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Synthesis and COX-2 inhibitory properties of *N*-phenyl- and *N*-benzyl-substituted amides of 2-(4-methylsulfonylphenyl)cyclopent-1-ene-1-carboxylic acid and of their pyrazole, thiophene and isoxazole analogs ☆

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Abstract

Some *N*-phenyl- (**7a–10a**) and *N*-benzyl-substituted (**7b–10b**) amido analogs of cyclooxygenase (COX-2) selective tricyclic non-steroidal anti-inflammatory drugs have been synthesized with the aim to obtain information on the structural requirements for the COX-inhibitory activity. Compounds **7–10** were tested in vitro for their inhibitory properties only towards COX-2 enzyme by measuring prostaglandin E2 (PGE2) production on activated J774.2 macrophages. Some of the new compounds (**7a, 8a, 9a** and **9b**) showed a modest activity, with percentage inhibition values near 30% at a concentration of 10 μM. These data have been tentatively explained by a conformational study which indicates that at least the *N*-phenyl-substituted amides **7a–9a** present steric hindrances which may prevent a good interaction with COX-2 active site.

Keywords: Anti-inflammatory drug; COX-2 inhibitor; NSAID

1. Introduction

Prostaglandins are endogenous substances involved in different processes of physiological nature, and are potent mediators of inflammation. Prostaglandins are produced, together with other prostanoids, in the arachidonic acid metabolism, whose first step, consisting of the oxidative conversion of arachidonic acid into prostaglandin H2, is catalyzed by cyclooxygenase (COX) [1,2]. This enzyme exists at least as two isoforms, one constitutive (COX-1) and the other inducible (COX-2) [2,3]. Thus, while COX-1 is constitutively expressed and is involved in the synthesis and supply of the necessary arachidonic acid metabolites for a maintenance of gastric and renal functions as well as for an adequate vascular homeostasis, COX-2 is expressed only after an inflammatory stimulus, releasing metabolites that are used to induce inflammation and pain [4–6]. Most of the non-steroidal anti-inflammatory drugs (NSAIDs) act by reducing prostaglandin biosynthesis through the inhibition of the COX reaction [7,8]. In spite of their beneficial action, their activity is associated with deleterious side effects, and continuous administration of these drugs leads to nefrotoxicity and gastric ulcerations [9,10]. The therapeutic anti-inflammatory action of NSAIDs is produced by inhibition of COX-2, while the unwanted side effects arise from the inhibition of COX-1 activity. Recently, some new NSAIDs possessing a good selectivity towards COX-2 have been described. The common structural backbone of these COX-2 selective inhibitors (**A**, Fig. 1) consists of two aryl groups linked to adjacent atoms of a central ring (X) which may be

Abbreviations: NSAID, non-steroidal anti-inflammatory drug; COX, cyclooxygenase; MAOMM, methyleneaminoxymethyl moiety.

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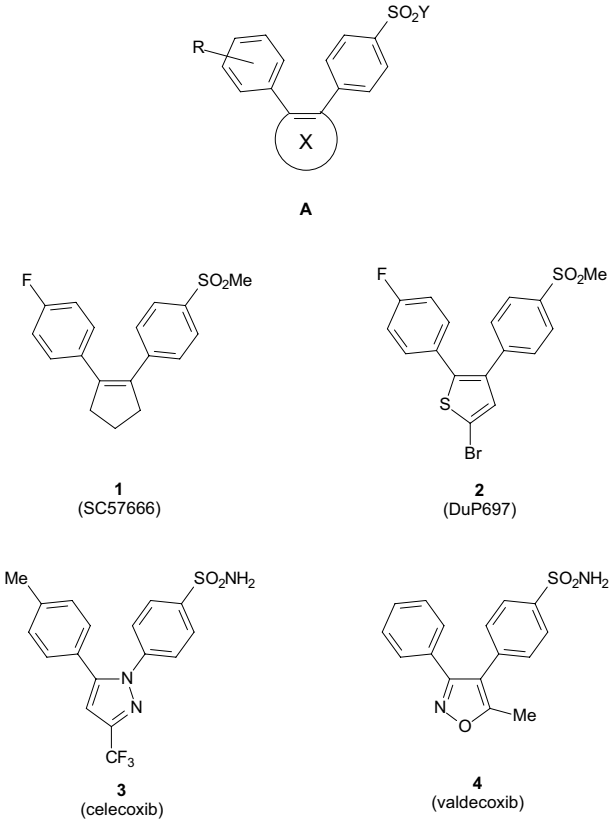


