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Synthesis and prostaglandin synthase inhibitory activity of new aromatic *O*-alkyloxime ethers substituted with methylsulfonamido or methylsulfonyl groups on their aliphatic portion

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Abstract

Some aromatic *O*-alkyloxime ethers substituted with methylsulfonamido (**7**) or methylsulfonyl (**8**) groups on their aliphatic portions were prepared as analogues of structurally related cyclooxygenase (COX) inhibitors (**6**) bearing a carboxylic group typical of the classic non-steroidal anti-inflammatory drugs (NSAIDs) in the place of the sulfurated moiety. In addition, also analogues of compounds **8** in which the aliphatic chain is further lengthened by 1 (**9**), 2 (**10**), or 3 (**11**) carbon atoms were synthesized. All compounds (**7**–**11**) were tested in vitro towards COX2, and compounds **7**–**9** towards COX1, by measuring prostaglandin E2 (PGE_2) production in activated J774.2 macrophages and U937 cell lines, respectively. While all new compounds were found to possess little or no activity on the COX2 isoenzyme, some of these (**7a**–**7d**, **8a**, **8d**, **9e** and **9f**) appeared to possess an appreciable activity on COX1, with % inhibition values at a concentration of 1 μM ranging from 30% of **8a** to 76% of **9e**. The COX1 selectivity of the new compounds was tentatively explained by means of a docking study of one of the more active compounds tested on both COX isoenzymes (**7d**), which indicated a different number of hydrogen bonding interactions with the Arg120 of the active sites of the two enzymes, and therefore, an energetically favored interaction (3.5 kcal/mol) with COX1, compared with COX2.

Keywords: NSAIDs; Ciclooxygenase inhibitors; Anti-inflammatory drugs; Methylsulfonyl inhibitors; Methylsulfonamido inhibitors

1. Introduction

Most non-steroidal anti-inflammatory drugs (NSAIDs) act through the inhibition of prostaglandin synthase (cyclooxygenase, COX) [1,2]. This enzyme exists as two isoforms, known as COX1 and COX2. The COX1 isoform is constitutively expressed in most tissues and is involved in the physiological production of prostaglandins, which are gastric cytoprotective agents. On the contrary, the COX2 isoform is induced by some of the inflammation mediators [3–8].

Most NSAIDs in current use belong to the class of arylacetic (**1**) or arylpropionic acids (**2**), and are not selective inhibitors of both constitutive (COX1) and inducible (COX2) cyclooxygenase. The typical side-effect of these NSAIDs consists of gastrointestinal damage, commonly attributed to their lack of COX2 selectivity [8,9]. Only a few anti-inflammatory drugs, such as nimesulide (**3**) [10], and more recently tricyclic compounds such as, for example, celecoxib (**4**) [11] and rofecoxib (**5**) [12], have proved to possess a more or less appreciable selectivity towards COX2 (Fig. 1).

Unlike the case of classic non-selective NSAIDs, which include in their structures a carboxylic function, in the selective drugs **3**–**5**, one of their two aryl groups is substituted by a sulfurated moiety, such as the sulfonamidic one of **3** and **4**, or the methylsulfonic one of **5**. Pending an unequivocable definition of the structure–activity relationships in the field of these drugs, it may

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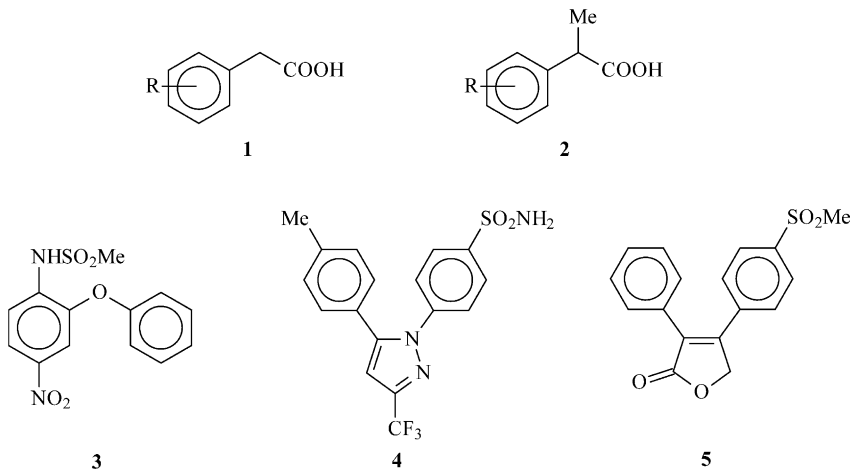


Fig. 1. General structures of non-selective arylacetic (1) or arylpropionic acids (2) and formulas of selective COX2 inhibitors (3-5).

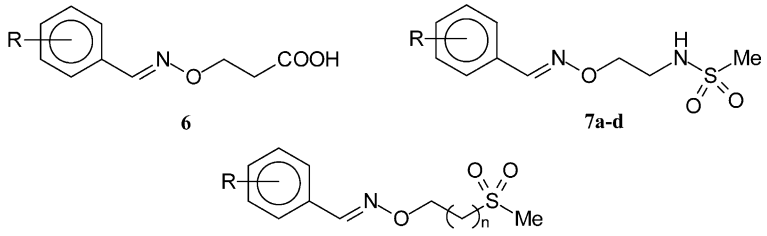
be reasonable to attribute the COX2 selectivity of compounds **3**–**5**, among other molecular factors, to the presence of these groups in their structures. This hypothesis appears to be supported by X-ray [13] and docking studies [14] of the interaction with COX2 of some of these selective inhibitors bearing the sulfurated moiety in the highest oxidation state, which indicate the presence of a strong hydrogen-bonding interaction between this moiety and the Arg513 of the enzyme active site.

