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Synthesis, biological evaluation, and docking studies of novel heterocyclic diaryl compounds as selective COX-2 inhibitors

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ABSTRACT

Three novel series of diaryl heterocyclic derivatives bearing the 2-oxo-5*H*-furan, 2-oxo-3*H*-1,3-oxazole, and 1*H*-pyrazole moieties as the central heterocyclic ring were synthesized and their in vitro inhibitory activities on COX-1 and COX-2 isoforms were evaluated using a purified enzyme assay. The 2-oxo-5*H*-furan derivative **6b** was identified as potent COX inhibitor with selectivity toward COX-1 (COX-1 IC_{50} = 0.061 μM and COX-2 IC_{50} = 0.325 μM; selectivity index (SI) = 0.19). Among the 1*H*-pyrazole derivatives, **11b** was found to be a potent COX-2 inhibitor, about 38 times more potent than Rofecoxib (COX-2 IC_{50} = 0.011 μM and 0.398 μM, respectively), but showed no selectivity for COX-2 isoform. Compound **11c** demonstrated strong and selective COX-2 inhibitory activity (COX-1 IC_{50} = 1 μM, COX-2 IC_{50} = 0.011 μM; SI = ~92). Molecular docking studies of compounds **6b** and **11b–d** into the binding sites of COX-1 and COX-2 allowed to shed light on the binding mode of these novel COX inhibitors.

1. Introduction

Cyclooxygenases (COXs), which catalyze the first step in arachidonic acid metabolism,[1] are the molecular targets of the nonsteroidal antiinflammatory drugs (NSAIDs).[2–4] COX enzymes exist at least in three isoforms, namely cyclooxygenase-1 (COX-1), a constitutive enzyme, cyclooxygenase-2 (COX-2), an isoform induced in response to a variety of pro-inflammatory stimuli,[5,6] and cyclooxygenase-3 (COX-3), present mainly in the cerebral cortex and human heart.[7] It was thought that while the antiinflammatory effect of NSAIDs occurs as a result of COX-2 inhibition, many of the undesirable side effects including; gastric irritation were due to the inhibition of COX-1 isoform.[8] Therefore, several new inhibitors directed towards COX-2 without interfering with COX-1 enzymatic activity were developed during the last two decades. These molecules, termed coxibs (i.e., Celecoxib,[9] Rofecoxib,[10] Valdecoxib,[11] and Etoricoxib[12]) showed reduced gastrointestinal side effects as compared to traditional NSAIDs.[13] However, coxibs were recently withdrawn from the market because of an increased risk of cardiovascular side effects particularly observed with Rofecoxib as a result of its long-term use in clinic. Despite this apparent failure, there is still growing interest for the design of novel COX-2 inhibitors owing to the reduced gastric and renal side effects as compared to conventional NSAIDs and also due to the additional therapeutic benefits of COX-2 inhibitors in several diseases including certain types of cancer.[14–18]

Selective COX-2 inhibitors so far can be divided into three classes, namely (i) vicinal diaryl heterocycle inhibitors (coxibs), (ii) sulfonanilide inhibitors, and (iii) modified classical nonselective NSAIDs. The pharmacophore of diaryl heterocycle inhibitors is characterized by a vicinal diaryl substitution about a central carbocyclic or heterocyclic ring system in which one of the aryl groups is substituted by a methylsulfonyl or sulfonamido group at *para* position.[19]

Although modifications on established nonselective NSAIDs[20] such as lengthening of the carboxyl side chain[21] of Indomethacin, could let to effective COX-2 selective inhibitors, most of the successful efforts have so far been directed to the diaryl heterocycle class. In particular, structural variation of the central ring in the tricyclic series is still a popular area of research.[22]

Therefore, we focused our attention to the design and synthesis on three different diaryl heterocyclic ring systems in which the 2-oxo-5*H*-furan, 2-oxo-3*H*-1,3-oxazole, and 1*H*-pyrazole moieties served as the central ring template bearing 3-methyl-2-oxo-3*H*-benzoxazole and 4-substituted phenyl group as vicinal aryl

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moieties. Several 2-oxo-3*H*-benzoxazole derivatives have been previously reported as analgesic and antiinflammatory agents, and some of them were reported to reduce PGE_2 formation in the exudates of paw edema.[23–26] Therefore, we aimed to take advantage of 3-methyl-2-oxo-3*H*-benzoxazole moiety as replacement of one of the aryl groups within the diaryl heterocyclic system to insure some additional structural benefits for COX-2 inhibition (Fig. 1).

2. Results and discussion

2.1. Synthesis

We focused our synthetic efforts on three different diaryl heterocyclic ring systems as illustrated in Schemes 1–4. The starting compound, 3-methyl-2-oxo-3*H*-benzoxazole (**2**), was prepared by methylation with dimethyl sulfate of 2-oxo-3*H*-benzoxazole (**1**) which was readily synthesized via the reaction of *o*-aminophenol and urea. Compound **2** was then converted to 6-bromoacetyl-3-methyl-2-oxo-3*H*-benzoxazole (**3**) and to 6-acetyl-3-methyl-2-oxo-3*H*-benzoxazole (**4**) via Friedel–Crafts acylation under microwave conditions (Scheme 1).

To obtain the 2-oxo-5*H*-furans (**6a–e**), compound **3** was reacted with appropriate phenylacetic acid derivatives to obtain 2-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl phenyl acetates (**5a–e**). For the ring closure reaction, compounds **5a–e** were heated in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to furnish the 3-phenyl-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-5*H*-furans (**6a–e**) (Scheme 2).

Reaction of compound **3** with sodium formate afforded 2-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl formate (**7i**) which was converted without purification to 6-hydroxyacetyl-3-methyl-2-oxo-3*H*-benzoxazole (**7**) by acid catalyzed hydrolysis. Compound **7** was reacted with appropriate phenyl isocyanates to yield 2-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl phenyl carbamates (**8a–e**) which were then cyclized to provide the corresponding 2-oxo-3*H*-1,3-oxazoles (**9a–e**) in refluxing acetic acid. The methylsulfonyl derivative (**9f**) was prepared by oxidation of methylthio derivative with *m*-chloroperbenzoic acid (70%) in dichloromethane (Scheme 3).

4,4,4-Trifluoro-1-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1,3-dioxobutane (**10**) was obtained from the reaction between compound **4** and ethyl trifluoroacetate in the presence of potassium *t*-butoxide. Subsequent, treatment of compound **10** with appropriate phenylhydrazine hydrochlorides led to 1-phenyl-3-trifluoromethyl-5-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1*H*-pyrazoles (**11a–e**) by the ring closure reaction under microwave conditions (Scheme 4).

Structures of all target compounds were elucidated by spectroscopic data and confirmed by elemental analyzes.

2.2. Biological evaluation

The 3,4-diaryl-2-oxo-5*H*-furans (**6a–e**), the 3,4-diaryl-2-oxo-3*H*-1,3-oxazoles (**9a–d** and **9f**), and the 1,5-diaryl-1*H*-pyrazoles (**11a–e**) were initially screened at 10 μM to determine their COX-2 inhibitory activities using purified enzyme assay.[27,28] Indomethacin and Rofecoxib were used as nonselective and COX-2 selective inhibitor standards, respectively, under the same assay conditions. The in vitro activity results are reported as a percentage of inhibition of the purified enzymes at 10 μM (Table 1). For the compounds exhibited inhibition more than 50% for COX-2, the inhibition of COX-1 at 10 μM and the IC_{50} values for both isoforms were also calculated from the concentration–response curves by means of the PRISM 4.0, GraphPad Software.[29]