Fig. 1. General formula (**A**) and structures of some COX-2 selective anti-inflammatory drugs (**1–4**).

homocyclic or heteroaromatic, one of which is substituted in the *p*-position with either an aminosulfonyl (Y = NH_2) or a methylsulfonyl (Y = Me) group. There are also examples of potent COX-2 inhibitors that possess cycloalkyl [11], alkoxy [12] or phenoxy [12,13] moieties in the non-sulfonyl containing 'aryl' region. The central rings most commonly found within this class of molecules are cyclopentene (**1**) [11], thiophene (**2**) [14], pyrazole (**3**) [15,16] and isoxazole (**4**) [17] (Fig. 1).

The limited chemical diversity of these molecules prompted us to search for new structures which, even though different from that of the COX-2 tricyclic inhibitors of type **A**, may possess analogs biopharmacological properties [18–21]. Therefore, some type **B** compounds were synthesized which may be viewed as analogs of 1,2-diarylsubstituted NSAIDs of type **A** (Fig. 2) in which the non-sulfurated aromatic ring is replaced with a methyleneaminoxymethyl moiety (MAOMM). This kind of group (**b** in Fig. 3) was previously described as bioequivalent of aryls (**a**

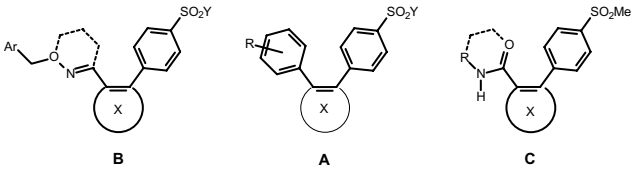


Fig. 2. General formulas of the tricyclic drugs (**A**) and of their MAOM (**B**) and amidic (**C**) analogs.

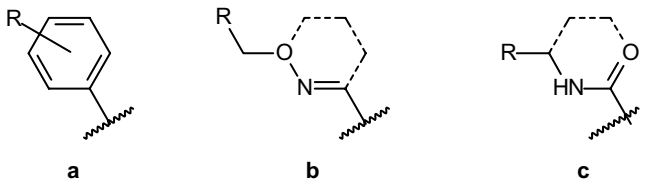


Fig. 3. Schematic representation of the possible bioisosterism between aryl (**a**) and methyleneaminoxymethyl (MAOM) (**b**) or amidic group (**c**).

in Fig. 3) in many other classes of drugs in which this aromatic system is considered important for the activity [21–27]. Some of the new oxime-ethers of type **B** in which X = cyclopentene (**5**) or thiophene (**6**) showed COX-2 inhibitory properties, with satisfactory level of activity and COX-2 vs. COX-1 selectivity [18,20].

These results suggested to widen the study of the effects on the inhibitory activity towards COX enzymes due to a further chemical manipulation at level of the non-sulfurated aromatic ring of compounds of type **A**. The amido group (**c** in Fig. 3) seemed to be suitable for this purpose since, as an aryl ring, it presents a nearly planar geometry; furthermore, the amido-moiety possess a π-system which may partially simulate the electronic system of an aryl.