In previous papers of ours [15,16], we described a series of substituted 3-(*E*)-benzylideneaminoxypropionic acids of type **6**, designed as analogues of arylacetic NSAIDs of type **1**, in which the aryl moiety is substituted by a methyleneaminoxymethyl group. Some of these compounds, assayed in vivo for their anti-inflammatory activity by the carrageenan-induced paw edema test in rats, proved to possess an appreciable activity. The in vitro tests carried out on COX1 and COX2 enzymes for **6a** and **6b** showed that these compounds possess a certain inhibitory activity towards both enzyme isoforms (unpublished data) (Fig. 2).

On the basis of these results, bearing in mind the possibility that sulfurated groups, like those present in compounds **3**–**5**, (Fig. 1) may confer a COX2 selectivity on molecules that are potentially capable of interacting with COXs, we synthesized some compounds of types **7** and **8**, (Fig. 2) which may be viewed as analogues of anti-inflammatory drugs of type **6**, in which the carboxylic function typical of the classic NSAIDs **1** and **2** is replaced by the methylsulfonamido or methylsulfonyl moiety, respectively. In addition, we also synthesized compounds of types **9**–**11**, which are analogues of compounds **8**, in which the ethylenic spacer between the aminoxy portion and the methylsulfonyl group is further lengthened by 1, 2, or 3 carbon atoms, respectively.

2. Chemistry

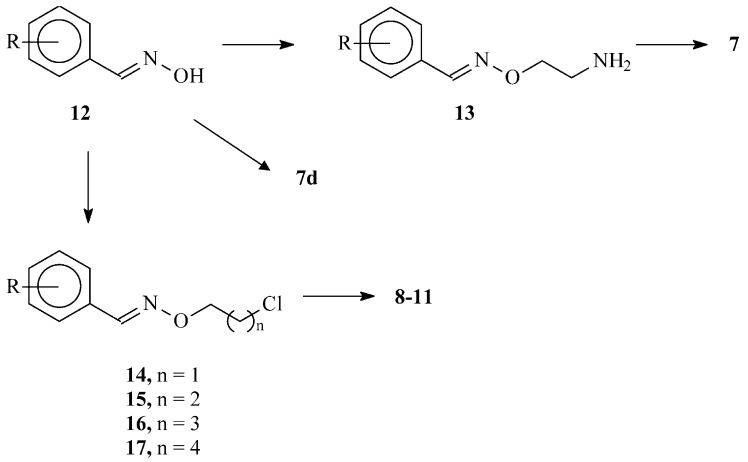
Compounds **7a**–**7c** were prepared as described in Scheme 1, starting from the appropriate substituted *E*-benzylaldoximes **12**, which were transformed into their sodium salt by treatment with EtONa, and were then allowed to react with 2-chloroethylamine to yield the corresponding substituted 2-benzylideneaminoxyethylamine derivatives **13a**–**13c**. Reaction of **13a**–**13c** with



8a-f n = 1
9a,b,d-f n = 2
10a,b,d-f n = 3
11a,b,f n = 4

a: R = H; **b**: R = *p*-Cl; **c**: R = *o*-Cl; **d**: R = *p*-F; **e**: R = *m*-F; **f**: R = 3,4-OCH_2O-.

Fig. 2. General structures of 3-(E)-benzylideneaminoxypropionic acids (6) and their methylsulfonamido- (7) and methylsulfonyl (8-11) analogues.



a: R = H; **b**: R = *p*-Cl; **c**: R = *o*-Cl; **d**: R = *p*-F; **e**: R = *m*-F; **f**: R = 3,4-OCH_2O-.

Scheme 1.

mesylchloride in the presence of triethylamine afforded the final methylsulfonamides **7a**–**7c**, which were purified by column chromatography on silica gel. Compound **7d** was directly obtained by reaction of the sodium salt of the 4-fluorobenzaldoxime with 2-[(methanesulfonyl)amino]-1-methanesulfonylethane.

The methylsulfonyl derivatives **8**–**11** were synthesized starting from the sodium salt of the appropriate oxime of type **12**, which were treated with 2-chloroethyl-1-tolylsulfonate, in the case of the preparation of **14a**–**14f**, or with 3-chloro-1-bromopropane, 4-chloro-1-bromobutane or 5-chloro-1-bromopentane in the case of the preparation of compounds of type **15**, **16**, **17**, respectively. The displacement of the chlorine atom of **14**–**17** with sodium methanesulfinate afforded the crude methylsulfones **8**–**11**, which were purified by crystallization from the proper solvent.

The configuration around the C=N double bond of the intermediates **13**–**17** and of the final products **7**–**11** was assigned on the basis of the knowledge of the configuration of the starting benzaldoximes (*E*), and of the chemical shift of the proton linked to the oximic carbon, which is in accordance with the *syn* relationship with the oximic oxygen [17].

3. Biological studies

The in vitro inhibitory activity towards the COX1 and COX2 enzymes of compounds **7**–**11** was evaluated at a dose of 1 μM by measuring prostaglandin E2 (PGE_2) production in U937 cell lines for COX1 and activated J774.2 macrophages for COX2. Results are reported in Table 1, together with those obtained in the same tests for one of the more active compounds of type **6** (**6b**) and celecoxib (**4**), chosen as a reference drug.

Table 1
In vitro inhibitory activity of methylsulfonamido- (**7**) and methysulfonyl- (**8**–**11**) alkyloxime ethers towards COX1 and COX2

Compound	R	Cyclooxygenase inhibitory activity (% inhibition)[a] at a dose of 1 μM	
		COX1	COX2
7a	H	50	2
7b	*p*-Cl	48	12
7c	*o*-Cl	54	6
7d	*p*-F	53	6
8a	H	30	6
8b	*p*-Cl	18	8
8c	*o*-Cl	0	0
8d	*p*-F	33	0
8e	*m*-F	0	13
8f	3,4-OCH_2O–	0	15
9a	H	21	19
9b	*p*-Cl	15	19
9d	*p*-F	0	6
9e	*m*-F	76	34
9f	3,4-OCH_2O–	40	19
10a	H	nt	0
10b	*p*-Cl	nt	5
10d	*p*-F	nt	0
10e	*m*-F	nt	6
10f	3,4-OCH_2O–	nt	0
11a	H	nt	4
11b	*p*-Cl	nt	0
11f	3,4-OCH_2O–	nt	14
6b	*p*-Cl	38	30
Celecoxib (**4**)		5	66

[a] S.E.M. was lower than 10% in all experiments. nt, not tested.

