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Original article

Synthesis and biological evaluation of 4,5-diphenyloxazolone derivatives on route towards selective COX-2 inhibitors

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ABSTRACT

A series of 3-unsubstituted/substituted-4,5-diphenyl-2-oxo-3*H*-1,3-oxazole derivatives were prepared as selective cyclooxygenase-2 (COX-2) inhibitors. Among the synthesized compounds, 4-(4-phenyl-3-methyl-2-oxo-3*H*-1,3-oxazol-5-yl)benzensulfonamide (compound **6**) showed selective COX-2 inhibition with a selectivity index of >50 (IC_{50}COX-1 = >100 μm, IC_{50}COX-2 = 2 μm) in purified enzyme (PE) assay. Compound **6** also exhibited selective COX-2 inhibition in human whole blood assay. Molecular docking studies showed that **6** can be docked into the COX-2 binding site thus providing the molecular basis for its activity.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAID) are among the most frequently prescribed medications being the drugs of the first choice for treatment of the inflammatory and rheumatic diseases. The common mechanism of NSAIDs involves the nonselective inhibition of cyclooxygenases (COXs) thereby preventing the biosynthesis of prostaglandins (PG) which are the important lipid mediators of inflammation as well as numerous homeostatic physiological functions [1]. As it is now well appreciated, COXs exist in two isoforms, namely COX-1 and COX-2 [2], while the existence of a third isoforom (COX-3) is still into debate. In general terms, COX-1 is the constitutive isoform providing normal production of PGs having roles in homeostasis and gastroprotection, whereas COX-2 is induced by proinflammatory stimuli at inflammatory sites [3]. The discovery of inducible COX-2 at sites of inflammation led to the development of selective COX-2 inhibitors with the hope of dimished gastrointestinal side effects associated with traditional NSAIDs [4,5]. However, recent studies have shown that COX-2 inhibitors are associated with increased thromboembolic phenomena in specific patient populations such as cardiovascular disease patients challenging the benefits of selective COX-2 inhibition [6–8]. Moreover, there is currently no clear evidence that COX-2 inhibitors represent an independent risk factor in patients at low demographic risk of cardiovascular diseases and therefore, clinical rationale for developing compounds with selective COX-2 inhibition still remains to be established [8,9]. Meantime, considerable interest in the further potential clinical utilities of COX-2 inhibitors has emerged [10–12]. Recent studies indicating the place of COX-2 inhibitors in cancer chemotherapy and neurological diseases such as Alzheimer's [13,14] and Parkinson [13,14] diseases still continues to attract investigations on development of COX-2 inhibitors.

The structural information on the tricyclic COX-2 selective inhibitors, for example vicinal diaryl substitution about a central heterocyclic ring, is described in detail in literature and having the characteristic sulfonyl group on one of the aryl rings are believed to play a crucial role on selectivity [15,16]. For this purpose, vicinal diaryl substituents on a central five- or six-membered ring template such as pyrazole, 2-(5*H*)-furanone, isoxazole, pyridine have been extensively investigated as selective COX-2 inhibitors [4] (Fig. 1). In addition, some studies for developing COX-2 inhibitors have concentrated on the preparation of the amide derivatives of currently used NSAIDs such as indomethacin [17,18] and

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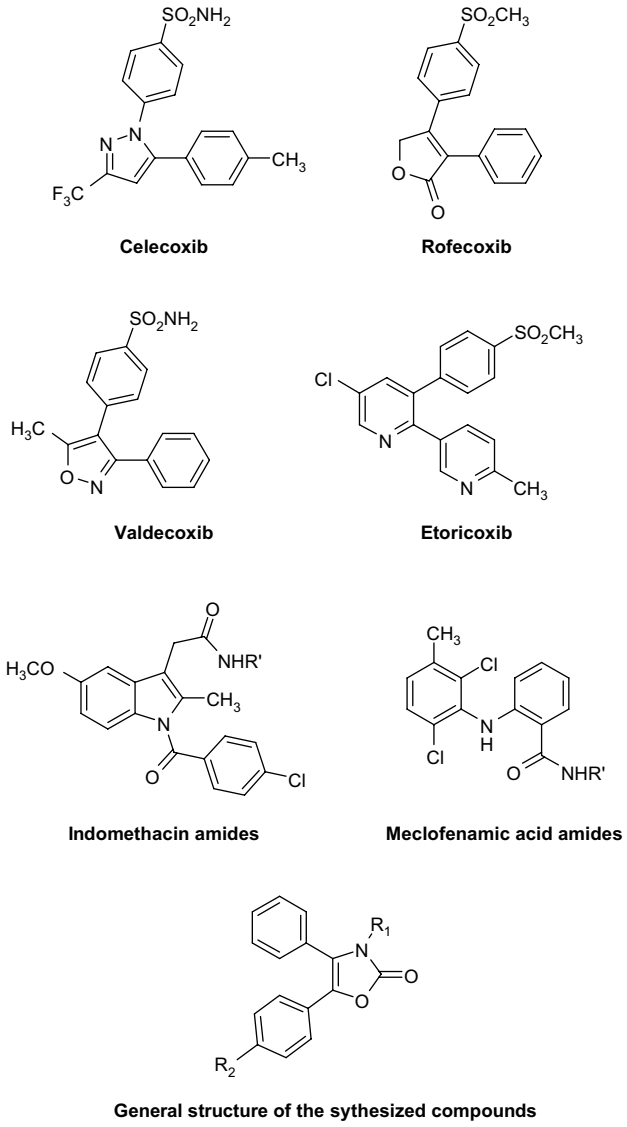


Fig. 1. Representative examples of selective COX-2 inhibitor compounds and the general structure of the synthesized 4,5-diphenyl-2-oxo-3*H*-1,3-oxazole derivatives.

meclofenamic acid [19] (Fig. 1) and found that neutralization of these NSAIDs by preparing the corresponding amide derivatives resulted into compounds that selectively inhibited COX-2 but not COX-1. As a part of our ongoing program to acquire structure–function relationship data for COX-2 inhibitors, we hereby describe the synthesis and the preliminary biological evaluation of a group of diphenyl substituted oxazolone derivatives possessing a sulfonyl group at the *para*-position of C-5 phenyl moiety in conjunction with a variety of substituents at the nitrogen atom of the central oxazolone ring (Fig. 1).