On the basis of these considerations, we have designed and synthesized some compounds of type **C** (Fig. 2) in which the central core may be the cyclopentene (**7**) as in SC57666 (**1**), the thiophene (**8**) as in Dup697 (**2**), the pyrazole (**9**) as in celecoxib (**3**) or the isoxazole (**10**) as in valdecoxib (**4**) (Fig. 4). As R substituent we have chosen the phenyl (**a**) or the benzyl (**b**) group which have been resulted important for the activity as substituents of the oximic oxygen in type **B** compounds (Fig. 2).

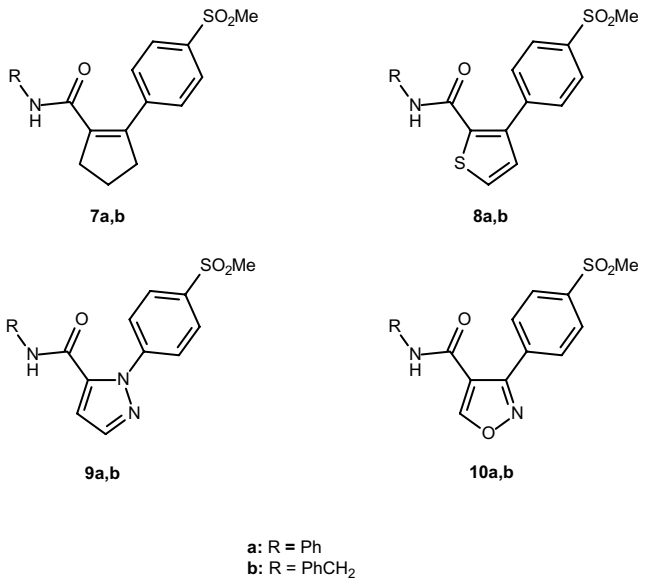
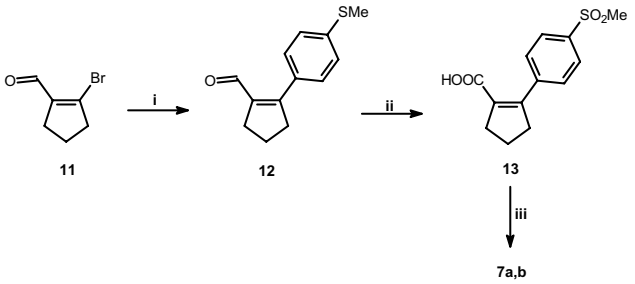


Fig. 4. Structures of the *N*-phenyl and *N*-benzyl-substituted amides of 2-[4-(methylsulfonyl)phenyl]cyclopent-1-ene-1-carboxylic acid (**7**) and of their pyrazole, thiophene and isoxazole analogs (**8–10**).

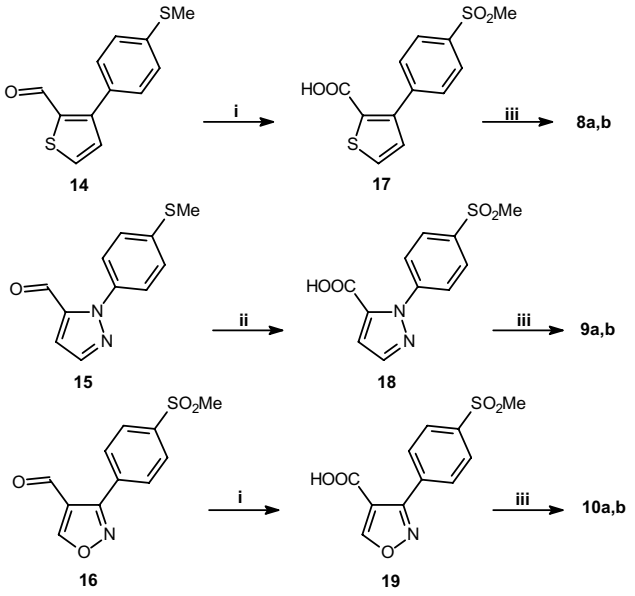


Scheme 1. Synthesis of *N*-phenyl- (**7a**) and *N*-benzyl-substituted (**7b**) amides of 2-[4-(methylsulfonyl)phenyl]cyclopent-1-ene-1-carboxylic acid. **i**: 4-(MeS)PhB(OH)$_2$, Pd(PPh$_3$)$_4$, Na$_2$CO$_3$ 2 M, EtOH–toluene, reflux; **ii**: NaClO$_2$, H$_2$O$_2$, NaH$_2$PO$_4$, CH$_3$CN; **iii**: PhNH$_2$ or PhCH$_2$NH$_2$, HBT, EDC, THF, r.t., 24 h.