4. Results and discussion

As far as the COX2 enzyme is concerned, only the methylsulfonyl compound **9e** proved to possess an activity slightly higher than that of the carboxylic compound **6b**, with a percentage inhibition value of 34% vs 30% of **6b**. The other new compounds, both of the methylsulfonamidic (**7**) and methylsulfonic (**8**–**11**) types, proved to be scarcely active (**7b**, **8e**, **8f**, **9a**, **9b** and **9f**), with percentage inhibition values lower than 20%, or practically inactive (**7a**, **7c**, **7d**, **8a**–**8d**, **9d**, **10a**, **10b**, **10d**–**10f**, **11a**, **11b** and **11f**).

As it regards the COX1 enzyme, all the methylsulfonamidic compounds **7a**–**7d** exhibited a good activity, with percentage inhibition values ranging from 48% of **7b** to 54% of **7c**; on the contrary, among the methylsulfonyl derivatives (**8**, **9**), only **9e** and **9f** proved to possess a significant activity (percent inhibition values of 76 and 40%, respectively), while the other compounds were scarcely active (**8a**, **8b**, **8d**, **9a** and **9b**) or completely inactive (**8c**, **8e**, **8f** and **9d**). Further research into the COX-inhibitory properties of the new compounds did not appear to be justifiable, considering their lack of any appreciable activity or selectivity towards COX2, which is the real target of an NSAID.

In order to suggest a rationalization of these results, a docking study was performed between **7d**, one of the most active compounds towards COX1 but practically inactive at the level of the COX2, and both COX isoenzymes. Fig. 3 illustrates the results obtained using the MACROMODEL program [18]. As it can be seen, the docking of **7d** with the active site of COX1 (**a**) and COX2 (**b**) appears to be very similar, with the methylsulfonamidic portion oriented in both cases towards Arg120. Furthermore, while in the COX1 site both oxygen atoms of the sulfonyl moiety of **7d** are at distances (2.8 and 2.6 Å) compatible with the existence of two hydrogen bonds with the Arg120, in the COX2 site, only one of the two sulfonyl oxygens is at a distance from the Arg120 (2.9 Å vs 5.8 of the other oxygen) which permits the formation of a hydrogen bond. In addition, the docking of **7d** with COX2 does not present any interactions between the sulfonyl group and Arg513, which are typical of some selective COX2 inhibitors [13,14]. Also, a comparison of the overall interaction energy of **7d** involving the Arg120 residue of the two isoenzymes, indicates a more favorable interaction ($\Delta E = 3.5$ kcal/mol) for COX1 than for COX2. A possible explanation of this result may be found in the presence in the COX2 site of an ionic interaction chain between Arg513–Glu524–Arg120 which causes this last amino acid to be less available to act as a hydrogen bond donor towards the ligand **7d**.

With the aim of confirm the validity of the results obtained by theoretical way for **7d**, also the docking of the COX2 selective inhibitor celecoxib (**4**) with both COX1 and COX2 isoenzymes was evaluated (see Fig. 3c and d, respectively), using the same computational methods utilized for **7d** [18]. As expected, in the COX2 active site (Fig. 3d) the sulfonamidic group of celecoxib is oriented towards Arg513, at a distance of 2.80 Å, compatible with the existence of a strong hydrogen bond; in the docking of celecoxib with COX1 active site (see Fig. 3c) this group interacts with Arg120.

The practical result of this work consists in the recognition of certain preferential COX1 inhibitors which, even if unsuitable for therapeutic use, may be utilized in biopharmacological studies. In addition, this work suggests that the preference or selectivity towards the COX2 isoenzyme of methylsulfonamidic or methylsulfonic NSAIDs such as nimesulide or celecoxib does not depend solely on the presence of these moieties in their molecular structures, but also on the fact that they possess an overall molecular profile which allows these groups to occupy a spatial position, during the interaction of the inhibitor with the enzyme catalytic site, which permits interactions with amino acidic residues specific for the COX2 active site.

5. Experimental

5.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. ^{1}H NMR spectra of all compounds were obtained with a Varian Gemini 200 instrument operating at 200 MHz, in a ca. 2% solution of $CDCl_3$. Analytical TLCs were carried out on 0.25 mm layer silica gel plates containing a fluorescent indicator; spots were detected under UV light (254 nm). Column chromatography was performed using 70–230-mesh silica gel. Mass spectra were detected with HP-5988 A spectrometer (EI, 70 eV). Evaporations were made in vacuo (rotating evaporator). The not commercially available oximes used for the preparation of compounds **13**, **14**, **15**, **16** and **17** were synthesized as described in Ref. [19]. Na_2SO_4 was always used as the drying agent. Elemental analyses were performed in our analytical laboratory and agreed with the theoretical values to within $\pm 0.4\%$.

5.1.1. Preparation of substituted 2-(benzylideneaminoxy)-ethylamines hydrochlorides (*13a*–*13c*·*HCl*)

A stirred solution of the sodium salt of the appropriate oxime (**12a**–**12c**), prepared by refluxing the oxime (1.42 mmol) with EtONa (2.84 mmol) in anhydrous EtOH (14 ml), was treated dropwise with a solution of 2-chloroethylamine hydrochloride (1.42 mmol) in anhydrous EtOH (5 ml). When the addition was complete the reaction mixture was refluxed for 6 h, cooled, filtered