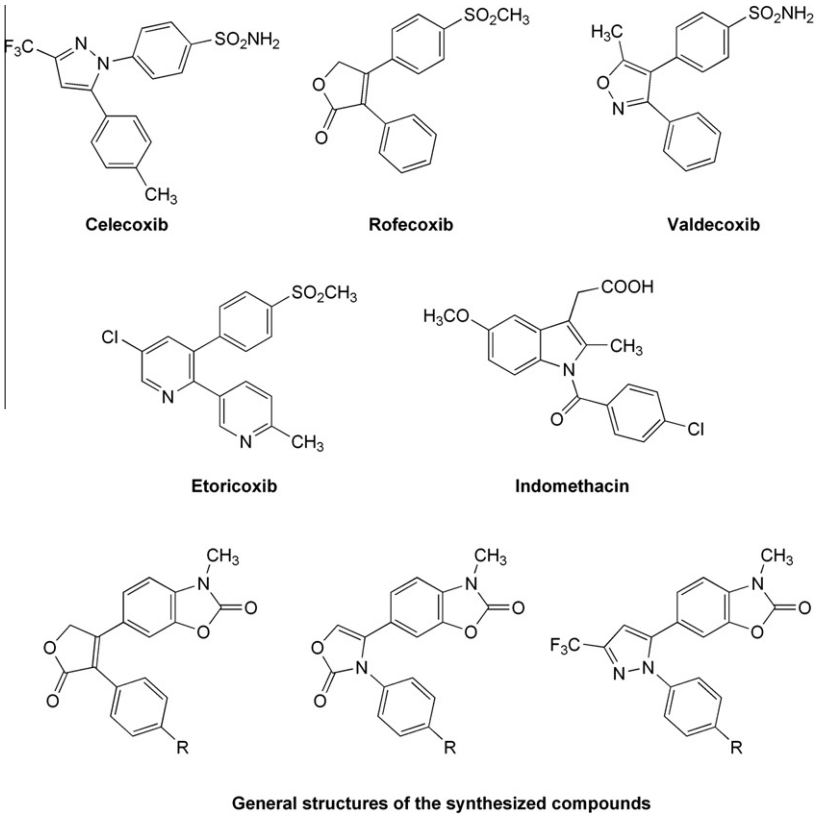
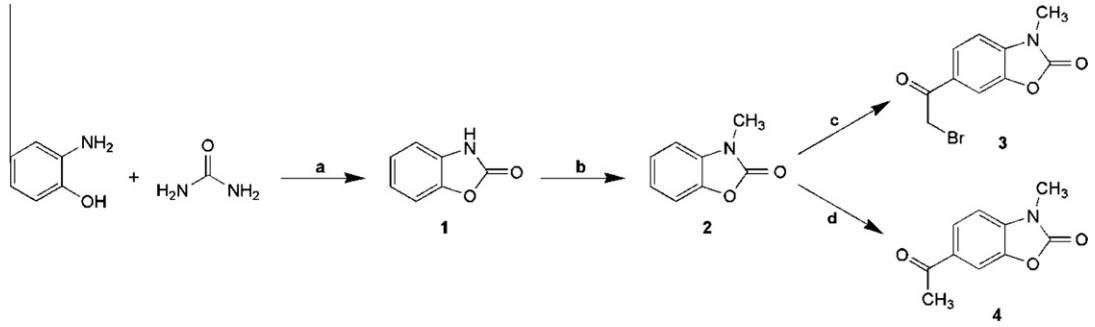
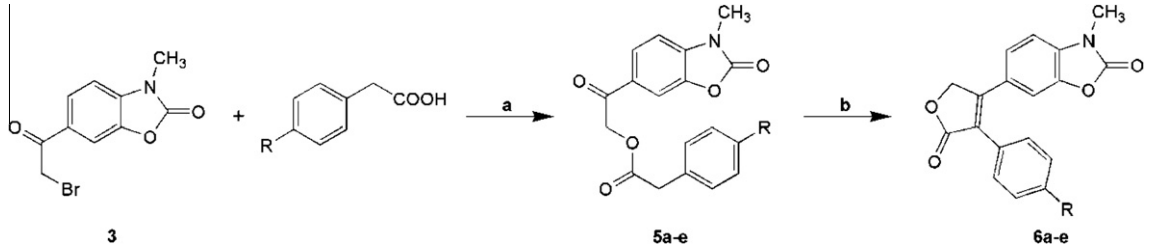


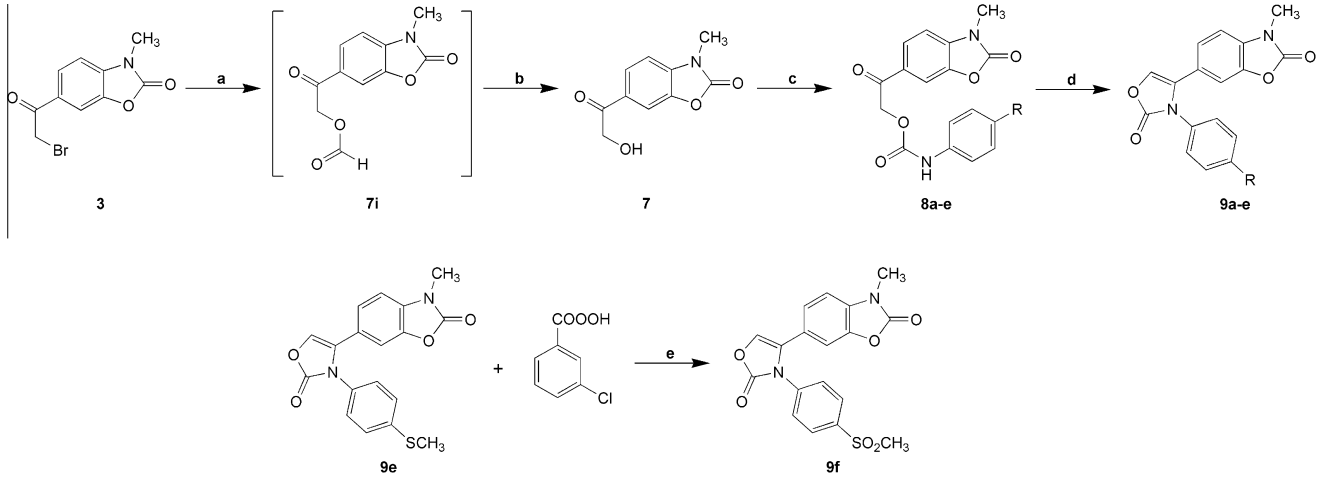
Figure 1. Structures of marketed COX-2 inhibitors and general structures of the synthesized compounds.



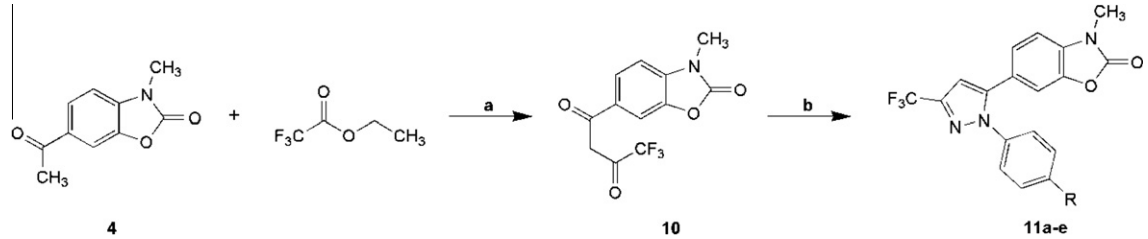
Scheme 1. Reagents and conditions: (a) MW irradiation, 140 °C, 10 min; (b) dimethyl sulfate, NaOH, 30 min; (c) bromoacetic acid, polyphosphoric acid (PPA), MW irradiation, 120 °C, 70 min; (d) acetic acid, PPA, MW irradiation, 90 °C, 20 min.



Scheme 2. Reagents and conditions: (a) TEA, CH_3CN, 4 h, reflux; (b) DBU, CH_3CN, 1–2 h, rt.



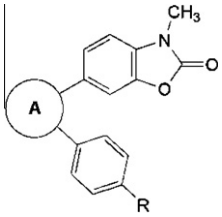
Scheme 3. Reagents and conditions: (a) sodium formate, CH_3CN, 4 h, reflux; (b) HCl, reflux; (c) appropriate phenyl isocyanate derivative, toluene, 7–8 h reflux; (d) acetic acid, 3–4 h reflux; (e) CH_2Cl_2, 5 h, rt.

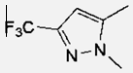


Scheme 4. Reagents and conditions: (a) potassium *t*-butoxide, ethyl trifluoroacetate, benzene, 5 h reflux; (b) appropriate phenylhydrazine HCl derivative, HCl, MW irradiation, 90 °C, 1 h.