2. Chemistry

As far as the *N*-phenyl- (**7a**) and the *N*-benzyl-substituted (**7b**) cyclopentene amides are concerned, they were prepared as reported in Scheme 1. The cross-coupling reaction between the 2-bromocyclopent-1-ene-1-carbaldehyde **11** [18] with *p*-thiomethylphenylboronic acid in toluene–EtOH solution in the presence of Pd(PPh$_3$)$_4$ and aqueous Na$_2$CO$_3$ afforded the 2-[4-(methylthio)phenyl]cyclopent-1-ene-1-carbaldehyde **12**. Compound **12** was oxidized in CH$_3$CN by a one-step procedure both at level of methylthio- and aldehydic groups with NaClO$_2$–H$_2$O$_2$ and aqueous solution of NaH$_2$PO$_4$ to the methylsulfonyl-carboxylic acid **13** which was then condensed with aniline or benzylamine to give the amides **7a** and **7b**, respectively.

The heteroaromatics phenyl- and benzyl amides **8–10** were prepared as shown in Scheme 2. The methylthiophenyl carbaldehydes of thiophene (**14**), pyrazole (**15**) and isoxazole (**16**) [20] were oxidized by a reaction with NaClO$_2$–H$_2$O$_2$ and a solution of NaH$_2$PO$_4$ (for the preparation of **17** and **19**) or by oxone® in THF–MeOH (for the preparation of **18**). The reaction of the carboxylic acids **17–19** with aniline or benzylamine in the presence of *N*-hydroxybenzotriazole (HBT) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide (EDC) yielded the corresponding amides **8–10** of type **a** or **b**, respectively.



Scheme 2. Synthesis of the thiophene (**8a,b**), pyrazole (**9a,b**) and isoxazole (**10a,b**) analogs of **7a,b. i**: NaClO$_2$, H$_2$O$_2$, NaH$_2$PO$_4$, CH$_3$CN; **ii**: oxone, THF–MeOH, r.t., 6 h; **iii**: PhNH$_2$ or PhCH$_2$NH$_2$, HBT, EDC, THF, r.t., 24 h.

3. Biological results and conclusion

For the new compounds **7–10** the inhibitory activity towards COX-2, which constitutes the ideal target of an anti-inflammatory drug, was evaluated in vitro by measuring the PGE2 production on activated J774.2 macrophages. The results were reported in Table 1 together with those obtained in the same type of test with celecoxib (**3**) and with the previously studied cyclopentenyl and thienyl oxime-ethers **5** and **6**. As it can be seen, only compounds **7a, 8a, 9a** and **9b**, at a concentration of 10 μM showed a modest activity, with percentage inhibition values close to 30%, while the other analogs resulted practically inactive. In the same experimental conditions, the benzyloxyimino-ethers **5** and **6** showed IC$_{50}$ values in the μM range.

These data indicate that in the field of the analogs of the tricyclic anti-inflammatory drugs, an amidic group, either *N*-phenyl- or *N*-benzyl-substituted, as the ones present in type **C** compounds **7–10**, is less effective than MAOMM in replacing the aryl lacking of sulfurated moiety of compounds of type **A**.