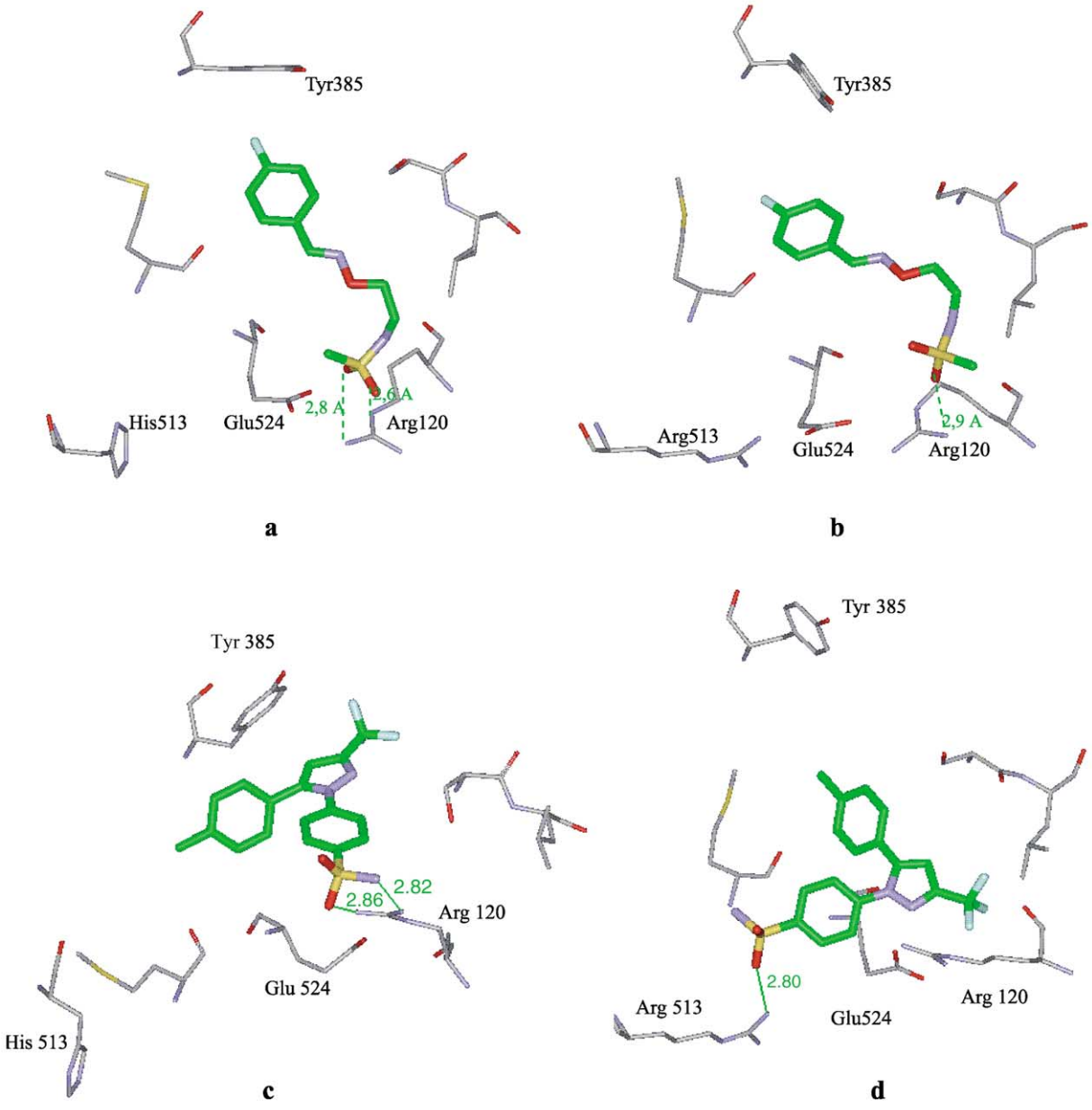


Fig. 3. Docking interactions between compound **7d** or celecoxib **4** and the catalytic site of COX1 (**a, c**) and COX2 (**b, d**), respectively.

and evaporated. The residue was take up with Et_2O, treated with an excess of $Et_2O \cdot HCl$ to yield crude **13a**–**13c** [20], as hydrochlorides, which were purified by, crystallization from MeOH/Et_2O. **13a**·HCl (15%): m.p. 124–125 °C; ^1H NMR δ 3.50 (t, 2H, $J = 5.0$ Hz), 4.40 (t, 2H, $J = 5.0$ Hz), 7.30–7.81 (m, 5H) and 8.31 ppm (s, 1H). Anal. $C_9H_{12}N_2O \cdot HCl$ (C, H, N). **13b**·HCl (20%): m.p. 184–185 °C; ^1H NMR δ 3.40 (t, 2H, $J = 5.0$ Hz), 4.40 (t, 2H, $J = 5.0$ Hz), 7.40 (d, 2H, $J = 8.0$ Hz), 7.61 (d, 2H, $J = 8.0$ Hz) and 8.30 ppm (s, 1H). Anal. $C_9H_{11}ClN_2O \cdot HCl$ (C, H, N). **13c**·HCl (35%): m.p. 113–114 °C; ^1H NMR δ 3.59 (t, 2H, $J = 5.0$ Hz), 4.50 (t, 2H, $J = 5.0$ Hz), 7.30–8.00 (m, 4H) and 8.60 ppm (s, 1H). Anal. $C_9H_{11}ClN_2O \cdot HCl$ (C, H, N).

5.1.2. *Preparation of substituted N-(2-(benzylideneaminoxy)ethyl)-methanesulfonamides* ***7a–7c***