Only compound **6b** from the 3,4-diaryl-2-oxo-5*H*-furan class (**6a–e**), significantly inhibited the activity of both COX-2 and COX-1 at 10 μM screening concentration. The calculated IC_{50} values of compound **6b** for COX-2 and COX-1 were found to be

Table 1
In vitro purified COX-2 and COX-1 enzyme inhibition assay data for the synthesized compounds



A	Compound	R	% Inhibition[a]		IC_{50} (μM)		SI
			COX-2	COX-1	COX-2	COX-1	
	**6a**	H	0.13 ± 6.16	nt[b]	—	—	—
	6b	OCH_3	**59.77 ± 0.78**	80.94 ± 3.77	0.325	0.061	0.19
	6c	Cl	48.07 ± 0.58	nt	—	—	—
	6d	F	11.60 ± 7.35	nt	—	—	—
	6e	SO_2CH_3	0.40 ± 2.46	nt	—	—	—
	**9a**	H	−22.34 ± 7.90	nt	—	—	—
	9b	OCH_3	47.10 ± 1.05	nt	—	—	—
	9c	Cl	36.96 ± 3.13	nt	—	—	—
	9d	F	0.00 ± 2.60	nt	—	—	—
	9f	SO_2CH_3	15.55 ± 5.25	nt	—	—	—
	**11a**	H	21.27 ± 1.34	nt	—	—	—
	11b	OCH_3	**94.90 ± 0.32**	95.61 ± 0.65	0.011	0.004	0.36
	11c	Cl	**93.19 ± 0.40**	91.21 ± 1.14	0.011	1	91.90
	11d	F	**66.82 ± 1.20**	55.47 ± 8.57	1	6	6
	11e	SO_2CH_3	29.23 ± 1.19	nt	—	—	—
—	Indomethacin	—	89.94 ± 0.29	71.89 ± 6.31	0.537	0.069	0.13
—	Rofecoxib[c]	—	59.34 ± 3.39	13.66 ± 6.66	0.398	>100	253

[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.

0.325 μM and 0.061 μM, respectively. The selectivity index (SI) defined as $[IC_{50}(COX\text{-}1)]/[IC_{50}(COX\text{-}2)]$ for compound **6b** is 0.19 as compared to Indomethacin (SI = 0.13) and Rofecoxib (SI = 250).

Generally, none of the compounds having the 2-oxo-3*H*-1,3-oxazole as a central ring showed the desired activity profile of COX-2. Only for compound **9b** the COX-2 inhibitor activity was found to be moderate (47%).

From the 1*H*-pyrazole series, compounds **11b**, **11c**, and **11d** significantly inhibited the activity of both COX-1 and COX-2 enzymes. Although compound **11b** is about 38 times more potent than Rofecoxib when tested in the in vitro COX-2 purified enzyme assay, it did not show any considerable selectivity towards COX-2. Additionally, compound **11c** was about 36 times more potent than Rofecoxib in the in vitro COX-2 purified enzyme assay and it showed some selectivity for COX-2. Compound **11d** is about 2.5 times less potent than Rofecoxib and was proved to be COX-2 selective.

2.3. Molecular modeling

Molecular docking studies of compounds **6b** and **11b–d** in the active sites of COX-1 (PDB code: 1PGF)[30] and COX-2 (PDB code: 6COX)[31] were performed in order to get further insight into the nature of interactions between the compounds and the active site amino acids to rationalize the obtained biological results.

Docking of compound **6b**, 2-oxo-5*H*-furan derivative, into COX-2 active site, showed an orientation similar to that of Rofecoxib as reported previously.[32] The carbonyl oxygen of the furanone moiety of compound **6b** is placed close to the side chain of Ser530 to consider the possible formation of hydrogen bond whereas the phenyl ring has been accommodated in the hydrophobic pocket (Fig. 2a, blue residues), and that the polar and bulky benzoxazole moiety is inserted into the selectivity pocket (Fig. 2a, green residues) that is appropriate to accommodate such fragments. On the other hand, the ligand is slightly destabilized by the insertion of methoxyphenyl group into the hydrophobic pocket. Binding energy and docking energy of −10.51 kcal/mol and −11.49 kcal/mol are obtained, respectively. Figure 2b reports the binding mode of compound **6b** in COX-1 active site (binding energy: −11.03 kcal/mol; docking energy: −10.95 kcal/mol). It is interesting to note that the binding orientation of compound **6b** in the active site of COX-1 is different to the orientation of the same compound in the binding site of COX-2. At this regard, it should be observed that very recently Rimon et al.[33] have reported the X-ray structure of Celecoxib bound to COX-1. Although the disposition of compound **6b** in our best pose is different from that Celecoxib in the X-ray structure of COX-1, our result confirms that the synthesized diaryl heterocyclic derivatives can productively be docked into the active site of COX-1. Whether this binding results or not into a functional inhibition is more complicated to predict, since it may be related to kinetic of dissociation rather than to the stability of the complex. Given our biological results, it can be speculated that the orientation we found for compound **6b** into COX-1 is able to interfere with the metabolism of arachidonic acid, at difference of what observed for Celecoxib in Rimon et al.[33]

The docking study showed that compound **11b** is bound to the primary binding site of COX-2 with the trifluoromethyl moiety which interacts with the polar zone (so-called 'trifluoromethyl zone') residue Arg120 (Fig. 3). The benzoxazole moiety lies in the selectivity pocket and the methoxyphenyl ring is located in a hydrophobic region (binding energy: −11.26 kcal/mol; docking energy: −12.49 kcal/mol).

A similar placement of compounds **11c** and **11d** has also been observed during their dockings in the active site of COX-2. Conformational superposition of SC-558 (from the X-ray crystal structure of SC-558:COX-2 complex) and compounds **11b–d** (from the

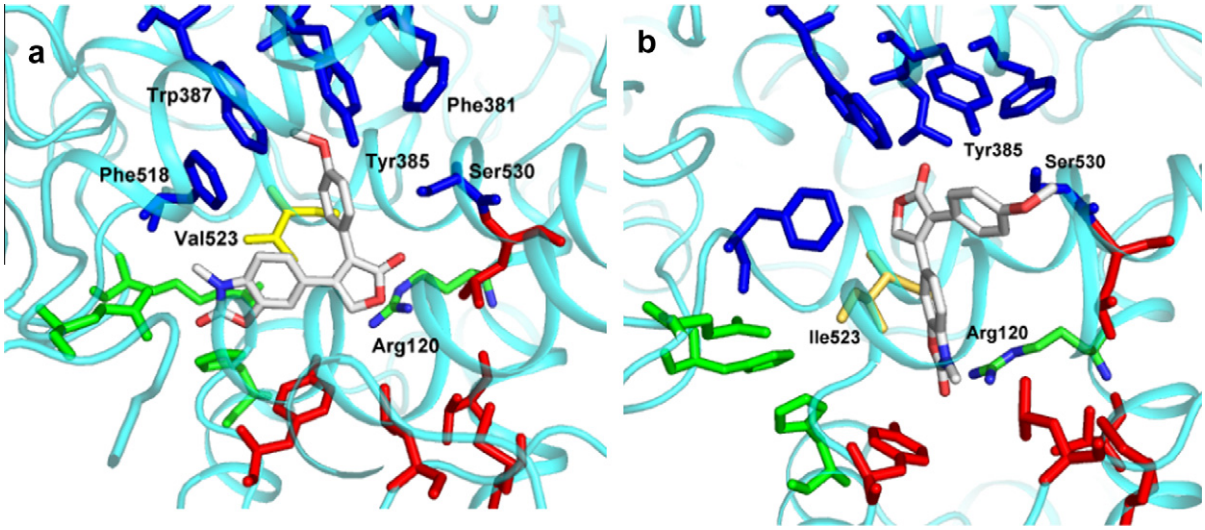


Figure 2. (a) Structure of compound **6b** docked into the binding site of COX-2 (blue: hydrophobic pocket, red: polar zone, yellow: responsible for the COX-2 selectivity, green: selectivity pocket). (b) Structure of compound **6b** docked into the binding site of COX-1.