2. Chemistry

The synthetic routes for the synthesized compounds are outlined in Schemes 1–3. The starting compound, 4,5-diphenyl-2-oxo-3*H*-1,3-oxazole (**1**), was readily prepared by the reaction of benzoin and urethane under distillation conditions as shown in Scheme 1 [20]. Compound **1** was then reacted with dimethyl sulfate to obtain 4,5-diphenyl-3-methyl-2-oxo-3*H*-1,3-oxazole (**2**) [20–22]. Treatment of **1** and **2** with chlorosulfonic acid yielded the sulfonyl chloride derivatives (**3**, **4**) which were subsequently reacted with ammonium hydroxide to yield the sulfonamide derivatives (**5**, **6**) (Scheme 1). The methylsulfonyl derivative (**8**) was obtained by the methylation of the sodium salt of **4** with dimethyl sulfate as demonstrated in Scheme 1.

The preparation of the amide derivatives **15**–**21** are outlined in Scheme 2. Alkylation of **1** with ethyl bromoacetate generated ethyl 2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)ethanoate (**9**) [23]. Subsequent hydrolysis of the ester linkage under basic conditions afforded 2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)ethanoic acid (**10**) [24,25]. Amidation of **10** with appropriate secondary and tertiary amines in the presence of ethyl chloroformate in dichloromethane at room temperature, resulted in the synthesis of amide derivatives **15**–**21** with quantitative yields (51–66%).

The sulfonamide derivative having an acetic acid substitution on the nitrogen of oxazolone was prepared as shown in Scheme 3. Firstly, ester derivative **9** was reacted with chlorosulfonic acid to yield sulfonyl chloride derivative (**11**) under mild reaction conditions. After protection of sulfonyl chloride with dibenzylamine and subsequent treatment with concentrated sulfuric acid resulted in the target sulfonamide derivative (**13**), which was readily hydrolyzed under acid conditions to obtain the desired acid derivative **14**.

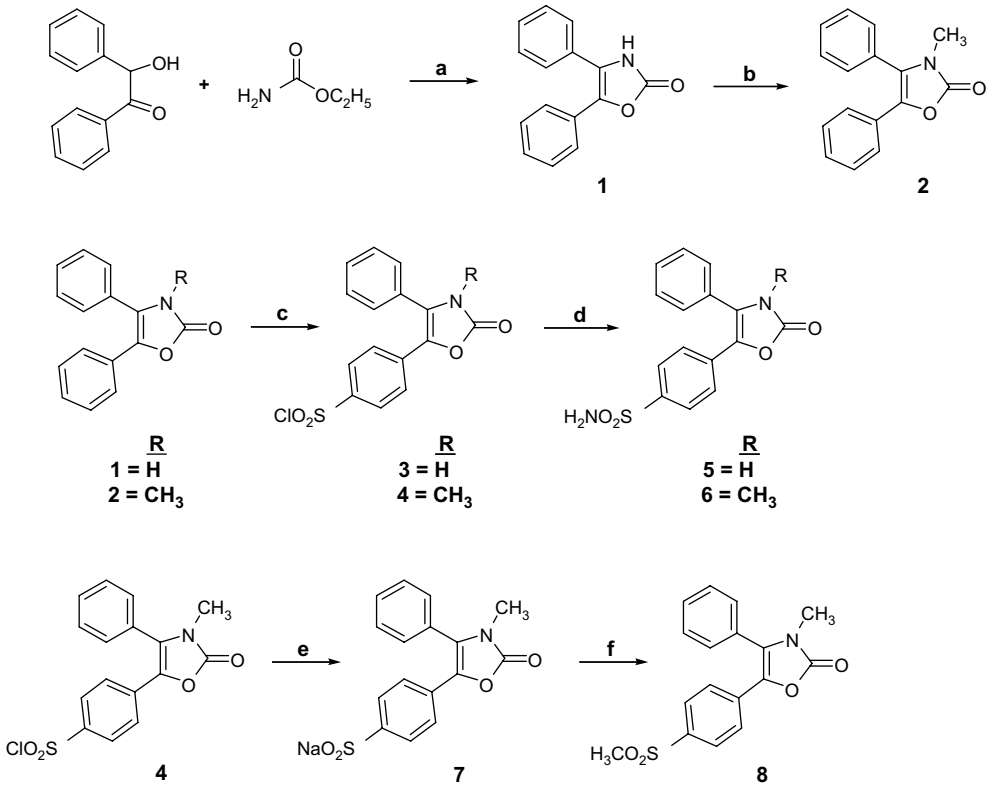
3. Results and discussion

The compounds reported herein were tested for their ability to inhibit COX-2 and/or COX-1 using the purified enzyme assay described by Futaki et al. [26] and Janusz et al. [27]. The in vitro activity results are reported as a percentage of inhibition of the purified enzymes at 10 μM (Table 1). For compounds which exhibited inhibition of more than 50% for COX-2, the inhibition of COX-1 at 10 μM and the IC_{50} values were also calculated from the concentration curves by means of the PRISM program. Furthermore, compound **6** was selected on the basis of its activity in the cell-free assay for evaluation of inhibition of human COX-2 and COX-1 using in vitro human whole blood assay described by Patrignani et al. [28].

In this preliminary study towards new potential COX-2 selective compounds as novel drug candidates for inflammatory and related diseases, we have introduced systematic modifications to the 4,5-diphenyloxazolone core structure. It is well established that 3,4-diaryloxazolones having a sulfone or sulfonamide on the 4-phenyl is a good template for selective COX-2 inhibition [29,30]. Thus, taking into account this structural feature, we planned a structure–activity relationship study using the 4,5-diphenyloxazolone core as a template. In particular, we envisaged a series of substitution on the N3-nitrogen atom of the heterocycle in order to introduce more flexibility to the template, while keeping the diphenyl portion unsubstituted or substituted with sulfone/sulfonamide which was required to maintain COX-2 selectivity. Results are shown in Table 1.

In general, none of the newly synthesized derivatives proved to be endowed with the desired activity profile at COX-2, as none but one of the compounds (compound **6**) inhibited at least 50% of the COX-2 isoform during preliminary screening.