A solution of methanesulfonylchloride (1.38 mmol) in Et_2O (10 ml) was added dropwise to a solution of the appropriate oxime ether **13a**–**13c** (1.38 mmol) and NEt_3 (6.46 mmol) in Et_2O (10 ml). The mixture was stirred at room temperature for 1 h, and the resulting suspension was filtered and evaporated to give crude **7a**–**7c**, which were purified by column chromatography on silica gel

using 15:85 AcOEt–hexane as the eluent and then crystallized from AcOEt–hexane mixture. **7a** (40%): m.p. 40–42 °C; ^{1}H NMR δ 2.97 (s, 3H), 3.25–3.58 (m, 2H), 4.28 (t, 2H, $J = 4.8$ Hz), 4.80 (br, 1H), 7.20–7.81 (m, 5H) and 8.07 ppm (s, 1H); MS m/z 243 (M^+). Anal. $C_{10}H_{14}N_2O_3S$ (C, H, N). **7b** (35%): m.p. 74–76 °C; ^{1}H NMR δ 3.03 (s, 3H), 3.36–3.76 (m, 2H), 4.40 (t, 2H, $J = 5.8$ Hz), 5.16 (br, 1H), 7.50 (d, 2H, $J = 8.0$ Hz), 7.63 (d, 2H, $J = 8.0$ Hz) and 8.16 ppm (s, 1H); MS m/z 276 (M^+). Anal. $C_{10}H_{13}ClN_2O_3S$ (C, H, N). **7c** (70%): m.p. 35–36 °C; ^{1}H NMR δ 2.97 (s, 3H), 3.52 (q, 2H, $J = 6.4$ Hz), 4.31 (t, 2H, $J = 6.4$ Hz), 4.80 (br, 1H), 7.22–7.91 (m, 4H) and 8.48 ppm (s, 1H); MS m/z 276 (M^+). Anal. $C_{10}H_{13}ClN_2O_3S$ (C, H, N).

5.1.3. Preparation of the 2-(4-fluorobenzylideneaminoxy)ethyl-methanesulfonamide 7d

A stirred solution of the sodium salt of 4-fluorobenzaldoxime (3.23 mmol) in THF (20 ml), prepared by treatment of **12d** with EtONa (3.30 mmol) in anydrous EtOH, was treated dropwise with a solution of 2-[(methanesulfonyl)amino]ethylmethanesulfonate (3.23 mmol) in THF (10 ml). The reaction mixture was stirred at room temperature for 24 h, the solvent was evaporated and the crude residue was purified by chromatography on silica gel column eluting with 1:1 AcOEt–hexane to yield pure **7d** (20%): m.p.77–78 °C (AcOEt–hexane); ^{1}H NMR δ 2.90 (s, 3H), 3.28–3.51 (m, 2H), 4.30 (t, 2H, $J = 5.9$ Hz), 4.76 (br, 1H), 7.09–7.57 (m, 4H) and 8.09 ppm (s, 1H); MS m/z 260 (M^+). Anal. $C_{10}H_{13}FN_2O_3S$ (C, H, N).

5.1.4. General procedure for the preparation of the substituted 2-(benzylideneaminoxy)-1-chloroethane derivatives 14a–14f

A suspension of the sodium salt of the appropriate oxime (12a–12f), prepared by refluxing the oxime (32 mmol) with EtONa (32 mmol) in anhydrous EtOH (85 ml), was added dropwise to a stirred solution of 2-chloroethyl-1-tolylsulfonate (32 mmol) in anhydrous EtOH (16 ml). After 12h at room temperature, the solvent was removed and the residue was taken up with $CHCl_3$. The organic phase was washed with H_2O, filtered and evaporated. The crude residue was then subjected to a column chromatography eluting with 1:8 AcOEt–hexane mixture to yield pure **14a**–**14f** which was directly used in the subsequent transformation. **14a** (40%): ^{1}H NMR δ 3.80 (t, 2H, $J = 6.0$ Hz), 4.40 (t, 2H, $J = 6.0$ Hz), 7.41–7.90 (m, 5H) and 8.31 ppm (s, 1H). Anal. $C_9H_{10}ClNO$ (C, H, N). **14b** [21] (35%): ^{1}H NMR δ 3.81 (t, 2H, $J = 6.0$ Hz), 4.42 (t, 2H, $J = 6.0$ Hz), 7.40 (d, 2H, $J = 10$ Hz), 7.70 (d, 2H, $J = 10$ Hz) and 8.20 ppm (s, 1H). **14c** (15%): ^{1}H NMR δ 3.80 (t, 2H, $J = 6.0$ Hz), 4.40 (t, 2H, $J = 6.0$ Hz), 7.20–8.01 (m, 4H) and 8.61 ppm (s, 1H). Anal. $C_9H_9Cl_2NO$ (C, H, N). **14d** (50%): ^{1}H NMR δ 3.80 (t, 2H, $J = 6.0$ Hz), 4.40 (t, 2H, $J = 6.0$ Hz), 7.00–7.80 (m, 4H) and 8.31 ppm (s, 1H). Anal. C_9H_9ClFNO (C, H, N). **14e** (40%): ^{1}H NMR δ 3.72 (t, 2H, $J = 6.0$ Hz), 4.39 (t, 2H, $J = 6.0$ Hz), 7.05–7.40 (m, 4H) and 8.10 ppm (s, 1H). Anal. C_9H_9ClFNO (C, H, N). **14f** (50%): ^{1}H NMR δ 3.80 (t, 2H, $J = 6.0$ Hz), 4.40 (t, 2H, $J = 6.0$ Hz), 6.00 (s, 2H), 6.66–7.00 (m, 3H) and 8.11 ppm (s, 1H). Anal. $C_{10}H_{10}ClNO_3$ (C, H, N).

5.1.5. General procedure for the synthesis of the substituted 3-(benzylideneaminoxy)-1-chloropropane 15a, 15b, 15d–15f 4-(benzylideneaminoxy)-1-chlorobutane 16a, 16b, 16d–16f 5-(benzylideneaminoxy)-1-chloropentane 17a, 17b, 17f derivatives