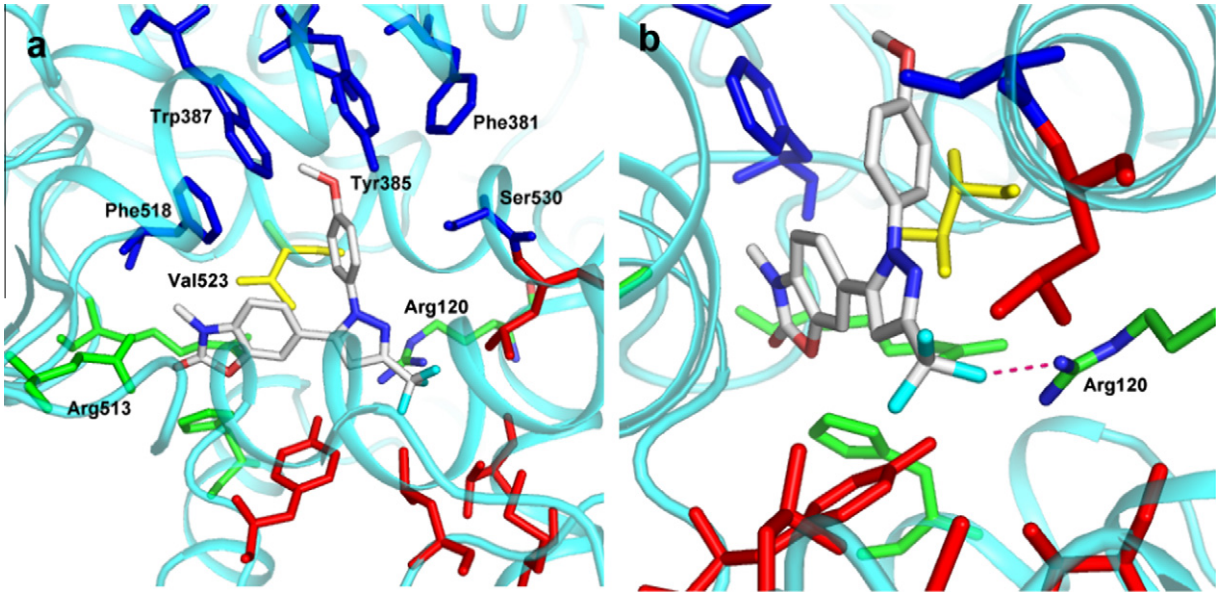


Figure 3. (a) Structure of compound **11b** docked into the binding site of COX-2. (b) Compound **11b** forms a H-bond with Arg120 (presented in magenta).

docking simulation) are shown in Figure 4. The superposition showed their hydrophilic and hydrophobic groups overlapped with each other. From the above mentioned data, the molecular modeling studies of the examined compounds **11b–d** showed that they bound to the COX-2 active site with position and orientation very similar to that of the crystal structure of SC-558 complex with COX-2. Consequently, these observations provided a good explanation for the observed potent inhibitory activity of compounds **11b–d**.

Docking studies were also carried out on compounds **6e** and **11e** with the aim of explaining their inactivity in inhibiting COX-2. As reported in Figure 5, the best obtained poses for both compounds are very similar to the crystallographic disposition of SC-558 in the binding pocket of COX-2. There are no apparent reasons for the inactivity of compounds **6e** and **11e**. The failure of static docking experiments in predicting the inactivity of these analogs, closely related to COX-2 active diaryl heterocyclic derivatives can be commented on the light of a recent paper by Limongelli et al.[34] who put forward not only the importance of the stability of the binding mode, but also the importance of the dynamic path of ligand(s) to reach the binding pose. In this context, Limongelli et al. proposed the existence of alternative binding modes of diaryl heterocyclic derivatives to COX-2. It can be speculated that the reason for the inactivity of compounds **6e** and **11e** may reside in their inability to achieve one or more alternative binding modes possibly relevant to functional inhibition.

3. Conclusion

The present study describes the synthesis of three new series of COX inhibitors in which the 2-oxo-5*H*-furan, 2-oxo-3*H*-1,3-oxazole, or 1*H*-pyrazole scaffolds are present in the central part, to which 3-methyl-2-oxo-3*H*-benzoxazole and 4-substituted phenyl moieties have been attached at vicinal positions. The results of biological evaluation revealed that especially compounds **11c** and **11d** belonging to 1*H*-pyrazole series exhibited the highest activity

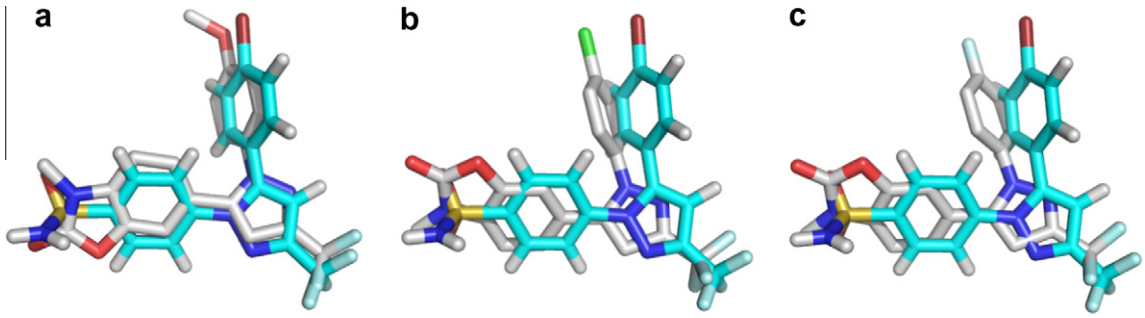


Figure 4. (a) The binding conformations found for **11b** (gray); (b) for **11c** (gray, binding energy: −11.35 kcal/mol, docking energy: −12.44 kcal/mol); (c) for **11d** (gray, binding energy: −10.88 kcal/mol, docking energy: −11.91 kcal/mol), in comparison with SC-558 (cyan) from the X-ray crystal structure of SC-558:COX-2 complex (PDB code: 6COX).

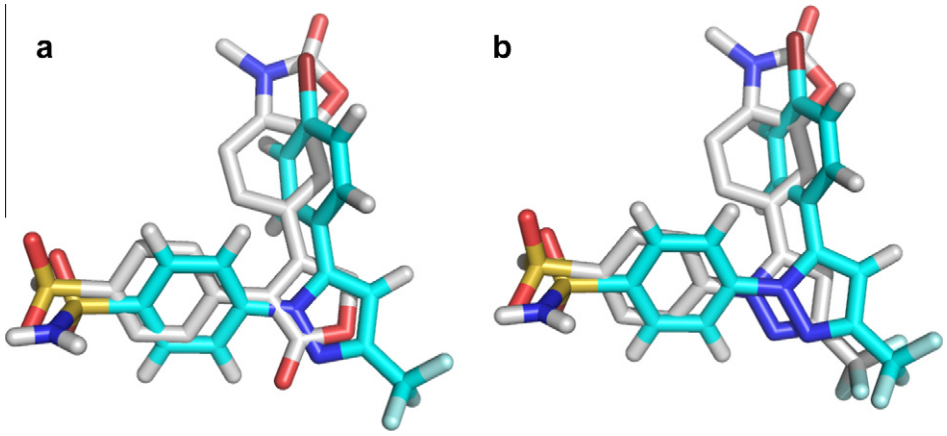


Figure 5. (a) The binding conformations found for **6e** (gray); (b) for **11e** (gray), in comparison with SC-558 (cyan) from the X-ray crystal structure of SC-558:COX-2 complex (PDB code: 6COX).

compared to the analog series. Compound **11c** was found to be more potent on COX-2, but has lower selectivity compared to Rofecoxib. In addition, the substitution of 1*H*-pyrazole as a suitable central ring template with the benzoxazole moiety can be suggested as an effective scaffold for further design of selective and potent COX-2 inhibitors.

4. Experimental

4.1. Chemistry

The chemicals were purchased from the commercial vendors and were used without purification. Thin-layer chromatography (TLC) was performed on Merck $60F_{254}$ plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or charring Dragendorff reagent.[35] All the melting points were taken on an Electrothermal 9300 capillary apparatus and are uncorrected. IR spectra were recorded on a Bruker Vector 22 spectrometer as KBr disks. ^{1}H NMR spectra were obtained with a Varian 400 MHz spectrometer in *d*-chloroform ($CDCl_3$) or d_6-dimethylsulfoxide (DMSO-d_6) and tetramethylsilane (TMS) was used as an internal standard. LC/MS spectra were performed using Waters Micromass ZQ by using ESI (+) method. Elemental analyzes were performed with LECO-932 (C, H, N, S-Elemental Analyzer). Microwave-assisted reactions were carried out with a Milestone MicroSYNTH Microwave Synthesis System.

4.1.1. 2-Oxo-3*H*-benzoxazole (1)

The dry flask charged with *o*-aminophenol (10.91 g, 0.1 mol) and urea (12.01 g, 0.2 mol) was placed in MicroSYNTH Microwave Synthesis System and irradiated at 400 W for 15 min while the temperature was set to 140 °C. After the reaction was completed, the flask was cooled to room temperature and the solid was solved in 5% solution of sodium hydroxide. After acidification with concentrated HCl the desired product was obtained. 11.61 g, 86% yield; mp 136 °C (Ref. 36; 137–138 °C).