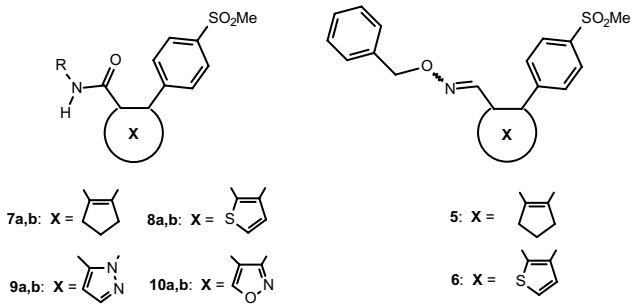
In order to attempt a possible rationalization of these results, a conformational study was carried out on compounds of the homogeneous series of the *N*-phenyl-substituted amides **7a, 8a** and **9a**, which includes the larger number of amidic compounds able to prove a certain ability to interact with COX-2 enzyme. Fig. 5 shows the superimposition of the most favorable conformation found for each of these compounds, together with those obtained for oxime-ether derivative **5** and for celecoxib **3**. From this figure it results clear that for compounds **7a–9a** the practically planar amidic group occupies a spatial region which roughly corresponds to the one which hosts both the aryl of celecoxib (**3**) and the methyleneaminoxy portion of the MAOMM of compound **5**. Nevertheless, amidic compounds **7a–9a** present an additional steric hindrance, due to *N*-phenyl substituent, in a spatial region, which is free in the more active compounds **3** and **5**. Therefore, the lower activity of the amidic compounds **7–9**, with respect to that of celecoxib (**3**) and the oxime-ethers **5** and **6**, may be tentatively attributed to this additional steric hindrance, which may at least partially prevent their good fit with the COX-2 active site.

4. Experimental procedures

4.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra of all com-

Table 1
Chemical and biological data for compounds **7a,b–10a,b**



Compound	R	m.p. (°C)	Recrystallizing solvent [a]	Formula [b]	In vitro inhibitory activity [c] COX-2
7a	PhNH	188–190	A	$C_{19}H_{19}NO_3S$	24%
7b	$PhCH_2NH$	120–122	B	$C_{20}H_{21}NO_3S$	7%
5 [d]	–				1.9
8a	PhNH	209–211	B	$C_{18}H_{15}NO_3S_2$	28%
8b	$PhCH_2NH$	164–166	C	$C_{19}H_{17}NO_3S_2$	4%
6 [e]	–				1.7
9a	PhNH	211–213	B	$C_{17}H_{15}N_3O_3S$	34%
9b	$PhCH_2NH$	180–182	B	$C_{18}H_{17}N_3O_3S$	26%
10a	PhNH	243–245	B	$C_{17}H_{14}N_2O_4S$	0
10b	$PhCH_2NH$	146–148	D	$C_{18}H_{16}N_2O_4S$	0
3 (celecoxib)					0.02

[a] A, *i*-PrOH; B, EtOH; C, $CHCl_3$–hexane; D, MeOH.
[b] All compounds were analyzed for C, H and N.
[c] Data are indicated as IC_{50} (μM) (for compounds **5, 6**) or as percentage of inhibition at a concentration of 10μM.
[d] See Ref. [18].
[e] See Ref. [20].

pounds were obtained with a Gemini 200 spectrometer 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 chromatographies were performed using 70–230 mesh silica gel. Mass spectra were detected with a Hewlett Packard 5988A spectrometer. Evaporations were made in vacuo (rotating evaporator). Na_2SO_4 was always used as the drying agent. Elemental analyzes were performed in our analytical laboratory and agreed with the theoretical values to within ±0.4%.