Compound **6** is endowed with a *N*-methyl substitution and with a *p*-sulfonamide on the 5-phenyl ring. We found that *N*-methyl derivative with the unsubstituted 5-phenyl ring (**2**) had a diminished COX-2 activity (~5% inhibition) with respect to the sulfonamide (**6**, 70%) or methylsulfonyl (**8**, 49%) analogs. This preliminary results indicated that the presence of *p*-sulfone/sulfamoyl is important for COX-2 activity and sulfone on the 5-phenyl ring was less effective with regard to sulfamoyl at the same position which was in close agreement with literature results for related compounds [29,30]. Within the sulfonamide analogs, introduction of larger substituents on the N3 position, i.e., ester (**13**) or acid (**14**), caused a decrease in the observed COX-2 activity at 10 μM screning dose as compared to *N*-methyl analog (**6**).

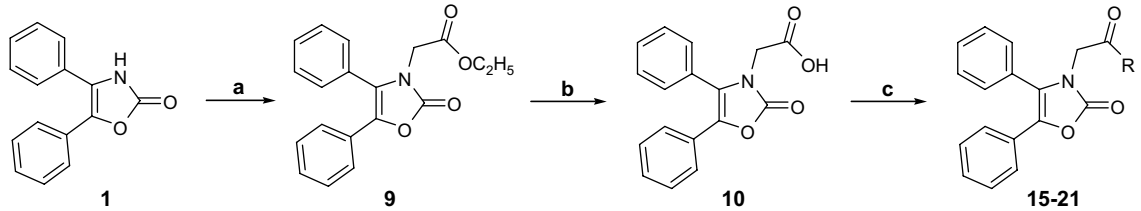


Scheme 1. Reagents: (a) dist.; (b) NaOH, dimethyl sulfate; (c) chlorosulfonic acid; (d) NH_4OH, reflux then concentrated HCl; (e) Na_2SO_3, $NaHCO_3$; (f) dimethyl sulfate, $NaHCO_3$, reflux.

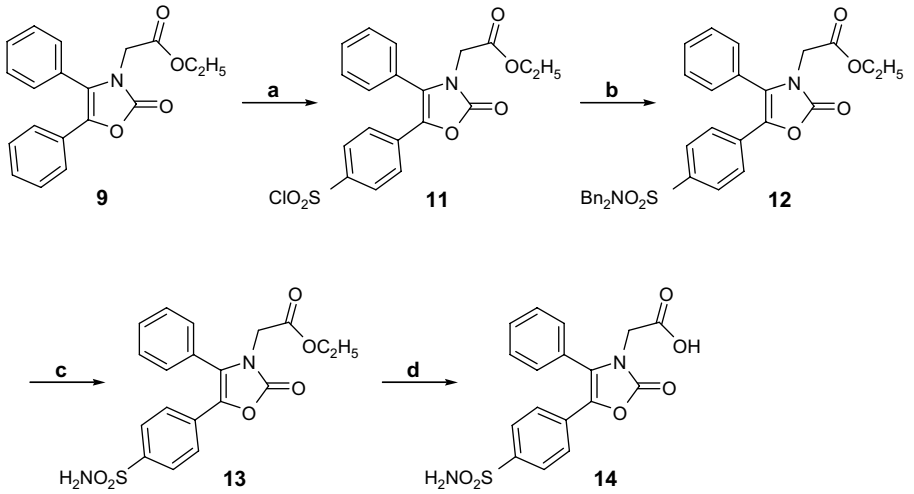
Recent studies evaluating ester and amide derivatives of indomethacin and meclofenamic acid resulted in highly potent and selective compounds and it was suggested that derivatization of the carboxylate moiety in moderately selective COX-1 inhibitors can be used as a novel strategy for generation of potent and selective COX-2 inhibitors [17,19,31]. On the basis of this background, we decided to introduce a carboxamide moiety in the 4,5-diphenyloxazolone scaffold by amidation of the acid derivative (**10**) in which the COX-2 inhibitor activity was shown to be moderate at 10 μM (∼42%) with the hope of obtaining better derivatives as selective COX-2 inhibitors. However, this was not the case and all of the amide derivatives (**15**–**21**) generally resulted in less potent COX-2 inhibition with respect to parent acid analog (**10**) in a range from 22 to 28% at this high screening concentration. This indicates that the impact of the substitution pattern of the nitrogen of oxazolone ring is low since the all tested derivatives show lower activity at 10 μM.

Since compound **6** was the only derivative that resulted in more than 50% COX-2 inhibition at 10 μM (∼70%), it was further evaluated for its inhibitory activity on COX-1 isoform and found that it insignificantly inhibited COX-1 isoform, 4% and 19% at 10 and 100 μM concentrations, respectively. Consequently, compound **6** was also tested in human whole blood assay at 10 μM and showed selective COX-2 inhibition under these assay conditions as well (85% for COX-2 and 7% for COX-1). Therefore, IC_{50} values of **6** for both isoforms was further calculated using in vitro purified enzyme assay from dose–response curve as >100 μM and 2 μM for COX-1 and COX-2, respectively, indicating that **6** behaves as a selective COX-2 inhibitor with a selectivity index (SI) of >50, which was about 5 times less potent than Rofecoxib ($IC_{50COx\text{-}1} > 100$ μM and $IC_{50COx\text{-}2} = 0.4$ μM, SI = 250).

With the aim of getting insights into the structural basis for its activity, compound **6** was docked into the active site of COX-2. The X-ray structure of COX-2 complexed with the selective inhibitor SC558 in the I222 space group (pdb code: 6cox) was selected for this purpose. The docking studies were carried out using Glide, as descibed in Section 5. In the top ranked pose, compound **6** adopts a disposition exactly matching that of SC558 (Fig. 2a). In particular, the unsubstituted phenyl moiety is inserted inside the COX-2 hydrophobic pocket (blue residues) while the sulfonamide group is positioned into the selectivity pocket (magenta residues) as the corresponding moiety of SC558. It can be appreciated from Fig. 2b how compound **6** forms two hydrogen bonds, one of them between the sulfonamide moiety and His90, situated at the bottom of the selectivity pocket, and the second one between the CO bond and Arg120 thus behaving in a similar manner like SC558 positioning the oxazolone with its carbonyl group coordinated to Arg120 in the



Scheme 2. Reagents: (a) K_2CO_3, ethyl bromoacetate, acetone, reflux; (b) KOH, water, reflux, then 1 N HCl; (c) ethyl chloroformate, Et_3N, amine, CH_2Cl_2.



