass spectral (MS) data were obtained on a Perkin Elmer, Clarus 600 GC/MS mass spectrometers. Carrageenan and dimethylsulfoxide (DMSO) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Celecoxib was obtained from Pfizer Inc., NY, USA. COX inhibitor screening assay kit was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Male Sprague–Dawley rats weighing 200 ± 30 g from Mansoura University Animal House (Mansoura, Egypt) were used.

All animal housing, care and treatment were strictly carried out in accordance with the University Guidelines for the Care and Use of Laboratory Animals. Food was withheld 12 h

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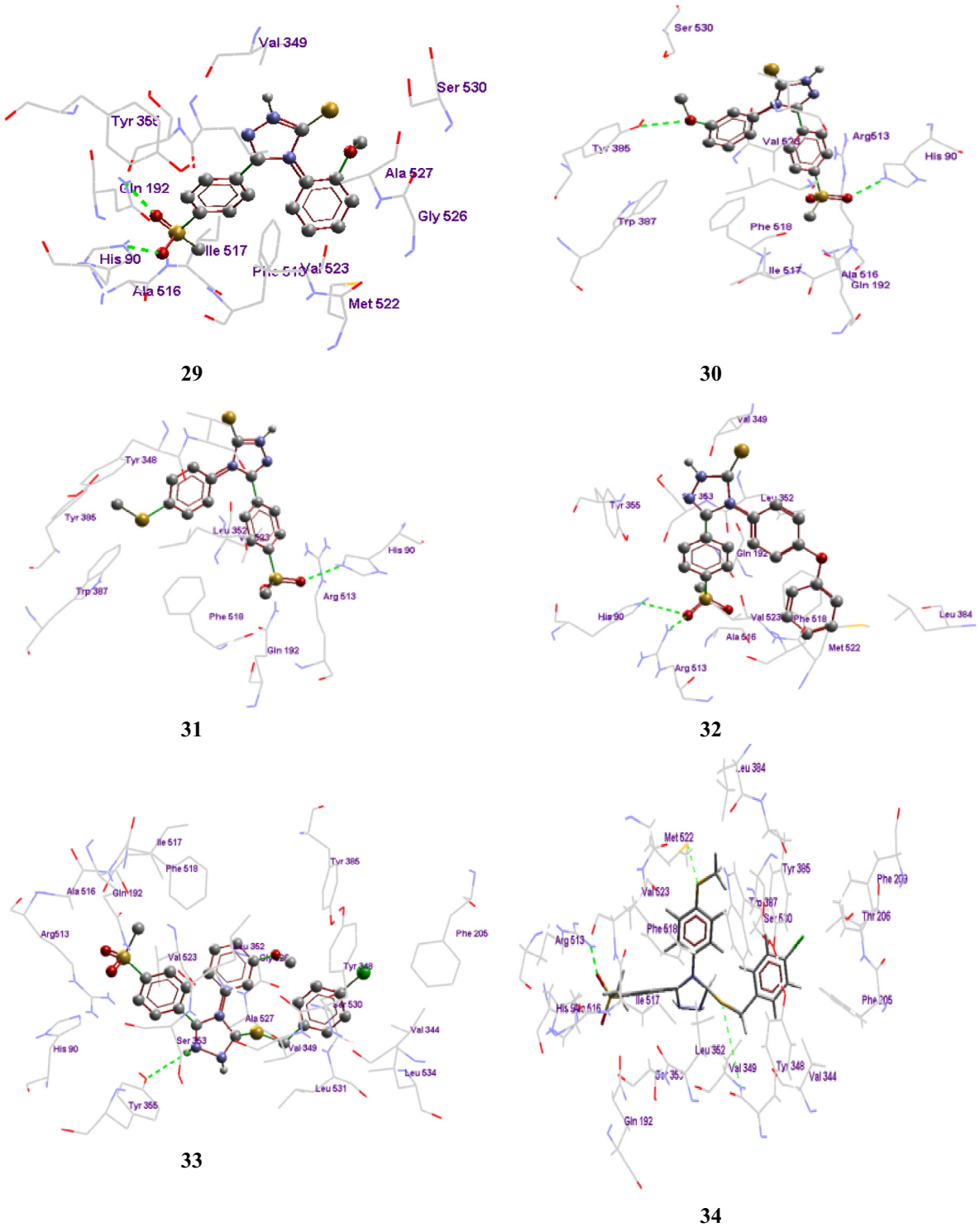