A suspension of sodium salt of the appropriate oxime **12**, prepared by refluxing the oxime (18 mmol) with EtONa (18 mmol) in anhydrous EtOH (30 ml), was evaporated and the residue was dissolved in DMF. The resulting solution was then added dropwise to a DMF solution (15 ml) of 1-bromo-3-chloropropane (17.8 mmol) for the preparation of **15**, 1-bromo-4-chlorobutane (17.8 mmol) for the preparation of **16**, or 1-bromo-5-chloropentane (17.8 mmol) for the preparation of **17**. The reaction mixture was stirred overnight at room temperature, then the solvent was removed and the residue was taken up with $CHCl_3$. The organic phase was washed with H_2O and evaporated to yield a crude residue, which was subjected to column chromatography on silica gel eluting with 1:8 AcOEt–hexane mixture to give pure **15a**, **15b**, **15d**–**15f**, **16a**, **16b**, **16d**–**16f**, **17a**, **17b**, and **17f**. **15a** (57%): ^{1}H NMR δ 2.01 (q, 2H, $J = 7.0$ Hz), 3.76 (t, 2H, $J = 6.0$ Hz), 4.40 (t, 2H, $J = 7.0$ Hz), 7.41–7.96 (m, 5H) and 8.30 ppm (s, 1H); MS m/z 197 (M^+). Anal. $C_{10}H_{12}ClNO$ (C, H, N). **15b** (58%): ^{1}H NMR δ 2.17 (q, 2H, $J = 7.0$ Hz), 3.65 (t, 2H, $J = 6.0$ Hz), 4.29 (t, 2H, $J = 7.0$ Hz), 7.29 (d, 2H, $J = 8.8$ Hz), 7.48 (d, 2H, $J = 8.8$ Hz) and 7.99 ppm (s, 1H); MS m/z 232 (M^+). Anal. $C_{10}H_{11}Cl_2NO$ (C, H, N). **15d** (57%): ^{1}H NMR δ 2.18 (q, 2H, $J = 6.4$ Hz), 3.67 (t, 2H, $J = 6.4$ Hz), 4.29 (t, 2H, $J = 6.4$ Hz), 7.06 (m, 2H), 7.55 (m, 2H) and 8.30 ppm (s, 1H); MS m/z 215 (M^+). Anal. $C_{10}H_{11}ClFNO$ (C, H, N). **15e** (57%): ^{1}H NMR δ 2.21 (q, 2H, $J = 6.4$ Hz), 3.75 (t, 2H, $J = 6.4$Hz), 4.40 (t, 2H, $J = 5.4$ Hz), 7.07–7.41 (m, 4H) and 8.09 ppm (s, 1H); MS m/z 215 (M^+). Anal. $C_{10}H_{11}ClFNO$ (C, H, N). **15f** (55%): ^{1}H NMR δ 2.16 (q, 2H, $J = 6.4$ Hz), 3.65 (t, 2H, $J = 6.4$ Hz), 4.25 (t, 2H, $J = 6.4$ Hz), 5.95 (s, 2H), 6.55–7.45 (m, 3H) and 7.94 ppm (s, 1H); MS m/z 241 (M^+). Anal. $C_{11}H_{12}ClNO_3$ (C, H, N). **16a** (48%): ^{1}H NMR δ 1.63–2.21 (m, 4H), 3.68 (t, 2H, $J = 6.4$ Hz), 4.30 (t, 2H, $J = 6.4$ Hz), 7.36–8.01 (m, 5H) and 8.30 ppm (s, 1H); MS m/z 211 (M^+). Anal. $C_{11}H_{14}ClNO$ (C, H, N). **16b** (58%): ^{1}H NMR δ 1.73–2.23 (m, 4H), 3.73 (t, 2H, $J =$ 7.0 Hz), 4.26 (t, 2H, $J = 7.0$ Hz), 7.28 (d, 2H, $J = 8.8$ Hz), 7.49 (d, 2H, $J = 8,8$ Hz) and 8.23 ppm (s, 1H); MS m/z 246 (M^+). Anal. $C_{11}H_{13}Cl_2NO$ (C, H, N). **16d** (55%): ^{1}H NMR δ 1.74–2.21 (m, 4H), 3.69 (t, 2H, $J =$

6.0 Hz), 4.18 (t, 2H, $J = 6.0$ Hz), 7.05–7.53 (m, 4H) and 8.04 ppm (s, 1H); MS *m/z* 229 (M^+). Anal. $C_{11}H_{13}ClFNO$ (C, H, N). **16e** (55%): ^{1}H NMR δ 1.66–2.23 (m, 4H), 3.66 (t, 2H, $J = 6.0$ Hz), 4.30 (t, 2H, $J = 6.0$ Hz), 7.13–7.83 (m, 4H) and 8.20 ppm (s, 1H); MS *m/z* 229 (M^+). Anal. $C_{11}H_{13}ClFNO$ (C, H, N). **16f** (60%): ^{1}H NMR δ 1.63–2.21 (m, 4H), 3.68 (t, 2H, $J = 6.0$ Hz), 4.25 (t, 2H, $J = 6.0$ Hz), 6.13 (s, 2H), 6.99–7.38 (m, 3H) and 8.18 ppm (s, 1H); MS *m/z* 255 (M^+). Anal. $C_{12}H_{14}ClNO_3$ (C, H, N). **17a** (60%): ^{1}H NMR δ 1.40–2.26 (m, 6H), 3.66 (t, 2H, $J = 7.0$ Hz), 4.36 (t, 2H, $J = 7.0$ Hz), 7.43–7.96 (m, 5H) and 8.30 ppm (s, 1H); MS *m/z* 225 (M^+). Anal. $C_{12}H_{16}ClNO$ (C, H, N). **17b** (40%): ^{1}H NMR δ 1.45–2.20 (m, 6H), 3.77 (t, 2H, $J = 6.8$ Hz), 4.25 (t, 2H, $J = 6.8$ Hz), 7.50 (d, 2H, $J = 8.0$ Hz), 7.70 (d, 2H, $J = 8.0$ Hz) and 8.30 ppm (s, 1H); MS *m/z* 259 (M^+). Anal. $C_{12}H_{15}Cl_2NO$ (C, H, N). **17f** (60%): ^{1}H NMR δ 1.30–2.10 (m, 6H), 3.53 (t, 2H, $J = 5.6$ Hz), 4.12 (t, 2H, $J = 5.6$ Hz), 5.94 (s, 2H), 6.70–7.14 (m, 3H) and 7.93 ppm (s, 1H); MS *m/z* 269 (M^+). Anal. $C_{13}H_{16}ClNO_3$ (C, H, N).