Scheme 3. Reagents: (a) chlorosulfonic acid, $CHCl_3$; (b) Bn_2NH, Et_3N, CH_2Cl_2; (c) H_2SO_4; (d) HCl.

lower entrance constriction as SC558 does with is CF_3 group. It can also be appreciated from Fig. 2 how the *N*-methyl group is positioned into a small, mainly hydrophobic cavity where larger groups won't fit, thus providing the basis for explaining the poor activity of compounds **9**–**21** (Table 1).

Fig. 3 reports the binding mode of **6** as compared to 5-iodoindomethacin complexed with COX-1 (PDB entry: 1PGG). In the most favourable orientation found by the docking program the sulfonamide moiety is projected towards the hydrophobic pocket (blue residues) as Ile523 (yellow residue) closes the entry to selectivity pocket in this enzyme. Because of the polarity of the sulfonamide moiety, it is quite unlikely that this disposition is actually a productive one, as it is also confirmed by the low value of the Gscore (−11.20 kcal/mol as compared to −13.50 kcal/mol of the best pose of **6** into COX-2). It can be concluded that compound **6** neatly fits the COX-2 binding site, giving a stable complex and acting as a good inhibitor of this isoform. On the other hand, the best obtained putative COX-1/compound **6** complex is much less stable thus providing a clear explanation for the lack of activity of **6** at COX-1.

4. Conclusion

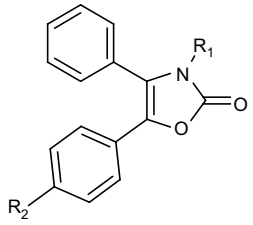
The synthesis of a series of 4,5-diphenyloxazolone derivatives substituted at N3 is described along with their preliminary evaluation as potential COX-2 inhibitors. Unfortunatelly, most of the compounds show no significant COX-2 inhibitory activity. Only compound **6** displayed potent and selective COX-2 inhibition. In conclusion, we feel that the preliminary in vitro activity results of this class of compounds may possess potential for design of future molecules with modifications on the aryl substituents as well as at N3 side chain to specifically inhibit one or more of these enzymes. Further studies are in progress.

5. Experimental

5.1. Materials and methods

All chemicals and solvents were purchased locally from Merck AG and Aldrich Chemicals. Melting points were determined with an Electrothermal-9200 Digital Melting Point Apparatus and are uncorrected. ^{1}H NMR spectra were recorded in DMSO-d_6 on a Varian Mercury 400, 400 MHz High Performance Digital FT-NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as δ (ppm). Microanalyses for C, H, and N were performed on a Leco-932 at Faculty of Pharmacy, Ankara University, Ankara, Turkey, and they were within the range of ±0.4% of the theoretical value. The synthesis of 4,5-diphenyl-2-oxo-3*H*-1,3-oxazole (**1**) [20], 4,5-diphenyl-3-methyl-2-oxo-3*H*-1,3-oxazole (**2**) [20–22], 4-(4-phenyl-2-oxo-3*H*-1,3-oxazol-5-yl)benzenesulfonyl chloride (**3**) [32], 4-(4-phenyl-2-oxo-3*H*-1,3-oxazol-5-yl) benzensulfonamide (**5**) [33], ethyl 2-(4,5-diphenyl-2-oxo-3*H*-1,

Table 1
In vitro inhibition of purified COX-2 by 4,5-diaryloxazolone derivatives.



Compound	R_1	R_2	COX-2 inhibition (%)[a]	COX-1 inhibition (%)[a]
2	CH_3	H	5.31 ± 8.25	nt[c]
5	H	SO_2NH_2	47.84 ± 3.05	nt
6	CH_3	SO_2NH_2	70.14 ± 1.71	3.56 ± 8.01 (10 μM) 19.28 ± 7.02 (100 μM)
8	CH_3	SO_2CH_3	48.60 ± 4.33	nt
9	$CH_2COOC_2H_5$	H	27.53 ± 1.30	nt
10	CH_2COOH	H	41.78 ± 15.95	nt
13	$CH_2COOC_2H_5$	SO_2NH_2	32.84 ± 5.29	nt
14	CH_2COOH	SO_2NH_2	33.17 ± 3.75	nt
15	$CH_2CONH(CH_2)_7CH_3$	H	23.22 ± 3.65	nt
16	$CH_2CONHCH_2CH_2C_6H_5$	H	22.22 ± 2.95	nt
17	$CH_2CONH(4\text{-}ClC_6H_4)$	H	26.53 ± 2.67	nt
18	$CH_2CONH(4\text{-}OCH_3C_6H_4)$	H	24.38 ± 1.60	nt
19	CH_2CO(piperidin-1-yl)	H	20.56 ± 1.30	nt
20	CH_2CONH(thiazol-2-yl)	H	28.19 ± 5.31	nt
21	CH_2CO(morpholin-1-yl)	H	23.05 ± 4.40	nt
Indomethacin			91.44 ± 0.24	71.89 ± 6.31
Rofecoxib[b]			66.78 ± 2.83	13.66 ± 6.66

[a] Data are indicated as percentage of inhibition at 10 μM ± SEM ($n = 4$).
[b] Rofecoxib was assayed at 100 μM and 1 μM for COX-1 and COX-2, respectively.
[c] nt: not tested.

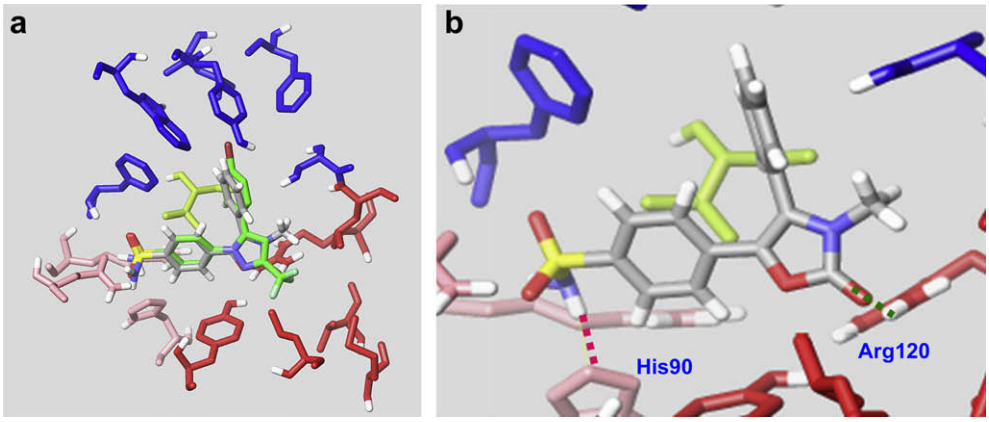


Fig. 2. (a) The best pose obtained for compound **6** (gray) inside the COX-2 binding pocket compared with SC558 (green). Note the very similar orientation of the two ligands. (b) Compound **6** makes two hydrogen bonds with Arg120 and His90. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3-oxazol-3-yl)ethanoate (**9**) [23], 2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)ethanoic acid (**10**) [24,25] was previously reported.