Figure 5 Docking of the triazole analogs; **33** and **34** at the binding pocket of COX-2.

before the experiment, with free access to water. Tested compounds were first dissolved in (DMSO) and diluted into reaction buffer (*in vitro* experiment) using celecoxib as reference standard.

Immediately before *in vivo* experiment, tested compounds were first dissolved in (DMSO) and diluted 0.5% carboxymethylcellulose. Carrageenan was dissolved as a 1% in saline and left overnight.

All modeling experiments were conducted with Hyperchem 6.03 package from Hypercube and Moelgro molecular viewer (Hyperchem, 1999; Molegro Virtual Docker MVD, 2007).

4.1. Synthesis of (±)-3-(substituted phenyl)-5-phenyl-2-thioxoimidazolidin-4-ones (20–23)

(±)-α-Phenylglycine (1.5 g; 9.9 mmol) is suspended in a mixture of 1:1 pyridine/H_2O (60 ml), warmed to 40 °C and adjusted to pH 9 with sodium carbonate solution 1N. The required isothiocyanate derivative (14.9 mmol) is added portion wise over 1 h with stirring. Three hours later, the solvents are evaporated under reduced pressure, and the crude residue is dissolved in water (100 ml). The solution was extracted three times with toluene (3 × 50 ml) and then acidified with hydrochloric acid 1N solution. The formed precipitate is extracted with ethyl acetate (3 × 50 ml). The organic layers were combined, washed with water, dried over anhydrous magnesium sulfate and evaporated under vacuum. The obtained residue was purified by applying preparative thin layer chromatography using $CHCl_3$:CH_3OH (7:3, v/v) to obtain the target 3, 5-diaryl-2-thioxoimidazolidin-4-ones (**20**–**23**).

4.1.1. (±)-3-(2-Methoxyphenyl)-5-phenyl-2-thioxoimidazolidin-4-one 20

Yield: 73%, mp: 193–194 °C. MS: *m/z*; M^+ 298 (5%). ^{1}H NMR (*DMSO-d*$_6$), δ 3.71 (s, 3H, OCH_3), 5.49 (s, 1H, —NH—C*H*—CO—), 7.02 (d, 1H, ArH), 7.10–7.23 (m, 3H, ArH), 7.30–7.45 (m, 5H, ArH),10.82 (brd, 1H, NH). Analysis for $C_{16}H_{14}N_2O_2S$ (298.36): C, 64.41; H, 4.73; Found: C, 64.20; H, 4.50.

4.1.2. (±)-3-(4-Methoxyphenyl)-5-phenyl-2-thioxoimidazolidin-4-one 21

Yield: 95%, mp: 197–199 °C. MS: *m/z*; M^+ 298 (7%). ^{1}H NMR (*DMSO-d*$_6$), δ 3.79 (s, 3H, OCH_3), 5.57 (s, 1H, —NH—C*H*—CO—), 6.82 (d, 2H, *J* = 8.5 Hz, ArH), 7.20 (d, 2H, 7.5 Hz, ArH), 7.35–7.45 (m, 5H, ArH), 10.97 (brd, 1H, NH). ^{13}C NMR: δ 55.9, 63.1, 114.5, 126.3, 126.4, 127.6, 129.5, 130.5, 135.0, 159.7, 173.32 (C═O), 183.7 (C═S). Analysis for $C_{16}H_{14}N_2O_2S$ (298.36): C, 64.41; H, 4.73; Found: C, 64.33; H, 4.43.