4.1.2. 3-Methyl-2-oxo-3*H*-benzoxazole (2)

This was carried out by the described method,[37] and the reaction product was obtained with a yield of about 90%; mp 83 °C (Ref. 38; 83–84 °C).

4.1.3. 6-Bromoacetyl-3-methyl-2-oxo-3*H*-benzoxazole (3)

The dry flask charged with 3-methyl-2-oxo-3*H*-benzoxazole (14.90 g, 0.1 mol), bromoacetic acid (16.68 g, 0.12 mol), and PPA (200 g) was placed in MicroSYNTH Microwave Synthesis System and irradiated at 300 W for 70 min while the temperature was set to 120 °C. The reaction mixture was poured into ice-cold water. The precipitated mixture was filtered off, dried, and purified by washing with toluene. 21.6 g, 80% yield; mp 178 °C (Ref. 39; 178–180 °C).

4.1.4. 6-Acetyl-3-methyl-2-oxo-3*H*-benzoxazole (4)

The dry flask charged with 3-methyl-2-oxo-3*H*-benzoxazole (7.45 g, 0.05 mol), acetic acid (3.15 mL, 0.055 mol), and PPA (100 g) was placed in MicroSYNTH Microwave Synthesis System and irradiated at 300 W for 22 min while the temperature was set to 90 °C. The reaction mixture was poured into ice-cold water. The precipitated mixture was filtered off, dried, and purified by recrystallization from ethanol–water mixture. 8.5 g, 89% yield; mp 167–168 °C (Ref. 40; 166–168 °C).

4.2. General procedure for the preparation of 2-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl phenyl acetates (5a–e)

A solution of 6-bromoacetyl-3-methyl-2-oxo-3*H*-benzoxazole (2.70 g, 0.01 mol), the respective phenylacetic acids (0.01 mol), and triethylamine (1.39 mL, 0.01 mol) in acetonitrile was stirred for 4 h at reflux, diluted with water. The precipitated mixture was filtered off, dried, and recrystallized from the appropriate solvent.

4.2.1. 2-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl phenyl acetate (5a)

Recrystallized from ethanol. 1.79 g, 55% yield; mp 128–129 °C; IR (KBr) 3066, 3030, 2959, 2926, 1775, 1753, 1697, 1625, 1236 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.90 (dd, 2H, $^3J_{H-H}$ = 8.4 Hz, $^4J_{H-H}$ = 1.6 Hz), 7.88 (d, 1H, $^4J_{H-H}$ = 1.6 Hz), 7.40 (d, 1H, $^3J_{H-H}$ = 8.2 Hz), 7.36–7.24 (m, 5H), 5.49 (s, 2H), 3.83 (s, 2H), 3.38 (s, 3H). Anal. Calcd for $C_{18}H_{15}NO_5$: C, 66.46; H, 4.65; N, 4.31. Found: C, 66.02; H, 4.58; N, 4.40.

4.2.2. 2-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl (4-methoxyphenyl) acetate (5b)

Recrystallized from ethanol. 2.61 g, 74% yield; mp 136.9 °C; IR (KBr) 3074, 3035, 2956, 2838, 1772, 1734, 1691, 1610, 1233, 1029 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.92 (d, 1H, $^3J_{H-H}$ = 8.4 Hz), 7.89 (s, 1H), 7.41 (d, 1H, $^3J_{H-H}$ = 8.2 Hz), 7.24 (d, 2H, $^3J_{H-H}$ = 8.4 Hz), 6.90 (d, 2H, $^3J_{H-H}$ = 8.4 Hz), 5.49 (s, 2H), 3.75 (s, 2H), 3.74 (s, 3H), 3.39 (s, 3H). Anal. Calcd for $C_{19}H_{17}NO_6$: C, 64.22; H, 4.82; N, 3.94. Found: C, 63.93; H, 4.86; N, 4.06.

4.2.3. 2-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl (4-chlorophenyl) acetate (5c)

Recrystallized from ethanol. 2.54 g, 71% yield; mp 154–155 °C; IR (KBr) 3084, 2946, 1783, 1749, 1686, 1611, 1212 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.90 (dd, 1H, $^3J_{H-H}$ = 8.4 Hz, $^4J_{H-H}$ = 1.2 Hz), 7.89 (s, 1H, $^4J_{H-H}$ = 1.2 Hz), 7.42–7.33 (m, 5H), 5.52 (s, 2H), 3.86 (s, 2H), 3.38 (s, 3H). Anal. Calcd for $C_{18}H_{14}ClNO_5$: C, 60.09; H, 3.92; N, 3.89. Found: C, 59.41; H, 3.77; N, 4.06.

4.2.4. 2-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl (4-fluorophenyl) acetate (5d)

Recrystallized from ethanol. 3.14 g, 92% yield; mp 160.4 °C; IR (KBr) 3084, 3064, 2936, 1780, 1750, 1687, 1610, 1217 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.91 (dd, 1H, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 1.6 Hz), 7.89 (s, 1H), 7.41 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.37 (dd, 2H, $^3J_{H-H}$ = 7.2 Hz, $^4J_{H-F}$ = 5.4 Hz), 7.17 (m, 2H), 5.52 (s, 2H), 3.86 (s, 2H), 3.39 (s, 3H). Anal. Calcd for $C_{18}H_{14}FNO_5$: C, 62.97; H, 4.11; N, 4.08. Found: C, 62.52; H, 4.09; N, 4.18.

4.2.5. 2-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl (4-methylsulfonylphenyl) acetate (5e)

Recrystallized from ethanol. 3.58 g, 89% yield; mp 152–153 °C; IR (KBr) 3064, 3015, 2925, 1776, 1755, 1694, 1622, 1303, 1152, 1236 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.92–7.88 (m, 4H), 7.61 (d, 2H, $^3J_{H-H}$ = 8.4 Hz), 7.40 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 5.53 (s, 2H), 4.01 (s, 2H), 3.37 (s, 3H), 3.22 (s, 3H). Anal. Calcd for $C_{19}H_{17}NO_7S$: C, 56.57; H, 4.25; N, 3.47; S, 7.95. Found: C, 55.80; H, 4.10; N, 3.81; S, 7.72.

4.3. General procedure for the preparation of 3-phenyl-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-5*H*-furanes (6a–e)

The solution of the respective 2-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl phenyl acetate (0.01 mol) and DBU (1.6 mL, 0.011 mol) in acetonitrile was stirred for 1–2 h at room temperature. The precipitated mixture was filtered off, dried, and recrystallized from an appropriate solvent.

4.3.1. 3-Phenyl-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-5H-furane (6a)

Recrystallized from ethanol. 1.41 g, 46% yield; mp 244 °C; IR (KBr) 3064, 2938, 2875, 1776, 1743 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.46–7.40 (m, 3H), 7.35–7.31 (m, 3H), 7.29 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.24 (dd, 1H, $^3J_{H-H}$ = 8.0 Hz, $^4J_{H-H}$ = 1.6 Hz), 5.40 (s, 2H), 3.32 (s, 3H). Anal. Calcd for $C_{18}H_{13}NO_4$: C, 70.35; H, 4.26; N, 4.56. Found: C, 69.74; H, 4.02; N, 4.61.

4.3.2. 3-(4-Methoxyphenyl)-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-5*H*-furane (6b)

Recrystallized from *n*-butanol. 1.72 g, 51% yield; mp 204 °C; IR (KBr) 3063, 2980, 2880, 1776, 1744, 1243, 1064 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.35 (s, 1H), 7.30–7.27 (m, 4H), 6.99 (d, 2H, $^3J_{H-H}$ = 6.8 Hz), 5.35 (s, 2H), 3.79 (s, 3H), 3.33 (s, 3H). Anal. Calcd for $C_{19}H_{15}NO_5$: C, 67.65; H, 4.48; N, 4.15. Found: C, 67.20; H, 4.32; N, 4.23.