*5.1.1. 4-(4-Phenyl-2-oxo-3*H*-1,3-oxazol-5-yl)benzensulfonamide (**5**)*

4-(4-Phenyl-2-oxo-3*H*-1,3-oxazol-5-yl)benzensulfonyl chloride (**3**) (0.003 mol) was added to 30 mL of cold concentrated ammonium hydroxide, and the mixture was heated to reflux and stirred for 1 h. After cooling to room temperature, the mixture was acidified with concentrated HCl to give a solid precipitate. The product was collected by suction filtration, washed with water, dried, and crystallized from ethanol (yield 58%, m.p. 267 °C).

*5.1.2. Ethyl 2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)ethanoate (**9**)*

The mixture of 4,5-diphenyl-2-oxo-3*H*-1,3-oxazole (**1**) (5 mmol), potassium carbonate (5.5 mmol) and ethyl bromoacetate (5.5 mmol) in 30 mL acetone was heated to reflux and stirred for 6 h. 100 g ice water was added to the cooled (0–10 °C) reaction mixture. After stirring for 1 h, the precipitated solid product was collected by suction filtration, washed with water, dried, and recrystallized from ethanol (yield 85%, m.p. 64 °C).

*5.1.3. 2-(4,5-Diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)ethanoic acid (**10**)*

Ethyl 2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)ethanoate (**9**) (0.05 mol) and potassium hydroxide (0.05 mol) in 200 mL water was refluxed for 4 h. After cooling to room temperature, the mixture was acidified with 1 N HCl to give a solid precipitate. The product was collected by suction filtration, washed with water, dried, and crystallized from ethanol/water (yield 85%, m.p. 184 °C).

*5.1.4. 4-(4-Phenyl-3-methyl-2-oxo-3*H*-1,3-oxazol-5-yl)benzensulfonamide (**6**)*

4,5-Diphenyl-3-methyl-2-oxo-3*H*-1,3-oxazole (**2**) (0.005 mol) was added slowly to 0.025 mol of chlorosulfonic acid at 10–15 °C. The reaction mixture was stirred for 15 min after all the 4,5-diphenyl-3-methyl-2-oxo-3*H*-1,3-oxazole (**2**) was added. The temperature was then raised to 60 °C and maintained at this temperature for 2 h. The reaction mixture was poured onto ice. The product was removed by filtration and dissolved in ether. The ethereal solution was washed with water and dried with anhydrous sodium sulfate and evaporated to dryness (yield 66%). 0.003 mol of the solid residue (4-(4-phenyl-3-methyl-2-oxo-3*H*-1,3-oxazol-5-yl)benzensulfonyl chloride (**4**)) was added to 30 mL of cold concentrated ammonium hydroxide, and the mixture was heated to reflux and stirred for 1 h. On cooling, the ammonium salt of the 4-(4-phenyl-3-methyl-2-oxo-3*H*-1,3-oxazol-5-yl)benzensulfonamide separated. This was removed by filtration and dissolved in water and converted to the free sulfonamide by acidification with 1 N HCl to give a solid precipitate. The product was collected by suction filtration, washed with water, dried and recrystallized from isopropanol to yield 45%; m.p. 210–211 °C. ^{1}H NMR (DMSO-d_6) δ: 7.67–7.65 (2H, d, 5-phenyl H^3, H^5), 7.56–7.54 (5H, m, 4-phenyl), 7.31 (2H, s, SO_2-$\underline{NH_2}$), 7.28–7.26 (2H, d, 5-phenyl H^2, H^6), 2.95 (3H, s, N-$\underline{CH_3}$). Anal. Calc. for $C_{16}H_{14}N_2O_4S$: C, 58.17; H, 4.27; N, 8.48; S, 9.71. Found: C, 57.97; H, 4.29; N, 8.46; S, 9.52%.

*5.1.5. 4-Phenyl-3-methyl-5-[4-(methylsulfonyl)phenyl]-2-oxo-3*H*-1.3-oxazole (**8**)*

The mixture of sodium sulfite (10 mmol) and sodium bicarbonate (10.5 mmol) in water (15 mL) was heated at 80 °C and sulfonyl chloride (**4**) (5.3 mmol) was added in portions of 20 mg