4.1.3. (±)-3-(2-Methylthiophenyl)-5-phenyl-2-thioxoimidazolidin-4-one 22

Yield: 15%, mp: 214–215 °C. MS: *m/z*; M^+; 314(10%). ^{1}H NMR (*DMSO-d*$_6$), δ 2.48 (s, 3H, SCH_3), 5.56 (s, 1H, —NH—C*H*—CO—), 7.22–7.30 (m, 5H, ArH), 7.32–7.35 (m, 2H, ArH), 7.38–7.44 (m, 2H, ArH), 10.95 (brd, 1H, NH). Analysis for $C_{16}H_{14}N_2OS_2$ (314.43): C, 61.12; H, 4.49; Found C, 61.20; H, 4.34.

4.1.4. (±)-3-(4-Phenoxyphenyl)-5-phenyl-2-thioxoimidazolidin-4-one 23

Yield: 50%, mp: 250–252 °C. MS: *m/z*; M^+; 360(4%). ^{1}H NMR (*DMSO-d*$_6$), δ 5.61 (s, 1H, imidazolidine-H), 7.09–7.12 (m, 4H, ArH), 7.19–7.22 (m, 1H, ArH), 7.36 (d, 2H, *J* = 8.5 Hz, ArH), 7.39–7.49 (m, 7H, ArH), 11.04 (brd, 1H, NH). ^{13}C NMR: δ 63.2, 118.5, 119.9, 124.6, 127.6, 128.7, 129.3, 129.5, 130.7, 131.0, 134.9, 156.3, 157.6, 173.2 (C═O), 183.4 (C═S). Analysis for $C_{21}H_{16}N_2O_2S$ (360.43): C, 69.98; H, 4.47; Found C, 69.85; H, 4.27.

4.2. Synthesis of 1-(4-methylsulfonyl benzoyl)-4-substituted phenyl-thiosemi-carbazide (25–28)

To a solution of 4-methylsulfonylbenzoic acid hydrazide **24** that was prepared as reported (El-Emam and Ibrahim, 1991) (0.32 g, 1.5 mmol) in absolute ethanol (5 ml), the appropriate isothiocyanate (1.55 mmol) was added and the mixture was stirred at room temperature for 24 h. The formed precipitate was filtered, dried and recrystallized from ethanol to give the desired products **25**–**28** as white solids.

4.2.1. 1-(4-Methylsulfonyl benzoyl)-4-(2-methoxyphenyl) thiosemicarbazide 25

Yield: 59%, mp: 180 °C. MS: *m/z*; M^+; 379 (10%). ^{1}H NMR (*DMSO-d*$_6$), δ 3.28 (s, 3H, SO_2CH_3), 3.75 (s, 3H, OCH_3), 6.94–7.17 (m, 4H, ArH), 8.02–8.16 (m, 4H, ArH), 9.32 (brd, 1H, NH), 9.90 (brd, 1H, NH), 10.83 (brs, 1H, NH). Analysis for $C_{16}H_{17}N_3O_4S_2$ (379.45): C, 50.64; H, 4.52; Found: C, 50.60; H, 4.49.

4.2.2. 1-(4-Methylsulfonyl benzoyl)-4-(3-methoxyphenyl) thiosemicarbazide 26

Yield: 75%, mp: 175 °C. MS: *m/z*; M^+; 379 (18%). ^{1}H NMR (*DMSO-d*$_6$), δ 3.28 (s, 3H, SO_2CH_3), 3.75 (s, 3H, OCH_3), 6.75–7.24 (m, 4H, ArH), 8.08–8.18 (m, 4H, ArH), 9.80 (brd, 2H, 2 NH), 10.80 (brd, 1H, NH). Analysis for $C_{16}H_{17}N_3O_4S_2$ (379.45): C, 50.64; H, 4.52; Found: C, 50.69; H, 4.35.