*5.1.6. General procedure for the synthesis of the substituted 1-(methanesulfonyl)-2-(benzylideneaminoxy) ethane **8a–8f**, 1-(methanesulfonyl)-3-(benzylideneaminoxy) propane **9a**, **9b**, **9d–9f**, 1-(methanesulfonyl)-4-(benzylideneaminoxy)butane **10a**, **10b**, **10d–10f**, and 1-(methanesulfonyl)-5-(benzylideneaminoxy) pentane **11a**, **11b**, **11f** derivatives*

Sodium methanesulfinate (2.23 mmol) and tetrabutylammonium bromide (2.23 mmol) were added to a stirred solution of the appropriate *O*-chloroalkyl-oxime ether **14–17** (2.15 mmol) in DMF (10 ml). The resulting solution was stirred at 85 °C for 5 days and then cooled, diluted with water and extracted with $CHCl_3$. The organic layers were washed several times with water, filtered and evaporated to yield a crude residue which was purified crystallized from the proper solvent to give pure **8–11**. **8a** (40%): m.p. 63–64 °C (*i*-PrOH); ^{1}H NMR δ 3.0 (s, 3H), 3.50 (t, 2H, $J = 5.9$ Hz), 4.60 (t, 2H, $J = 5.9$ Hz), 7.3–7.9 (m, 5H) and 8.20 ppm (s, 1H); MS *m/z* 227 (M^+). Anal. $C_{10}H_{13}NO_3S$ (C, H, N). **8b** (55%): m.p. 86–87 °C (*i*-PrOH); ^{1}H NMR δ 3.0 (s, 3H), 3.5 (t, 2H, $J = 5.8$ Hz), 4.70 (t, 2H, $J = 5.8$ Hz), 7.50 (d, 2H, $J = 8.8$ Hz), 7.70 (d, 2H, $J = 8.8$ Hz) and 8.20 ppm (s, 1H); MS *m/z* 261 (M^+). Anal. $C_{10}H_{12}ClNO_3S$ (C, H, N). **8c** (45%): m.p. 93–94 °C (*i*-PrOH). ^{1}H NMR δ 3.0 (s, 3H), 3.5 (t, 2H, $J = 6.0$ Hz), 4.70 (t, 2H, $J = 6.0$ Hz), 7.20–8.10 (m, 4H) and 8.70 ppm (s, 1H); MS *m/z* 261 (M^+). Anal. $C_{10}H_{12}ClNO_3S$ (C, H, N). **8d** (60%): m.p. 47–48 °C (*i*-PrOH). ^{1}H NMR δ 3.0 (s, 3H), 3.50 (t, 2H, $J = 6.0$ Hz), 4.60 (t, 2H, $J = 6.0$ Hz), 7.00–7.90 (m, 4H) and 8.20 ppm (s, 1H); MS *m/z* 245 (M^+). Anal. $C_{10}H_{12}FNO_3S$ (C, H, N). **8e** (45%): m.p. 46–47 °C (*i*-PrOH). ^{1}H NMR δ 2.98 (s, 3H), 3.46 (t, 2H, $J = 5.6$ Hz), 4.60 (t, 2H, $J = 5.6$ Hz), 7.01–7.39 (m, 4H) and 8.09 ppm (s, 1H); MS *m/z* 245 (M^+). Anal. $C_{10}H_{12}FNO_3S$ (C, H, N). **8f** (45%): m.p. 67–68 °C (*i*-PrOH). ^{1}H NMR δ 3.0 (s, 3H), 3.40 (t, 2H, $J = 6.0$ Hz), 4.60 (t, 2H, $J = 6.0$ Hz), 6.0 (s, 2H), 7.66–7.40 (m, 3H) and 8.16 ppm (s, 1H); MS *m/z* 271 (M^+). Anal. $C_{11}H_{13}NO_5S$ (C, H, N). **9a** (30%): m.p. 63–65 °C (hexane). ^{1}H NMR δ 1.80–2.61 (m, 2H), 2.90 (s, 3H), 3.0–3.45 (m, 2H), 4.27 (t, 2H, $J = 5.8$ Hz), 7.75–7.80 (m, 5H) and 8.05 ppm (s, 1H); MS *m/z* 241 (M^+). Anal. $C_{11}H_{15}NO_3S$ (C, H, N). **9b** (30%): m.p. 95–96 °C (hexane). ^{1}H NMR δ 1.95–2.45 (m, 2H), 2.91 (s, 3H), 2.90–3.36 (m, 2H), 4.29 (t, 2H, $J =$ 6.4 Hz), 7.23 (d, 2H, J = 8.8 Hz), 7.47 (d, 2H, $J = 8.8$ Hz) and 7.99 ppm (s, 1H); MS *m/z* 275 (M^+). Anal. $C_{11}H_{14}ClNO_3S$ (C, H, N). **9d** (30%): m.p. 55–56 °C (hexane). ^{1}H NMR δ 2.15–2.37 (m, 2H), 2.95 (s, 3H), 2.87–3.26 (m, 2H), 4.25 (t, 2H, $J = 6.0$ Hz), 7.01–7.60 (m. 4H) and 8.05 ppm (s, 1H); MS *m/z* 259 (M^+). Anal. $C_{11}H_{14}FNO_3S$ (C, H, N). **9e** (25%): m.p. 58–59 °C (hexane). ^{1}H NMR δ 2.18–2.44 (m, 2H), 2.79 (s, 3H), 2.81–3.66 (m, 2H), 4.28 (t, 2H, $J = 6.4$ Hz), 7.16–7.36 (m, 4H) and 8.01 ppm (s, 1H); MS *m/z* 259 (M^+). Anal. $C_{11}H_{14}FNO_3S$ (C, H, N). **9f** (30%): m.p. 110–111 °C (hexane). ^{1}H NMR δ 2.15–2.34 (m, 2H), 2.91 (s, 3H), 2.97–3.25 (m, 2H), 4.23 (t, 2H, $J = 5.6$ Hz), 5.96 (s, 2H), 6.69–7.22 (m, 3H) and 7.94 ppm (s, 1H); MS *m/z* 285 (M^+). Anal. $C_{12}H_{15}NO_5S$ (C, H, N). **10a** (20%): m.p. 39–40 °C (*i*-Pr_2O). ^{1}H NMR δ 1.70–2.25 (m, 4H), 2.88 (s, 3H), 2.90–3.31 (m, 2H), 4.19 (t, 2H, $J = 5.6$ Hz), 7.22–7.48 (m, 5H) and 8.03 ppm (s, 1H); Anal. $C_{12}H_{17}NO_3S$ (C, H, N). **10b** (30%): m.p. 84–85 °C (*i*-Pr_2O). ^{1}H NMR δ 1.65–2.30 (m, 4H), 2.88 (s, 3H), 2.85–3.25 (m, 2H), 4.18 (t, 2H, J = 5.6 Hz), 7.28 (d, 2H, $J = 9.6$ Hz), 7.49 (d, 2H, $J = 9.6$ Hz) and 7.98 ppm (s, 1H); Anal. $C_{12}H_{16}Cl\ NO_3S$ (C, H, N). **10d** (30%): m.p. 73–74 °C (*i*-Pr_2O). ^{1}H NMR δ 1.88–2.15 (m, 4H), 2.90 (s, 3H), 3.04–3.12 (m, 2H), 4.19 (t, 2H, $J = 5.6$ Hz), 7.02–7.59 (m, 4H) and 8.04 ppm (s, 1H); Anal.$C_{12}H_{16}FNO_3S$ (C, H, N). **10e** (25%): m.p. 40–41 °C (*i*-Pr_2O). ^{1}H NMR δ 1.70–2.25 (m, 4H), 2.89 (s, 3H), 2.91–3.20 (m, 2H), 4.20 (t, 2H, $J = 5.6$ Hz), 6.90–7.40 (m, 4H) and 8.0 ppm (s, 1H); Anal. $C_{12}H_{16}\ FNO_3S$ (C, H, N). **10f** (20%): m.p. 110–111 °C (*i*-Pr_2O). ^{1}H NMR δ 1.65–2.30 (m, 4H), 2.88 (s, 3H), 2.90–3.21 (m, 2H), 4.15 (t, 2H, $J = 6.4$ Hz), 5.95 (s, 2H), 6.50–7.35 (m, 3H) and 7.93 ppm (s, 1H); Anal. $C_{13}H_{17}\ NO_5S$ (C, H, N). **11a** (20%): m.p. 44–45 °C (hexane). ^{1}H NMR δ 1.40–2.20 (m, 6H), 2.87 (s, 3H), 3.02–3.31 (m, 2H), 4.16 (t, 2H, $J = 5.6$ Hz), 7.22–7.51 (m, 5H) and 8.02 ppm (s, 1H); Anal. $C_{13}H_{19}NO_3S$ (C, H, N). **11b** (20%): m.p. 72–73 °C (hexane). ^{1}H NMR δ 1.40–2.20 (m, 6H), 2.87 (s, 3H), 2.90–3.21 (m, 2H), 4.16 (t, 2H, $J = 6.4$ Hz), 7.28 (d, 2H, $J = 9.6$ Hz), 7.48 (d, 2H, $J = 9.6$ Hz) and 7.98 ppm (s, 1H); Anal. $C_{13}H_{18}ClNO_3S$ (C, H, N). **11f** (20%): m.p. 78–79 °C (hexane). ^{1}H NMR δ 1.35–2.10 (m, 6H), 2.87 (s, 3H), 2.90–3.17 (m, 2H), 4.12 (t, 2H, $J = 5.2$ Hz), 5.95