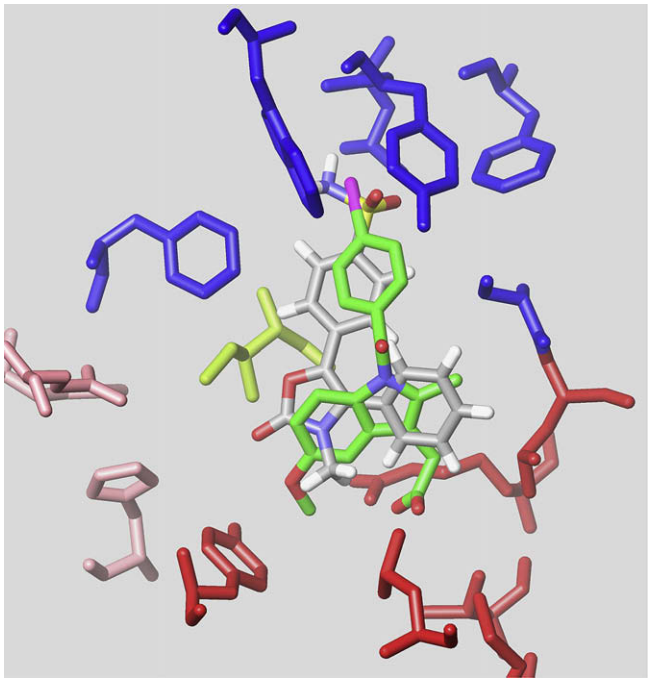


Fig. 3. The best pose found for compound **6** (gray) inside the COX-1 binding site, compared with the orientation of 5-iodoindomethacin (green) in the crystal structure 1PGG. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with stirring, during 3 h. After the addition was completed, the mixture was heated and stirred at 80 °C for 1 h. The crude sodium sulfinate solution was allowed to cool for overnight. The solid sodium 4-(4-phenyl-3-methyl-2-oxo-3*H*-1,3-oxazol-5-yl)benzensulfinate (**7**) which separated was collected by filtration and mixed with 10 mmol of $NaHCO_3$ and 8.15 mmol of dimethyl sulfate in 2 mL water. After heating under reflux for 20 h, the mixture was cooled and water was added (20 mL). The precipitated solid product was collected by suction filtration, washed with water, dried, and crystallized from acetone/water to yield 20%; m.p. 196–198 °C. ^{1}H NMR (DMSO-d_6) δ: 7.81–7.79 (2H, d, 5-phenyl H^3, H^5), 7.62–7.56 (5H, m, 4-phenyl), 7.37–7.34 (2H, d, 5-phenyl H^2, H^6), 3.16 (3H, s, $-SO_2\underline{CH_3}$), 2.97 (3H, s, N–$\underline{CH_3}$). Anal. Calc. for $C_{17}H_{15}NO_4S$: C, 61.99; H, 4.59; N, 4.25; S, 9.74. Found: C, 61.92; H, 4.70; N, 4.31; S, 9.71%.

*5.1.6. General procedure for the synthesis of 2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)acetamide derivatives (**15–21**)*

2-(4,5-Diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)ethanoic acid (**10**) (0.01 mol) in 40 mL dichloromethane at 0 °C (ice-bath) was treated with triethylamine (0.015 mol) and 0.01 mol of ethyl chloroformate. After stirring the reaction mixture at 0 °C for further 20 min, 0.011 mol of appropriate amine derivative (0.013 mol) was added, and the final mixture was stirred at room temperature for overnight. After evaporation to dryness, the product was solidified with ice-cold water and crystallized from the appropriate solvent.

5.1.6.1. N-*octyl-2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)acetamide (**15**).* Recrystallized from ethanol to yield 61%; m.p. 112–114 °C. ^{1}H NMR (DMSO-d_6) δ: 8.01 (1H, t, –NH–), 7.56–7.50 (3H, m, 4-phenyl H^2, H^4, H^6), 7.46–7.43 (2H, m, 4-phenyl H^3, H^5), 7.31–7.16 (5H, m, 5-phenyl), 3.95 (2H, s, $-\underline{CH_2}$–CO–), 2.97 (2H, q, –NH–$\underline{CH_2}$–), 1.30–1.13 (12H, m, $-\underline{CH_2}$–), 0.86 (3H, t, $-\underline{CH_3}$). Anal. Calc. for $C_{25}H_{30}N_2O_3$: C, 73.86; H, 7.44; N, 6.89. Found: C, 73.97; H, 7.56; N, 6.88%.

5.1.6.2. N-*Phenylethyl-2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)acetamide (**16**).* Recrystallized from ethanol to yield 56%; m.p. 168–170 °C. ^{1}H NMR (DMSO-d_6) δ: 8.17 (1H, t, –NH–), 7.58–7.53 (3H, m, 4-phenyl H^2, H^4, H^6), 7.45–7.43 (2H, m, 4-phenyl H^3, H^5), 7.31–7.10 (10H, m, phenyl, 5-phenyl), 3.95 (2H, s, $-\underline{CH_2}$–CO–), 3.21 (2H, q, –NH–$\underline{CH_2}$–), 2.61 (2H, t, $-\underline{CH_2}$–C_6H_5). Anal. Calc. for $C_{25}H_{22}N_2O_3$: C, 75.36; H, 5.57; N, 7.03. Found: C, 74.94; H, 5.59; N, 6.99%.

5.1.6.3. N-*(4-Chlorophenyl)-2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)acetamide (**17**).* Recrystallized from ethanol to yield 63%; m.p. 207–208 °C. ^{1}H NMR (DMSO-d_6) δ: 10.25 (1H, s, –NH–), 7.55–7.53 (3H m, 4-phenyl H^2, H^4, H^6), 7.50–7.48 (4H, m, 4-phenyl H^3, H^5, 4-Cl-phenyl H^2, H^6), 7.36–7.34 (2H, d, 4-Cl-phenyl H^3, H^5), 7.30–7.19 (5H, m, 5-phenyl), 4.23 (2H, s, $-\underline{CH_2}$–CO–). Anal. Calc. for $C_{23}H_{17}ClN_2O_3$: C, 68.23; H, 4.23; N, 6.92. Found: C, 68.15; H, 4.27; N, 6.90%.

5.1.6.4. N-*(4-Methoxyphenyl)-2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-il)acetamide (**18**).* Recrystallized from ethanol to yield 55%. m.p. 150–151 °C. ^{1}H NMR (DMSO-d_6) δ: 9.96 (1H, s, –NH–), 7.55–7.53 (3H, m, 4-phenyl H^2, H^4, H^6), 7.49–7.48 (2H, m, 4-phenyl H^3, H^5), 7.37–7.35 (2H, d, *J*: 8.8 Hz, 4-CH_3O-phenyl H^2, H^6), 7.30–7.20 (5H, m, 5-phenyl), 6.87–6.85 (2H, d, *J*: 8.8 Hz, 4-CH_3O-phenyl H^3, H^5), 4.18 (2H, s, $-\underline{CH_2}$–CO–), 3.70 (3H, s, $-O\underline{CH_3}$). Anal. Calc. for $C_{24}H_{20}N_2O_4$: C, 71.99; H, 5.03; N, 7.00. Found: C, 72.01; H, 5.11; N, 6.97%.