4.2.3. 1-(4-Methylsulfonyl benzoyl)-4-(4-methylthiophenyl) thiosemicarbazide 27

Yield: 100%, mp: 200 °C. MS: *m/z*; M^+; 395 (5%). ^{1}H NMR (*DMSO-d*$_6$), δ 2.47 (s, 3H, SCH_3), 3.28 (s, 3H, SO_2CH_3), 7.24–741 (m, 4H, ArH), 8.08–8.19 (m, 4H, ArH), 9.79 (brs, 2H, NH), 10.80 (brs, 1H, NH). Analysis for (395.52): C, 48.59; H, 4.33; Found: C, 48.55; H, 4.35.

4.2.4. 1-(4-Methylsulfonyl benzoyl)-4-(4-phenoxyphenyl) thiosemicarbazide 28

Yield: 68%, mp: 200 °C. MS: *m/z*; M^+; 441 (14%). ^{1}H NMR (*DMSO-d*$_6$), δ 3.28 (s, 3H, SO_2CH_3), 6.95–7.25 (m, 4H, ArH), 7.35–7.43 (m, 5H, ArH), 8.03–8.23 (m, 4H, ArH), 9.83 (brd, 2H, NH), 10.82 (brd, 1H, NH). Analysis for $C_{21}H_{19}N_{23}O_4S2$ (441.52): C, 57.13; H, 4.34; Found: C, 57.35; H, 4.350.

4.3. Synthesis of 5-[4-(methylsulfonyl)phenyl]-4-substituted phenyl-2,4-dihydro-3H-1, 2, 4-triazol-3-thiones (29–32)

A mixture of compounds (**25**–**28**, 1.5 mmol) and saturated aqueous sodium carbonate solution (15 ml) were heated under reflux with stirring for 12 h. The obtained solution was cooled and neutralized with hydrochloric acid 10%. The formed precipitate was filtered, washed with water, dried and then recrystallized from ethanol to give compounds **29**–**32**.

4.3.1. 5-(4-Methylsulfonylphenyl)-2-methoxyphenyl-2,4-dihydro-3H-1,2,4-triazol-3-thione 29

Yield: 45%, mp: > 300 °C. MS: *m/z*; M^+; 361 (3%). ^{1}H NMR (*DMSO-d*$_6$), δ 3.21(s, 3H, SO_2CH_3), 3.55(s, 3H, OCH_3), 7.13–

7.79 (m, 4H, ArH), 7.85–8.06 (m, 4H, ArH), 14.24 (brd, 1H, NH). Analysis for $C_{16}H_{15}N_3O_3S$ (361.44): C, 53.17; H, 4.18; Found C, 53.22; H, 4.45.

4.3.2. 5-(4-Methylsulfonylphenyl)-3-methoxyphenyl-2,4-dihydro-3H-1,2,4-triazol-3-thione 30

Yield: 60%, mp: 267 °C. MS: *m/z*; M^+; 361 (7%). ^{1}H NMR (*DMSO-d*$_6$), δ 3.22 (s, 3H, SO_2CH_3), 3.74 (s, 3H, OCH_3), 6.92–7.33 (m, 4H, ArH), 7.55–7.92 (m, 4H, ArH), 14.28 (brd, 1H, NH). ^{13}C NMR: δ 43.6 (SO_2CH_3), 56.0 (OCH_3), 115.3, 115.7, 121.2, 127.6, 129.5, 130.7, 131.0, 135.7, 142.6, 149.6, 160.2, 169.5. Analysis for $C_{16}H_{15}N_3O_3S$ (361.44): C, 53.17; H, 4.18; Found C, 53.25; H, 4.30.

4.3.3. 5-(4-Methylsulfonylphenyl)-4-methylthiophenyl-2,4-dihydro-3H-1,2,4-triazol-3-thione 31

Yield: 92%, mp: 255 °C. MS: *m/z*; M^+; 377 (1%). ^{1}H NMR (*DMSO-d*$_6$), δ2.51(s, 3H, SCH_3), 3.23(s, 3H, SO_2CH_3), 7.34–7.65 (m, 4H, ArH), 7.70–7.93 (m, 4H, ArH), 14.27 (brs, 1H, NH). ^{13}C NMR: δ 14.8 (SCH_3), 43.6(SO_2CH_3), 126.5, 127.6, 129.5, 129.6, 131.0, 131.2, 141.0, 142.7, 149.7, 169.7. Analysis for $C_{16}H_{15}N_3O_2S_3$ (377.5): C, 50.91; H, 4.01; Found C, 50.85; H, 4.00.