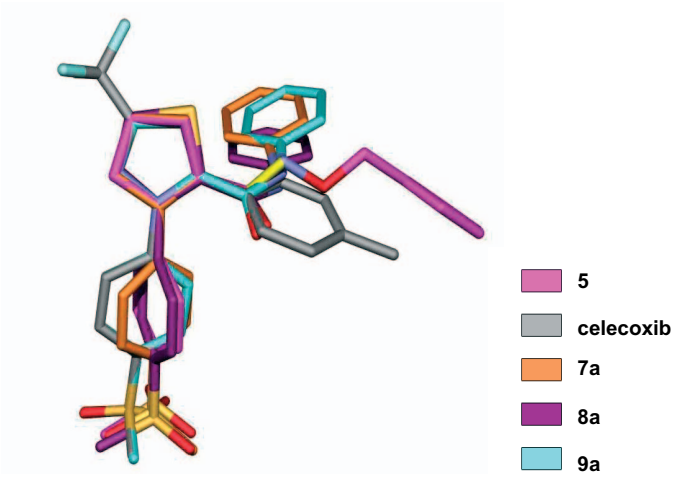


Fig. 5. Superimposition of the *N*-phenyl-substituted amides **7a–9a**, with celecoxib (**3**) and cyclopentene MAOM derivative **5**, in their lowest energy conformations.

*4.1.1. Synthesis of 2-[(4-methylthio)phenyl]cyclopent-1-ene-1-carbaldehyde **12***

A mixture of 2-bromocyclopent-1-ene-1-carbaldehyde **11** (1.0 g, 5.72 mmol) and 4-methylthiophenylboronic acid (0.96 g, 5.72 mmol) in a 1:1 toluene–EtOH mixture (32 ml) and aqueous 2 M Na_2CO_3 (12 ml) was treated with $Pd(PPh_3)_4$ (0.2 g) and then refluxed under argon with stirring for 12 h. The organic phase was then evaporated and the aqueous residue was taken up in AcOEt, washed with H_2O, filtered and evaporated to afford a crude residue which was subjected to column chromatography eluting with 2:8 hexane–AcOEt mixture to yield pure **12** (70%): ^1H NMR: δ 2.00 (q, 2H, J = 7.6 Hz, CH_2); 2.51 (s, 3H, SMe); 2.75–2.97 (m, 4H, CH_2); 7.27 (s, 4H, Ar); 9.83 (s, 1H, CHO).

*4.1.2. Synthesis of 2-(4-methylsulfonylphenyl)cyclopent-1-ene-1-carboxylic acid **13***

A solution of 2-(4-methylthiophenyl)cyclopent-1-ene-1-carbaldehyde **12** (0.10 g, 0.46 mmol) in CH_3CN (0.5 ml) was treated with NaH_2PO_4 (15 mg) in H_2O (0.2 ml) and with a

solution of H_2O_2 30% (0.2 ml). The resulting solution was then treated dropwise with $NaClO_2$ (0.03 g, 0.33 mmol) in H_2O (1 ml). After 5 h of vigorous stirring at r.t., the mixture was diluted with H_2O and neutralized with aqueous $NaHCO_3$ and extracted three times with $CHCl_3$. The aqueous phase was then acidified to pH 4 with a solution of HCl 10% and the solid residue which precipitated was then collected and purified by crystallization from *i*-PrOH (25%): m.p. 184–186 °C; ^{1}H NMR: δ 2.04 (m, 2H, CH_2); 2.80–2.91 (m, 4H, CH_2); 3.07 (s, 3H, SO_2Me); 7.48 and 7.89 (2d, 4H, J = 8.4 Hz, $ArSO_2Me$). Anal. $C_{13}H_{14}O_4S$ (C, H).

*4.1.3. Synthesis of 3-(4-methylsulfonylphenyl)thiophene-2-carboxylic acid **17***

Compound **17** was synthesized from **14** [20] following the same procedure described above for the preparation of **13**. Compound **17** (50%): m.p. 163–165 °C (*i*-PrOH); ^{1}H NMR: δ 3.11 (s, 3H, SO_2Me); 7.09 (s, 1H, H4 thiophene); 7.62–7.68 (m, 3H, H5 thiophene and $ArSO_2$); 7.97 (d, 2H, J = 8.4 Hz, $ArSO_2$). Anal. $C_{12}H_{10}O_4S_2$ (C, H).