*5.1.6.5. 3-[2-Oxo-2-(piperidin-1-yl)ethyl]-4,5-diphenyl-2-oxo-3*H*-1,3-oxazole (**19**).* Recrystallized from ethanol to yield 56%; m.p. 160–161 °C. ^{1}H NMR (DMSO-d_6) δ: 7.56–7.54 (3H, m, 4-phenyl H^2, H^4, H^6), 7.42–7.39 (2H, m, 4-phenyl H^3, H^5), 7.29–7.17 (5H, m, 5-phenyl), 4.25 (2H, s, $-CH_2$–CO–), 3.34 (1H, m, piperidine $H^{2(6)}$), 3.21 (3H, t, piperidine $H^{2(6)}$), 1.49–1.47 (2H, m, piperidine $H^{3(5)}$), 1.31–1.30 (2H, m, piperidine $H^{3(5)}$), 1.20–1.19 (2H, m, piperidine H^4). Anal. Calc. for $C_{22}H_{22}N_2O_3$: C, 72.91; H, 6.12; N, 7.73. Found: C, 72.90; H, 6.19; N, 7.70%.

5.1.6.6. N-*(1,3-Thiazol-2-yl)-2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)acetamide (**20**).* Recrystallized from ethanol to yield 33%; m.p. 185–186 °C. ^{1}H NMR (DMSO-d_6) δ: 12.34 (1H, s, NH), 7.54–7.44 (6H, m, 4-phenyl, thiazole H^4), 7.33–7.18 (6H, m, 5-phenyl, thiazole H^5), 4.35 (2H, s, $-CH_2$–CO–). Anal. Calc. for $C_{20}H_{15}N_3O_3S$: C, 63.65; H, 4.01; N, 11.13; S, 8.50. Found: C, 63.53; H, 4.15; N, 11.10; S, 8.34%.

*5.1.6.7. 3-[2-Oxo-2-(morpholin-1-yl)ethyl]-4,5-diphenyl-2-oxo-3*H*-1,3-oxazole (**21**).* Recrystallized from ethanol to yield 51%; m.p. 174–176 °C. ^{1}H NMR (DMSO-d_6) δ: 7.56–7.54 (3H, m, 4-phenyl H^2, H^4, H^6), 7.41–7.39 (2H, m, 4-phenyl H^3, H^5), 7.28–7.16 (5H, m, 5-phenyl), 4.30 (2H, s, $-CH_2$–CO–), 3.43–3.41 (2H, m, morpholine $H^{2(6)}$), 3.35–3.33 (4H, m, morpholine H^3, H^5, H, H), 3.28–3.27 (2H, m, morpholine $H^{2(6)}$). Anal. Calc. for $C_{21}H_{20}N_2O_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.38; H, 5.58; N, 7.68%.

*5.1.7. Ethyl 2-{4-phenyl-5-[4-(*N,N*-dibenzylaminosulfonyl)phenyl]-2-oxo-3*H*-1,3-oxazol-3-yl}ethanoate (**12**)*

Ethyl 2-(4,5-diphenyl-2-oxo-3*H*-1,3-oxazol-3-yl)ethanoate (**9**) (0.01 mol) was dissolved in chloroform (4 mL). Chlorosulfonic acid (0.05 mol) was added and the reaction mixture stirred at rt for 30 min. The mixture then was poured into a slush of 200 g of ice and then added 50 mL ether. Organic phase was separated, washed with water, dried with Na_2SO_4 and evaporated to dryness (yield 75%). 0.0075 mol of the solid residue (ethyl 2-{4-phenyl-5-[4-(chlorosulfonyl)phenyl]-2-oxo-3*H*-1,3-oxazol-3-yl}ethanoate) (**11**) was dissolved in 25 mL of CH_2Cl_2 and the resulting solution was added dropwise to a mixture of 0.01 mol of dibenzylamine and 0.01 mol of triethylamine in 15 mL CH_2Cl_2 and being stirred at room temperature. After the reaction was complete, CH_2Cl_2 was evaporated to dryness, acetone was added to the residue and the precipitate formed was filtered off. Acetone was evaporated and the residue was crystallized from ethanol to yield 90%; m.p. 110 °C. ^{1}H NMR (DMSO-d_6) δ: 7.76–7.74 (2H, d, 5-phenyl H^3, H^5), 7.62–7.60 (3H, m, 4-phenyl H^2, H^4, H^6), 7.49–7.47 (2H, m, 4-phenyl H^3, H^5), 7.34–7.32 (2H, d, 5-phenyl H^2, H^6), 7.18–7.17 (6H, dd, –N–benzyl H^2, H^4, H^6), 7.05–7.02 (4H, dd, –N–benzyl H^3, H^5), 4.27 (2H, s, $-\underline{CH_2}$–CO–), 4.25 (4H, s, C_6H_5–$\underline{CH_2}$–N–), 4.05 (2H, q, –O–$\underline{CH_2}$–CH_3), 1.08 (3H, t, $-CH_2$–$\underline{CH_3}$). Anal. Calc. for $C_{31}H_{26}N_2O_6S$: C, 68.02; H, 5.19; N, 4.81; S, 5.50. Found: C, 67.56; H, 5.38; N, 4.81; S, 5.26%.

*5.1.8. Ethyl 2-{4-phenyl-5-[4-(aminosulfonyl)phenyl]-2-oxo-3*H*-1,3-oxazol-3-yl}ethanoate (**13**)*

A suspension of 1 mmol of ethyl 2-{4-phenyl-5-[4-(*N*,*N*-dibenzylaminosulfonyl)phenyl]-2-oxo-3*H*-1,3-oxazol-3-yl}ethanoate (**12**) in 1.3 mL concentrated H_2SO_4 was stirred at room temperature for 20 min. The mixture was poured into ice. The resulting solid was filtered, washed with water, dried and crystallized from methanol/water to yield 28%; m.p. 163–164 °C. ^{1}H NMR (DMSO-d_6) δ: 7.73–7.71 (2H, d, 5-phenyl H^3, H^5), 7.60–7.58 (3H, m, 4-phenyl H^2, H^4, H^6), 7.48–7.45 (2H, m, 4-phenyl H^3, H^5), 7.36 (2H, s, disappeared after D_2O exchange, NH_2), 7.34–7.32 (2H, d, 5-phenyl H^2, H^6), 4.28 (2H, s, $-\underline{CH_2}$–CO–), 4.04 (2H, q, –O–$\underline{CH_2}$–CH_3), 1.08 (3H, t, $-CH_2$–$\underline{CH_3}$). Anal. Calc. for $C_{19}H_{18}N_2O_6S$: C, 56.71; H, 4.51; N, 6.96; S, 7.97. Found: C, 56.72; H, 4.61; N, 6.94; S, 7.99%.