4.3.4. 5-(4-Methylsulfonylphenyl)-4-phenoxyphenyl-2,4-dihydro-3H-1,2,4-triazol-3-thione 32

Yield: 54%, mp: 246 °C. MS(EI): *m/z*; M^+; 423 (3%). ^{1}H NMR (*DMSO-d*$_6$), δ 3.28 (s, 3H, SO_2CH_3), 7.04–7.41 (m, 4H, ArH), 7.44–7.75 (m, 4H, ArH), 7.88–7.99 (m, 5H, ArH), 14.31 (brd, 1H, NH). Analysis for $C_{21}H_{17}N_3O_3S2$ (423.51): C, 59.56; H, 4.05; Found: C, 59.50; H, 4.00.

4.4. Synthesis of 3-(alkyl or aralkyl thio)-4-substituted phenyl-5-(4-methyl-sulfonyl)-4H-1, 2, 4-triazoles (33 and 34)

The appropriate halides namely *p*-chlorobenzyl chloride (0.5 mmol) was added to a solution of (**30**, **31**, 0.5 mmol**)** in ethanolic sodium carbonate 2% (5 ml). The mixture was stirred and heated under reflux for 24 h. The solvent was evaporated under reduced pressure; the obtained residue was washed with water, dried and then recrystallized from ethanol to give the title compounds **33** and **34**.

4.4.1. 3-(4-Chloro-benzylthio)-4-(3-methoxyphenyl)-5-(4-methylsulfonyl)-4H-1,2,4-triazole 33

Yield: 38%, mp: 120 °C. MS: *m/z*; M^+; 486 (4%). ^{1}H NMR (*CDCl*$_3$), δ 3.03 (s, 3H, SO_2CH_3), 3.80 (s, 3H, OCH_3), 4.54 (s, 2H, CH_2Ph), 6.75–7.78 (m, 4H, ArH), 7.78–7.80 (m, 4H, ArH), 7.85–7.87 (m, 4H, ArH). Analysis for $C_{23}H_{20}ClN_3O_3S2$ (486.01): C, 56.84; H, 4.15; Found: C, 56.80; H, 4.11.

4.4.2. 3-(4-Chloro-benzylthio)-4-(4-methylthiophenyl)-5-(4-methylsulfonyl)-4H-1,2,4-triazole 34

Yield: 38%, mp: 175–8 °C. MS: *m/z*; M^+; 502 (7%). ^{1}H NMR (*CDCl*$_3$), δ2.51 (s, 3H, SCH_3), 3.02 (s, 3H, SO_2CH_3), 4.51 (s, 2H, CH_2Ph), 7.08–7.22 (m, 4H, ArH), 7.25–7.77 (m, 4H, ArH), 7.79–7.87 (m, 4H, ArH). ^{13}C NMR: δ 15.1 (SCH_3), 36.5 (CH_2Ph), 44.2 (SO_2CH_3), 127.0, 127.3, 127.7, 128.8, 129.0, 129.3, 130.1, 133.9, 134.6, 134.7, 141.9, 143.1, 152.9, 154.0. Analysis for $C_{23}H_{20}ClN_3O_2S3$ (502.07): C, 55.02; H, 4.02; Found: C, 55.10; H, 4.09.