4.3.3. 3-(4-Chlorophenyl)-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-5*H*-furane (6c)

Recrystallized from ethanol–water mixture. 2.35 g, 69% yield; mp 207 °C dec; IR (KBr) 3063, 2935, 1794, 1743, 1612 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.52 (d, 2H, $^3J_{H-H}$ = 8.2 Hz), 7.39–7.35 (m, 3H), 7.31 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.24 (dd, 1H, $^3J_{H-H}$ = 8.0 Hz, $^4J_{H-H}$ = 1.6 Hz), 5.39 (s, 2H), 3.33 (s, 3H). Anal. Calcd for $C_{18}H_{12}ClNO_4$: C, 63.26; H, 3.54; N, 4.10. Found: C, 62.66; H, 3.60; N, 4.45.

4.3.4. 3-(4-Fluorophenyl)-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-5*H*-furane (6d)

Recrystallized from acetic acid. 2.35 g, 69% yield; mp 207 °C dec; IR (KBr) 3070, 2927, 1793, 1745, 1214 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 7.41 (dd, 2H, $^3J_{H-H}$ = 8.4 Hz, $^4J_{H-F}$ = 5.6 Hz), 7.27 (s, 1H), 7.20 (dd, 1H, $^3J_{H-H}$ = 8.0 Hz, $^4J_{H-H}$ = 1.6 Hz), 7.16–7.06 (m, 2H), 6.95 (d, 1H, $^3J_{H-H}$ = 7.6 Hz), 5.18 (s, 2H), 3.42 (s, 3H). Anal. Calcd for $C_{18}H_{12}FNO_4$: C, 66.46; H, 3.72; N, 4.31. Found: C, 65.75; H, 3.61; N, 4.39; LC/MS (ES+) *m/z*: 326.14 [M+H], 348.14 [M+Na].

4.3.5. 3-(4-Methylsulfonylphenyl)-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-5*H*-furane (6e)

Recrystallized from ethanol. 2.19 g, 57% yield; mp 276.5 °C dec; IR (KBr) 3087, 3016, 2932, 1798, 1741, 1338, 1152, 1294 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.98 (d, 2H, $^3J_{H-H}$ = 6.4 Hz), 7.61 (d, 2H, $^3J_{H-H}$ = 6.6 Hz), 7.38 (d, 1H, $^4J_{H-H}$ = 1.2 Hz), 7.30 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.21 (dd, 1H, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 1.4 Hz), 5.43 (s, 2H), 3.32 (s, 3H), 3.26 (s, 3H). Anal. Calcd for $C_{19}H_{15}NO_6S$: C, 59.21; H, 3.92; N, 3.63; S, 8.32. Found: C, 59.17; H, 3.90; N, 3.72; S, 8.40.

4.3.6. 6-Hydroxyacetyl-3-methyl-2-oxo-3*H*-benzoxazole (7)

A solution of 6-bromoacetyl-3-methyl-2-oxo-3*H*-benzoxazole (8.10 g, 0.03 mol) and sodium formate (4.08 g, 0.06 mol) in acetonitrile was stirred for 4 h at reflux, diluted with water. The precipitated ester derivative was filtered off, washed with cold ethanol, and hydrolyzed by concentrated HCl. The crude was purified by recrystallization from water. 3.5 g, 56% yield; mp 176–177 °C; IR (KBr) 3447, 3399, 3090, 2929, 1788, 1764 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.88 (dd, 1H, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 1.2 Hz), 7.85 (d, 1H, $^4J_{H-H}$ = 1.2 Hz), 7.38 (d, 1H, $^3J_{H-H}$ = 8.4 Hz), 5.08 (s, 1H), 4.78 (s, 2H), 3.38 (s, 3H). Anal. Calcd for $C_{10}H_9NO_4$: C, 57.97; H, 4.38; N, 6.76. Found: C, 57.75; H, 4.45; N, 6.75.

4.4. General procedure for the preparation of 2-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl *N*-phenyl carbamates (8a–e)

A solution of 6-hydroxyacetyl-3-methyl-2-oxo-3*H*-benzoxazole (2.07 g, 0.01 mol), the respective phenyl isocyanates (0.03 mol) in toluene was stirred for 7–8 h at reflux. After that petroleum ether was added and the precipitated mixture was filtered off, dried, and recrystallized from an appropriate solvent.

4.4.1. 2-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl *N*-phenyl carbamate (8a)

Recrystallized from ethanol. 1.98 g, 61% yield; mp 178–179 °C; IR (KBr) 3301, 3133, 3035, 2946, 1750, 1725, 1699, 1231 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 9.99 (s, 1H), 7.96 (dd, 1H, $^3J_{H-H}$ = 8.4 Hz, $^4J_{H-H}$ = 1.6 Hz), 7.94 (d, 1H, $^4J_{H-H}$ = 1.6 Hz), 7.48 (d, 2H, $^3J_{H-H}$ = 7.6 Hz), 7.43 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.29 (t, 2H, $^3J_{H-H}$ = 7.5 Hz), 7.01 (t, 1H, $^3J_{H-H}$ = 7.2 Hz), 5.52 (s, 2H), 3.40 (s, 3H). Anal. Calcd for $C_{17}H_{14}N_2O_5$: C, 62.57; H, 4.32; N, 8.59. Found: C, 62.36; H, 4.36; N, 8.53.

4.4.2. 2-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl *N*-(4-methoxyphenyl) carbamate (8b)

Recrystallized from ethanol. 3.28 g, 92% yield; mp 183 °C; IR (KBr) 3446, 3396, 3074, 2926, 1764, 1699, 1611, 1093 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 9.76 (s, 1H), 7.94 (dd, 1H, $^3J_{H-H}$ = 8.0 Hz, $^4J_{H-H}$ = 1.2 Hz), 7.90 (d, 1H, $^4J_{H-H}$ = 1.2 Hz), 7.40 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.36 (d, 2H, $^3J_{H-H}$ = 9.2 Hz), 6.86 (d, 2H, $^3J_{H-H}$ = 8.8 Hz), 5.47 (s, 2H), 3.70 (s, 3H), 3.38 (s, 3H). Anal. Calcd for $C_{18}H_{16}N_2O_6$: C, 60.67; H, 4.53; N, 7.86. Found: C, 60.98; H, 4.44; N, 7.87.

4.4.3. 2-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl *N*-(4-chlorophenyl) carbamate (8c)

Recrystallized from ethanol. 2.98 g, 83% yield; mp 214–215 °C; IR (KBr) 3338, 3074, 2936, 1790, 1775, 1696, 1222 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 10.17 (s, 1H), 7.96 (dd, 1H, $^3J_{H-H}$ = 8.4 Hz, $^4J_{H-H}$ = 1.6 Hz), 7.94 (d, 1H, $^4J_{H-H}$ = 1.6 Hz), 7.50 (d, 2H, $^3J_{H-H}$ = 9.2 Hz), 7.44 (d, 1H, $^3J_{H-H}$ = 8.2 Hz), 7.36 (d, 2H, $^3J_{H-H}$ = 8.8 Hz), 5.53 (s, 2H), 3.40 (s, 3H). Anal. Calcd for $C_{17}H_{13}ClN_2O_5$: C, 56.60; H, 3.63; N, 7.77. Found: C, 56.10; H, 3.56; N, 7.77.

4.4.4. 2-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl *N*-(4-fluorophenyl) carbamate (8d)

Recrystallized from ethanol. 2.75 g, 80% yield; mp 204–205 °C; IR (KBr) 3336, 3084, 2946, 1787, 1772, 1733, 1222 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 10.04 (s, 1H), 7.96 (dd, 1H, $^3J_{H-H}$ = 8.4 Hz), 7.93 (s, 1H), 7.48 (dd, 2H, $^3J_{H-H}$ = 8.4 Hz, $^4J_{H-F}$ = 5.2 Hz), 7.43 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.14 (m, 2H), 5.51 (s, 2H), 3.40 (s, 3H). Anal. Calcd for $C_{17}H_{13}FN_2O_5$: C, 59.30; H, 3.81; N, 8.14. Found: C, 59.01; H, 3.82; N, 8.16.

4.4.5. 2-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl *N*-(4-methylthiophenyl) carbamate (8e)

Recrystallized from ethanol. 2.38 g, 64% yield; mp 185 °C; IR (KBr) 3310, 3064, 2926, 1783, 1715, 1692, 1223 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 9.99 (s, 1H), 7.93 (dd, 1H, $^3J_{H-H}$ = 8.0 Hz, $^4J_{H-H}$ = 1.2 Hz), 7.91 (d, 1H, $^4J_{H-H}$ = 1.2 Hz), 7.41 (d, 2H, $^3J_{H-H}$ = 8.4 Hz), 7.20 (d, 2H, $^3J_{H-H}$ = 8.0 Hz), 7.08 (d, 1H, $^3J_{H-H}$ = 8.8 Hz), 5.48 (s, 2H), 3.37 (s, 3H), 2.41 (s, 3H). Anal. Calcd for $C_{18}H_{16}N_2O_5S$: C, 58.05; H, 4.33; N, 7.52; S, 8.61. Found: C, 57.71; H, 4.45; N, 7.41; S, 8.54.