*5.1.9. 2-{4-Phenyl-5-[4-(aminosulfonyl)phenyl]-2-oxo-3*H*-1,3-oxazol-3-yl} ethanoic acid (**14**)*

Ethyl 2-{4-phenyl-5-[4-(aminosulfonyl)phenyll]-2-oxo-3*H*-1,3-oxazol-3-yl}ethanoat (**13**) (0.01 mol) was heated up to reflux temperature in concentrated HCl for 12 h. After cooling and diluted with water, the precipitate formed was filtered off, washed with

water, dried and recrystallized from ethanol/water to obtain a yield of 20%; m.p. 224–226 °C. ^{1}H NMR (DMSO-d_6) δ: 71–7.69 (2H, d, 5-phenyl H^3,H^5), 7.59–7.58 (3H, m, 4-phenyl H^2, H^4, H^6), 7.47–7.44 (2H, m, 4-phenyl H^3, H^5), 7.36 (2H, s, NH_2), 7.32–7.30 (2H, d, 5-phenyl H^2, H^6), 4.13 (2H, s, N–$\underline{CH_2}$–CO). Anal. Calc. for $C_{17}H_{14}N_2O_6S$: C, 54.54; H, 3.77; N, 7.48; S, 8.57. Found: C, 54.24; H, 4.12; N, 7.18; S, 7.83%.

5.2. Biological assays

5.2.1. Cyclooxygenase inhibition

5.2.1.1. In vitro assay of purified COX-1 and COX-2 with exogenous substrate (arachidonic acid). The method described by Futaki et al. [26] and by Janusz et al. [27] was followed with some modifications. Briefly, the assay was carried out in a final volume of 0.5 ml with Tris–HCl buffer (100 mM, pH 8) as the reaction medium containing hematin (1 μM) phenol (2 mM) as cofactors, and EDTA (5 mM). After adding 50 μL of the test compound (10 μM), the reference compound (1 or 100 μM) or the vehicle (DMSO, 1%), a unit of ovine purified COX-1 or COX-2 (Cayman Chemical) was suspended in the reaction medium and preincubated at 37 °C with continuous stirring for 10 min. The reaction was initiated with 50 μL of arachidonic acid (100 μM). After 5 min incubation in the same condition, the reaction was stopped by addition of 50 μL 1 N HCl followed by neutralization with 50 μL of Tris Base (1 M). 50 μL of the final solution were diluted to a final volume of 500 μL with the immunoenzymatic assay buffer, and 50 μL of the diluted sample were taken to test prostaglandin E2 (PGE2) by enzyme immunoassay (EIA) (Amersham). Detection was carried out in a microplate reader (Lansystems multiscan MS) at 450 nm, and data were processed by means of GENESIS-LITE program (Windows-based Microplate Software). Indometacin (COX-1, $IC_{50} = 0.069$ μM, COX-2, $IC_{50} = 0.537$ μM) and rofexocib (COX-1, $IC_{50} = >100$ μM, COX-2, $IC_{50} = 0.398$ μM) were used as nonselective COX inhibitor and selective COX-2 inhibitor references in the assays. Rofecoxib tested at 1 μM final concentration for COX-2 and 100 μM for COX-1. Results were expressed as the percent COX-1 or COX-2 inhibition relative to vehicle and calculated by:

$$\left([PGE_2]_{vehicle} - [PGE_2]_{drug}\right) \times 100/[PGE_2]_{vehicle}$$

For compounds with which COX-2 inhibition exceeded 50%, IC_{50} values were calculated by the GraphPad software PRISM. Selectivity indexes were defined as the ratio: [IC_{50}(COX-1)]/[IC_{50}(COX-2)].

5.2.1.2. Human whole blood COX-1 and COX-2 assay. The human whole blood assay, originally developed by Patrignani et al. [28], is considered to be the more biologically relevant way to assess the inhibition of the cyclooxygenase isoenzymes, COX-1 and COX-2, by a test compound [34]. In this assay, platelets stimulated upon coagulation are believed to be the main source of COX-1, whereas monocytes stimulated with LPS are thought to be the source of COX-2. COX-1 activity is determined by the production of thromboxane B_2 (TXB_2), while COX-2 activity is determined by the production of prostaglandin E_2 (PGE_2).

For testing the COX-1 activity, fresh blood from healthy volunteers who had not taken any NSAIDs for at least 7 days prior to blood extraction was collected in vacuutainer tubes without any anticoagulant. Aliquots of 500 μL of blood were incubated either with 1 μL of vehicle (DMSO) or 1 μL of test compound solution (10 μM) for 1 h at 37 °C. Plasma was separated by centrifugation (5 min at 13,000 rpm, 4 °C) and TXB_2 levels were measured using the Correlate-EIA™ TXB_2 Enzyme Immunoassay Kit from Assay Design Inc. (Ann Arbor, MI).

For COX-2, fresh blood from healthy volunteers who had not taken any NSAIDs for at least 7 days prior to blood extraction was collected in EDTA-containing tubes. Aliquots of 500 μL of blood were incubated either with 1 μL of vehicle (DMSO) or 1 μL of test compound solution (10 μM) in the presence of LPS (10 μg/mL) for 24 h at 37 °C to induce COX-2 expression. Plasma was separated by centrifugation (5 min at 13000 rpm, 4 °C) and PGE_2 levels were measured using the Correlate-EIA™ PGE_2 Enzyme Immunoassay Kit from Assay Design Inc. (Ann Arbor, MI).

5.3. Computational details

Docking studies have been performed using Glide program (Glide is available from Schrödinger Inc., http://www.schrodinger.com). With this purpose, crystal structure of both COX-1/iodoindomethacin (a nonselective inhibitor) and COX-2/SC558 (a selective inhibitor) complexes (PDB codes: 1PGG and 6COX, respectively) were obtained from the Protein Data Bank in order to prepare both proteins for docking studies. Compound **6** structure was built using Sybyl7.3 program (Sybyl is available from Tripos Inc., www.tripos.com), and after the proper minimization an optimization was exported to Glide for docking. Docking procedure was followed using the standard protocol implemented in Glide and the geometry of resulting complexes was studied using the Glide's Pose Viewer utility.

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