5. Biological evaluations

5.1. In vitro COX-1 and COX-2 inhibition assay

The assay includes both bovine COX-1 and human recombinant COX-2 enzymes to perform screening of isozyme-specific inhibitors using celecoxib was used as standard (Pradelles et al., 1985). The tested compounds were added to both COX-1 and COX-2 with the substrates and buffers to perform the COX reaction according to kit instructions. The COX-derived PGH_2 produced in the COX reaction is reduced to the more stable $PGF_{2\alpha}$ by $SnC1_2$, and the amount of $PGF_{2\alpha}$ was quantified via enzyme immunoassay (EIA) using a broadly specific antiserum that binds to all the major PG compounds according to kit instructions. The product of the enzymatic reaction has a distinct yellow color that was absorbed strongly at 412 nm. The intensity of the color, which determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of free PG present in the well during the incubation. Therefore, the more the inhibition of COX by any of the tested compounds, the less PG produced, and the more absorbance or color developed.

5.2. In vivo carrageenan-induced paw edema

This method was originally described by Winter et al. (1962) and modified by Mielens et al. (1968). It is a model of acute inflammation. Tested compounds were administered (150 mg kg^{-1}) 1 h before injecting 0.1 ml of 1% carrageenan into the subplantar region of the right hind paw. The left hind paw of each rat received a subplantar injection of equal volume of normal saline. Control group received the vehicle instead of tested compounds. After a further 3 h, the rats were humanely killed by cervical dislocation and the hind paws removed at the paw hairline (tibiotarsic articulation) and weighed.

5.3. Data analysis

In vitro experiment, calculations and analysis of data were carried out according to the reported instructions. The concentration of each sample was identified by extrapolating its $\%B/B_0$ on the standard curve, and then the % inhibition of COX-1 and COX-2 by each inhibitor was calculated.

For *in vivo* experiment, data were expressed as mean ± standard error of mean (SEM), where (*n*) equals the number of animals (rats). The carrageenan-induced paw edema was calculated as following:

$$\%\ \text{Increase in right paw edema} = \frac{\text{right paw weight} - \text{left paw weight}}{\text{left paw weight}} \times 100$$

Significant differences between groups were determined with one-way ANOVA with Dunnett's post-hoc test.

6. Computational molecular modeling

6.1. COX-2 enzyme structure

Starting coordinate of the cyclooxygenase-2 enzyme, "1CX2" in complex with a selective inhibitor, SC-558, code ID 1CX2, was obtained from the Protein Data Bank of Brookhaven National Laboratory, Fig. 2 (Kurumbail et al., 1996).

6.2. COX-1 enzyme structure

Starting coordinate of the cyclooxygenase-1 enzyme, "1PGF" in complex with a non-selective inhibitor, Iodoindomethacin "IMM" codeID 1PGF, was obtained from the Protein Data Bank of Brookhaven National Laboratory, Fig. 2 (Loll et al., 1996).

6.3. The selective COX-2 inhibitor SC-558

The crystal structure of SC558 selective inhibitor was depicted at the binding active site showing the putative hydrogen bonding with the conserved amino acid residues.

6.4. COX-1 non-selective IMM

The crystal structure of 1MM non-selective inhibitor was captured at the binding active site showing the putative hydrogen bonding with the surrounding amino acid residues.

6.5. Molecular structure of the synthesized imidazoles and triazoles

The imidazole or triazole analogs **(20–23** and **29–34)** were constructed from fragment libraries in the Hyperchem program followed by energy minimization using the "Amber force field". The partial atomic charges for each analog were assigned with the semiempirical mechanical calculation method "AM1" implemented in Hyperchem 6.03. Conformational search was performed around all the rotatable bonds with an increment of 100 using conformational search module as implemented in HyperChem 6.03 All the conformers were minimized until the Root Mean Square (RMS) deviation was 0.01 kcal/mol Å.

6.6. Docking and molecular geometrical optimization

Lowest energy conformer of each new analog "global-minima" was docked into the two enzymes COX-1 and COX-2 binding cavity 1CX2 & 1PGF. For each of the tested analogs, energy minimizations were performed using 1000 steps of steepest descent, followed by conjugate gradient minimization to a RMS energy gradient of 0.01 kcal/mol Å. Hydrogen bonds with a bond length up to 3.5 Å were considered. The active site of the enzyme was defined using a radius of 8.0 Å around the ligands SC-558 and IMM.

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