4.5. General procedure for the preparation of 3-phenyl-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-3*H*-1,3-oxazoles (9a–e)

The solution of the respective 2-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxoethyl *N*-phenyl carbamate (0.01 mol) in acetic acid was stirred for 3–4 h at reflux, diluted with water. The precipitated mixture was filtered off, dried, and recrystallized from an appropriate solvent.

4.5.1. 3-Phenyl-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-3*H*-1,3-oxazole (9a)

Recrystallized from ethanol. 2.98 g, 97% yield; mp 197–198 °C; IR (KBr) 3153, 3054, 1791, 1745 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.65 (s, 1H), 7.46–7.36 (m, 3H), 7.28 (dd, 2H, $^3J_{H-H}$ = 6.7 Hz, $^4J_{H-H}$ = 1.6 Hz), 7.20 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.13 (d, 1H, $^4J_{H-H}$ = 1.6 Hz), 6.95 (dd, 1H, $^3J_{H-H}$ = 8.0 Hz, $^4J_{H-H}$ = 1.6 Hz), 3.29 (s, 3H). Anal. Calcd for $C_{17}H_{12}N_2O_4$: C, 66.23; H, 3.92; N, 9.09. Found: C, 65.67; H, 3.94; N, 9.05.

4.5.2. 3-(4-Methoxyphenyl)-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-3*H*-1,3-oxazole (9b)

Recrystallized from ethanol. 3.10 g, 92% yield; mp 212–213 °C; IR (KBr) 3130, 2946, 1775, 1751 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.62 (s, 1H), 7.23–7.19 (m, 3H), 7.14 (d, 1H, $^4J_{H-H}$ = 1.6 Hz), 6.99–6.94 (m, 3H), 3.76 (s, 3H), 3.29 (s, 3H). Anal. Calcd for $C_{18}H_{14}N_2O_5$: C, 63.90; H, 4.17; N, 8.28. Found: C, 63.59; H, 4.10; N, 8.30.

4.5.3. 3-(4-Chlorophenyl)-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-3*H*-1,3-oxazole (9c)

Recrystallized from ethanol. 2.63 g, 77% yield; mp 218–220 °C; IR (KBr) 3143, 3094, 2946, 1767 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.66 (s, 1H), 7.51 (dd, 2H, $^3J_{H-H}$ = 6.8 Hz, $^4J_{H-H}$ = 2.0 Hz), 7.23 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.19 (d, 1H, $^4J_{H-H}$ = 1.6 Hz), 6.94 (dd, 1H, $^3J_{H-H}$ = 8.0 Hz, $^4J_{H-H}$ = 1.6 Hz), 3.30 (s, 3H). Anal. Calcd for $C_{17}H_{11}ClN_2O_4$: C, 59.57; H, 3.23; N, 8.17. Found: C, 59.02; H, 3.26; N, 8.12.

4.5.4. 3-(4-Fluorophenyl)-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-3*H*-1,3-oxazole (9d)

Recrystallized from ethanol. 2.45 g, 75% yield; mp 216 °C; IR (KBr) 3163, 3074, 1793, 1762 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.65 (s, 1H), 7.37–7.26 (m, 4H), 7.22 (d, 1H, $^3J_{H-H}$ = 8.4 Hz), 7.16 (d, 1H, $^4J_{H-H}$ = 1.6 Hz), 6.94 (dd, 1H, $^3J_{H-H}$ = 7.8 Hz, $^4J_{H-H}$ = 1.6 Hz), 3.30 (s, 3H). Anal. Calcd for $C_{17}H_{11}FN_2O_4$: C, 62.58; H, 3.40; N, 8.59. Found: C, 62.15; H, 3.38; N, 8.53.

4.5.5. 3-(4-Methylthiophenyl)-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-3*H*-1,3-oxazole (9e)

Recrystallized from ethanol. 2.15 g, 61% yield; mp 170 °C; IR (KBr) 3065, 2924, 2854, 1768 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.62 (s, 1H), 7.29–7.18 (m, 5H), 7.16 (d, 1H, $^4J_{H-H}$ = 1.6 Hz), 6.95 (dd, 1H, $^3J_{H-H}$ = 8.0 Hz, $^4J_{H-H}$ = 1.6 Hz), 3.28 (s, 3H), 2.46 (s, 3H). Anal. Calcd for $C_{18}H_{14}N_2O_4S$: C, 61.01; H, 3.98; N, 7.90; S, 9.05. Found: C, 61.33; H, 4.04; N, 7.89; S, 9.03.

4.5.6. 3-(4-Methylsulfonylphenyl)-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-3*H*-1,3-oxazole (9f)

The solution of 3-(4-methylthiophenyl)-4-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-2-oxo-3*H*-1,3-oxazole (3.54 g, 0.01 mol) and *m*-chloroperbenzoic acid (70%) (9.92 g, 0.04 mol) in dichloromethane was stirred for 5 h at room temperature, diluted with water and extracted with dichloromethane. The combined organic layer

was washed with saturated solution of sodium bicarbonate and dried with magnesium sulfate and the residue obtained after solvent evaporation was recrystallized from acetone–water mixture. 1.44 g, 41% yield; mp 264 °C dec; IR (KBr) 3152, 3070, 2956, 1797, 1750, 1327, 1151 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 7.94 (d, 2H, $^3J_{H-H}$ = 8.0 Hz), 7.39 (d, 2H, $^3J_{H-H}$ = 8.0 Hz), 7.05 (s, 1H), 7.03 (s, 1H), 6.91 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 6.86 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 3.42 (s, 3H), 3.07 (s, 3H). Anal. Calcd for $C_{18}H_{14}N_2O_6S$: C, 55.95; H, 3.65; N, 7.25; S, 8.30. Found: C, 56.37; H, 3.58; N, 7.47; S, 8.75.

4.5.7. 4,4,4-Trifluoro-1-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1,3-dioxobutane (10)

6-Acetyl-3-methyl-2-oxo-3*H*-benzoxazole (3.00 g, 0.015 mol) and potassium *t*-butoxide (4.2 g, 0.037 mol) were suspended in benzene and to the agitated reaction mixture ethyltrifluoro acetate (3.57 mL, 0.03 mol) was added in portions under ice-cold conditions. The mixture was let to stir for 30 min at 0 °C and then was stirred for 5 h at reflux. The solvent was removed and the residue was stirred with ice-cold water. After acidification with concentrated HCl, the solution was extracted with diethyl ether. The dried organic layer after solvent evaporation left behind an oily residue which was stirred with saturated solution of sodium bicarbonate for overnight at room temperature to afford the desired product. The solid was filtered off and recrystallized from carbon tetrachloride. 2.25 g, 53% yield; mp 313 °C dec; IR (KBr) 3514, 3090, 2890, 1775, 1635 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.72 (dd, 1H, $^3J_{H-H}$ = 7.8 Hz, $^4J_{H-H}$ = 1.6 Hz), 7.66 (d, 1H, $^4J_{H-H}$ = 1.6 Hz), 7.23 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 5.89 (s, 1H), 3.47 (s, 3H). Anal. Calcd for $C_{18}H_{14}N_2O_6S$: C, 46.95; H, 2.59; N, 4.50. Found: C, 46.95; H, 2.39; N, 4.60; LC/MS (ES+) *m/z*: 288.10 [M+H].

4.6. General procedure for the preparation of 1-phenyl-3-trifluoromethyl-5-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1*H*-pyrazoles (11a–e)

The dry flask charged with solution of 4,4,4-trifluoro-1-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1,3-dioxobutane (2.87 g, 0.01 mol), the respective phenylhydrazine HCl (0.011 mol) and concentrated HCl (2 mL, 0.055 mol) was placed in MicroSYNTH Microwave Synthesis System and irradiated at 300 W for 1 h while the temperature was set to 90 °C. The solvent was removed by evaporation. The residue was solved in chloroform and washed with water and the saturated solution of sodium bicarbonate, respectively. The combined organic layer was dried with magnesium sulfate and the residue obtained after solvent evaporation was recrystallized from an appropriate solvent.