(s, 2H), 6.79–7.20 (m, 3H) and 7.93 ppm (s, 1H); Anal. $C_{14}H_{19}NO_5S$ (C, H, N).

5.2. Biopharmacological methods

5.2.1. Enzyme assays

Compounds **7**–**11** were tested in intact cell assays to verify their capacity to inhibit PGE_2 production, considered as an index of activity on COX1 and COX2 enzymes. For the COX1 assay, 1.5×10^6 resting U937 human cells were incubated with the test compounds for 30 min in the presence of 10 μM arachidonic acid. Tubes were then centrifuged and the PGE_2 content in the supernatant was measured by a commercial immunoenzymatic assay (Amersham). The COX2 assay was performed in accordance with the method described by Mitchell et al. [22] with minor modifications, as suggested by Grossman et al. [23]. Murine J774.2 cells were pretreated for 1 h with 300 μM aspirin to inactivate endogenous constitutive COX1 and were then stimulated with LPS to induce COX2 expression. After overnight incubation, cells were treated for 45 min with different test compounds. Supernatants were then collected and PGE_2 was measured as described above. All compounds were tested in duplicate.

5.3. Docking studies

The docking studies of **7d** with COX1 and COX2 were carried out using the enzyme parameters obtained from the crystallographic structures of the complexes between ovine COX1 and murine COX2 with flurbiprofen (1CQE.pdb and 3PGH.pdb, respectively) [24,13]. The docking studies of celecoxib with the same isoenzymes were carried out using the enzyme parameters obtained from the crystallographic structures of the complex between ovine COX1 and flurbiprofen, for COX1, and murine COX2 complexed with SC-558 (1CX2.pdb) [25] (a selective COX2 inhibitor structurally related to celecoxib), for COX2. The complexes so obtained were minimized using MACROMODEL program [18] in order to solve any conflicts due to the rigidity of amino acids of the catalytic sites. The procedure used to refine the complexes involved 50 ps of molecular dynamics with a constraint of 0.02 kcal/mol on the protein backbone, while the ligand and the side chain were free. The simulation temperature was 300 K, the timestep 1.5 fs. The molecular dynamics simulations were followed by a minimization through Conjugate Gradient method with derivative convergence at 0.05 kJ/Å mol in which no kind of restraint was applied. The calculations were performed using AMBER forcefield with distance dependent electrostatic treatment and dielectric constant 4.0.

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