*4.1.4. Synthesis of 1-(4-methylsulfonylphenyl)pyrazole-5-carboxylic acid **18***

A cooled (0 °C) and stirred solution of 1-(4-methylthiophenyl)pyrazole-5-carbaldehyde **15** (0.67 g, 3.04 mmol) [20] in a 1:1 THF–MeOH mixture (18 ml) was treated dropwise with a solution of oxone® (4.12 g, 6.70 mmol) in H_2O (17 ml). The resulting mixture was vigorously stirred for 6 h, and then diluted with AcOEt and washed with water. The organic phase was extracted with a solution of $NaHCO_3$ and the aqueous extracts were combined and acidified to pH 1 and extracted with AcOEt. Evaporation of the organic phase gave a crude residue which was purified by crystallization from $CHCl_3$. Compound **18** (65%): m.p. 240–242 °C; ^{1}H NMR (DMSO): δ 3.31 (s, 3H, SO_2Me); 7.09 and 7.87 (2d, 2H, J = 2.7 Hz, H3 and H4 pyrazole); 7.78 and 8.03 (2d, 4H, J = 8.4 Hz, $ArSO_2Me$). Anal. $C_{11}H_{10}N_2O_4S$ (C, H, N).

*4.1.5. Synthesis of 3-(4-methylsulfonylphenyl)isoxazole-4-carboxylic acid **19***

Compound **19** was synthesized from **16** [20] following the same procedure described above for the preparation of **13**. Compound **19** (47%): m.p. 219–221 °C (*i*-PrOH); ^{1}H NMR: δ 3.11 (s, 3H, SO_2Me); 8.05 (s, 4H, Ar); 9.14 (s, 1H, H5 isoxazole). Anal. $C_{11}H_9NO_5S$ (C, H, N).

*4.1.6. Synthesis of 2-(4-methylsulfonylphenyl)cyclopent-1-ene-1-carboxylic acid phenylamide and 2-(4-methylsulfonylphenyl)cyclopent-1-ene-1-carboxylic acid benzylamide **7a,b***

A solution of **13** (0.33 g, 1.25 mmol) in THF (17 ml) was treated with the appropriate amine (1.25 mmol), HBT (0.25 g, 1.87 mmol) and EDC (0.42 g, 2.20 mmol). The resulting mixture was stirred at r.t. for 24 h. Evaporation of the solvent afforded to a crude residue which was taken up in AcOEt, and washed with aqueous solutions of NaOH 10% and HCl 10%. The organic phase was then dried and evaporated to yield a residue, which was purified by crystallization from the appropriate solvent. **7a** (30%) ^{1}H NMR: δ 2.23 (m, 2H, CH_2); 3.11 (s, 3H, SO_2Me); 2.90–3.32 (m, 4H, CH_2); 7.36–7.56 (m, 5H, Ph); 7.61 and 8.02 (2d, 4H, J = 8.2 Hz, $ArSO_2$). **7b** (75%) ^{1}H NMR: δ 2.25 (m, 2H, CH_2); 3.10 (s, 3H, SO_2Me); 2.92–3.35 (m, 4H, CH_2); 4.42 (d, 2H, J = 5.6 Hz, $PhCH_2$); 6.83 (bs, NH); 7.12–7.34 (m, 5H, Ph); 7.59 and 7.88 (2d, 4H, J = 8.2 Hz, $ArSO_2$).

*4.1.7. Synthesis of 3-(4-methylsulfonylphenyl)thiophene-2-carboxylic acid phenylamide and 3-(4-methylsulfonylphenyl)thiophene-2-carboxylic acid benzylamide **8a,b***

Compounds **8a,b** were synthesized from **17** following the same procedure described above for the preparation of **7a,b**. Compound **8a** (38%) ^{1}H NMR: δ 3.11 (s, 3H, SO_2Me); 7.12 and 7.57 (2d, 2H, J = 5.1 Hz, H4 and H5 thiophene); 7.20–7.32 (m, 5H, Ph); 7.74 and 8.05 (2d, 4H, J = 8.4 Hz, $ArSO_2$). **8b** (30%) ^{1}H NMR: δ 3.03 (s, 3H, SO_2Me); 4.46 (d, 2H, J = 5.7 Hz, $PhCH_2$); 5.65 (bs, NH); 7.06–7.50 (2d, 2H, J = 5.1 Hz, H4 and H5 thiophene); 7.11–7.31 (m, 5H, Ph); 7.59 and 7.88 (2d, 4H, J = 8.2 Hz, $ArSO_2$).