4.6.1. 1-Phenyl-3-trifluoromethyl-5-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1*H*-pyrazole (11a)

Recrystallized from ethanol–water mixture. 2.94 g, 82% yield; mp 160 °C; IR (KBr) 3132, 1771, 1613 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.46–7.32 (m, 5H), 7.29 (d, 1H, $^4J_{H-H}$ = 1.6 Hz), 7.19 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.18 (s, 1H), 7.07 (dd, 1H, $^3J_{H-H}$ = 8.0 Hz, $^4J_{H-H}$ = 1.6 Hz), 3.29 (s, 3H). Anal. Calcd for $C_{18}H_{12}F_3N_3O_2$: C, 60.17; H, 3.37; N, 11.69. Found: C, 59.97; H, 3.34; N, 11.55.

4.6.2. 1-(4-Methoxyphenyl)-3-trifluoromethyl-5-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1*H*-pyrazole (11b)

Recrystallized from ethanol–water mixture. 2.94 g, 82% yield; mp 177 °C; IR (KBr) 3133, 2966, 1773, 1611 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.30–7.27 (m, 3H), 7.24 (d, 1H, $^3J_{H-H}$ = 8.4 Hz), 7.15 (s, 1H), 7.08 (dd, 1H, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 1.6 Hz), 6.99 (d, 2H, $^3J_{H-H}$ = 8.4 Hz), 3.78 (s, 3H), 3.31 (s, 3H). Anal. Calcd for $C_{19}H_{14}F_3N_3O_3$: C, 58.61; H, 3.62; N, 10.79. Found: C, 58.46; H, 3.71; N, 10.69.

4.6.3. 1-(4-Chlorophenyl)-3-trifluoromethyl-5-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1*H*-pyrazole (11c)

Recrystallized from ethanol. 2.89 g, 75% yield; mp 178 °C; IR (KBr) 3133, 2956, 1768, 1611 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.54 (d, 2H, $^3J_{H-H}$ = 8.8 Hz), 7.40 (d, 2H, $^3J_{H-H}$ = 8.4 Hz), 7.38 (s, 1H), 7.28 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 7.22 (s, 1H), 7.10 (d, 1H, $^3J_{H-H}$ = 8.0 Hz), 3.34 (s, 3H). Anal. Calcd for $C_{18}H_{11}F_3N_3O_2$: C, 54.91; H, 2.82; N, 10.67. Found: C, 54.73; H, 2.80; N, 10.61.

4.6.4. 1-(4-Fluorophenyl)-3-trifluoromethyl-5-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1*H*-pyrazole (11d)

Recrystallized from ethanol. 1.92 g, 51% yield; mp 171 °C; IR (KBr) 3133, 3074, 2926, 1772, 1611 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 7.44 (dd, 2H, $^3J_{H-H}$ = 8.8 Hz, $^4J_{H-F}$ = 5.2 Hz), 7.35–7.29 (m, 3H), 7.26 (d, 1H, $^3J_{H-H}$ = 8.4 Hz), 7.21 (s, 1H), 7.09 (dd, 1H, $^3J_{H-H}$ = 8.4 Hz, $^4J_{H-H}$ = 1.6 Hz), 3.24 (s, 3H). Anal. Calcd for $C_{18}H_{11}F_4N_3O_2$: C, 57.30; H, 2.94; N, 11.14. Found: C, 57.10; H, 3.07; N, 11.12.

4.6.5. 1-(4-Methylsulfonylphenyl)-3-trifluoromethyl-5-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1*H*-pyrazole (11e)

Recrystallized from *n*-hexane. 2.56 g, 68% yield; mp 213 °C; IR (KBr) 3104, 3064, 2926, 1786, 1315, 1155, 1616 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) 8.00 (d, 2H, $^3J_{H-H}$ = 8.8 Hz), 7.63 (d, 2H, $^3J_{H-H}$ = 8.4 Hz), 7.44 (s, 1H), 7.30 (d, 1H, $^3J_{H-H}$ = 8.4 Hz), 7.27 (s, 1H), 7.13 (d, 1H, $^3J_{H-H}$ = 8.4 Hz), 3.29 (s, 3H), 2.51 (s, 3H). Anal. Calcd for $C_{19}H_{14}F_3N_3O_4S{\cdot}0.1C_6H_{14}$: C, 52.78; H, 3.48; N, 9.42; S, 7.19. Found: C, 52.83; H, 3.56; N, 9.53; S, 7.31.

4.7. Biological activity

4.7.1. COX inhibition-purified enzyme assay

All compounds were tested for their ability to inhibit COX-1 and/or COX-2 using purified enzyme (PE) assay.[27,28] For both of these in vitro assays, compounds were tested against COX-1 and/or COX-2 at 10 μM with n = 4 determinations. Compounds that showed a % inhibition >50% were also evaluated to calculate their COX-1 and COX-2 IC_{50} values. IC_{50} values were obtained by nonlinear regression from only one experiment with eight different concentrations with n = 4 determinations, using PRISM 4.0, GraphPad Software. For each assay selectivity indexes (SI) were calculated as the ratio [COX-1 IC_{50}/COX-2 IC_{50}].

4.7.2. Enzyme reactions

A commercial kit with ovine COX-1 and COX-2 enzymes (Manufacturer, Cayman Chemical Co., Ann Arbor, MI) was used to assess inhibition of each COX isoform. One unit of COX-1 or COX-2 enzyme in 50 μL was suspended in 0.35 mL of pH 8 Tris–HCl buffer (100 mM) containing hematin (1 μM), EDTA (5 mM), and phenol (2 mM) as co-factors. The reaction medium was preincubated at 37 °C for 15 min with 50 μL of the test compound, standard drugs or vehicle. Immediately, the enzymatic reaction was started adding 50 μL of 100 μM final concentration of arachidonic acid substrate and was incubated for 5 min at 37 °C. To stop the reaction, 1 N HCl (50 μL) was added. After that, 50 μL of Tris-base (1 M) was also added. Immediately, prostaglandin (PGE_2) production was quantified in samples diluted 1:10, by an specific enzyme-linked immunoassay (EIA) (Amersham Biosciences, RPN222) following manufacturer's instructions.

4.7.3. Drugs

For all drugs, including Indomethacin and Rofecoxib using as standard drugs, a 10 μM concentration in DMSO was prepared. Successive dilutions also in DMSO, were prepared to get the appropriate concentrations.

4.8. Computational methodology

4.8.1. Ligand preparation

Ligands were built and optimized using the SYBYL program.[41] To this purpose, appropriate fragments from the SYBYL libraries were used to build each molecule and partial atomic charges were calculated by means of the Gasteiger–Marsili method.[42] The Tripos force field[43] was used in the calculations and each molecule was optimized by means of the Powell method[44] until the energy gradient was smaller than 0.05 kcal/mol Å^2, and the optimized geometry was transferred to the AUTODOCK TOOLS (ADT) program,[45] in order to prepare the appropriate file needed for the docking study. For this purpose, the nonpolar hydrogen atoms were deleted and their partial atomic charges were merged onto the heavy atom to which they were bonded.

4.8.2. Enzyme setup

COX-1:Iodoindomethacin complex (PDB code: 1PGF)[29] or chain A of the crystal structure of COX-2 complexed with SC-558 (PDB code: 6COX)[30] were selected for the docking studies. The ligand and heme group were deleted and, polar hydrogen atoms, partial atomic charges and solvation parameters were added using the ADT program.

4.8.3. Docking procedure

Potential maps were generated using the AUTOGRID program,[46] available in the AUTODOCK 3.05 package, using a grid centered in the COX-2 or COX-1 binding pockets, respectively (coordinates: $x = -24.000$, $y = -1.060$, $z = 8.600$), with a size of $60 \times 60 \times 60$ points and a grid spacing of 0.375 Å. Hundred runs of GA were performed for each ligand using the standard conditions defined in the AUTODOCK program. Final geometries of each run were compared with the initial geometry and clustered as a function of the root mean square deviation in relation to the initial geometry. Free energy of binding and docking energies was also calculated by the AUTODOCK program for the most stable conformation of the most populated cluster for each docked compound. The standard deviation of each computed energy, reported in the text as the average value of the chosen is always below 0.01, since the differences between the geometries included in each cluster are very small.

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