*4.1.8. Synthesis of 3-(4-methylsulfonylphenyl)pyrazole-5-carboxylic acid phenylamide and 3-(4-methylsulfonylphenyl)pyrazole-5-carboxylic acid benzylamide **9a,b***

Compounds **9a,b** were synthesized from **18** following the same procedure described above for the preparation of **7a,b**. Compound **9a** (72%) ^{1}H NMR: δ 3.08 (s, 3H, SO_2Me); 6.88 and 7.78 (2d, 2H, J = 2.0 Hz, H3 and H4 pyrazole); 7.19–7.56 (m, 5H, Ph); 7.81 (bs, 1H, NH), 7.74 and 7.99 (2d, 4H, J = 8.6 Hz, $ArSO_2$). MS (*m/z*) 341 (M^+). **9b** (38%) ^{1}H NMR: δ 3.08 (s, 3H, SO_2Me); 4.57 (d, 2H, J = 5.9 Hz, $PhCH_2$); 6.39 (bs, NH); 6.74 (d, 1H, J = 1.8 Hz, H4 pyrazole); 7.29–7.41 (m, 5H, Ph); 7.71–7.77 (m, 3H, $ArSO_2$ and H3 pyrazole); 7.99 (d, 2H, J = 8.6 Hz, $ArSO_2$). MS (*m/z*) 355 (M^+).

*4.1.9. Synthesis of 3-(4-methylsulfonylphenyl)isoxazole-4-carboxylic acid phenylamide and 3-(4-methylsulfonylphenyl)isoxazole-4-carboxylic acid benzylamide **10a,b***

Compounds **10a,b** were synthesized from **19** following the same procedure described above for the preparation of **7a,b**. Compound **10a** (38%) ^{1}H NMR: δ 3.10 (s, 3H, SO_2Me); 7.31–7.48 (m, 5H, Ph); 8.02–8.07 (m, 4H, SO_2Me); 9.02 (s, 1H, H5 isoxazole). MS (*m/z*) 342 (M^+). **10b** (20%) ^{1}H NMR: δ 3.06 (s, 3H, SO_2Me); 4.54 (d, 2H, J = 5.7 Hz, $PhCH_2$); 7.23–7.37 (m, 5H, Ph); 7.92 and 7.98 (2d, 4H, J = 8.6 Hz, $ArSO_2$); 8.88 (s, 1H, H5 isoxazole). MS (*m/z*) 356 (M^+).

4.2. Conformational studies

A conformational optimization on compounds celecoxib (**3**), oxime-ether **5** and anilides **7a, 8a** and **9a** was performed

through the Gaussian 98 program [28] at AM1 semiempirical level. A superimposition of the five-membered rings of these compounds was then made and the results are shown in Fig. 5.

4.3. Enzyme assays

Compounds **7–10** were tested following the procedure previously described [18] in intact cell assays to verify their capacity to inhibit PGE2 production, considered as an index of activity on COX-2 enzymes. The COX-2 assay was performed in accordance with the method described by Mitchell et al. [29] with minor modifications, as suggested by Grossman et al. [30]. Murine J774.2 cells were pretreated for 1 h with 300 μM aspirin to inactivate endogenous constitutive COX-1 and were then stimulated with LPS to induce COX-2 expression. After overnight incubation, cells were treated for 45 min with the different test compounds. Supernatants were then collected and PGE2 was measured by a commercial immunoenzymatic assay (Amersham). All compounds were tested in duplicate. For each product, a stock solution was prepared in DMSO at a concentration of 100 mM.

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