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Eur. J. Med. Chem. 37 (2002) 585–594

EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY

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Original article

Aryl-substituted methyleneaminoxymethyl (MAOM) analogues of diarylcyclopentenyl cyclooxygenase-2 inhibitors: effects of some structural modifications on their biological properties

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Received 11 January 2002; accepted 12 April 2002

Abstract

The (*E*)-[2-(4-aminosulfonylphenyl)-1-cyclopentenyl-1-methyliden]-(arylmethyloxy)amines (**6a**,**b**), which are the sulfonamidic analogues of the previously described methylsulfonyl derivatives **5a**,**b**, and their corresponding sulfides (**7a**,**b**) and sulfoxides (**8a**,**b**) were synthesised in order to obtain information about the role played by these different sulphur-containing groups in the cyclooxygenase-2 inhibitory activity of this class of compounds. In addition, other chemical manipulations concerning the oxime-ether substituent of this type of derivatives were affected by preparing compounds **9a**,**b**, which present a methyl group on the oximic carbon of the oxime-ether chain of **5a**,**b**, and compounds **10** and **11**, in which the atomic sequence (C=NOCH$_2$) of the MAOMM of **8b** and **5b**, respectively, is inverted. Compounds **6**–**11** were tested in vitro for their inhibitory activity towards COX-1 and COX-2 by measuring prostaglandin E2 (PGE2) production in U937 cell lines and activated J774.2 macrophages, respectively. Some of the new compounds showed an appreciable in vitro COX-2 inhibitory activity, with IC_{50} values in the μM (**7a**,**b**, **8a** and **9b**) or sub-μM (**8b**) range. This last compound was also assayed in vivo for its antiinflammatory activity by means of the carrageenan-induced paw edema test in rats. No inhibitory effects were detected up to dose of 30 mg kg^{-1} orally administered.

Keywords: Antiinflammatory drug; COX-2 inhibitor; Aryl-substituted methyleneaminoxymethyl moiety; (*E*)-(2-aryl-1-cyclopentenyl-1-alkylidene)(arylmethyloxy)amine; (*E*)-[2-aryl-1-cyclopentenylmethyloxy)-(benzylidene)amine

1. Introduction

Most non-steroidal antiinflammatory drugs (NSAIDs) act through the inhibition of cyclooxygenase (COX) [1,2]. This enzyme exists in two isoforms, one constitutive (COX-1), which is involved in different physiological processes, such as gastric cytoprotection, and the other inducible (COX-2), which is expressed in the presence of a wide variety of inflammatory mediators, and which appears to play a major role in the production of prostaglandins associated with inflammation responses [3–8].

The target of an ideal NSAID is therefore that of inhibiting the production of COX-2 during the inflammation process, without modifying the physiological levels of constitutive COX-1 [9,10].

In the last decade, new COX-2 selective inhibitors have been described, in which the common molecular characteristic consists of two aromatic moieties attached to adjacent atoms in a bridging carbocyclic or heterocyclic five-membered ring; one of the aromatic groups is substituted in the *para* position by a methylsulfonyl (**1**, **2** and **3**) [11,12] or by an aminosulfonyl

Abbreviations: NSAID, non-steroidal antiinflammatory drug; COX, cyclooxygenase; MAOMM, methyleneaminoxymethylmoiety; PGE2, prostaglandin E2.

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0223-5234/02/$ - see front matter
PII: S0223-5234(02)01385-5

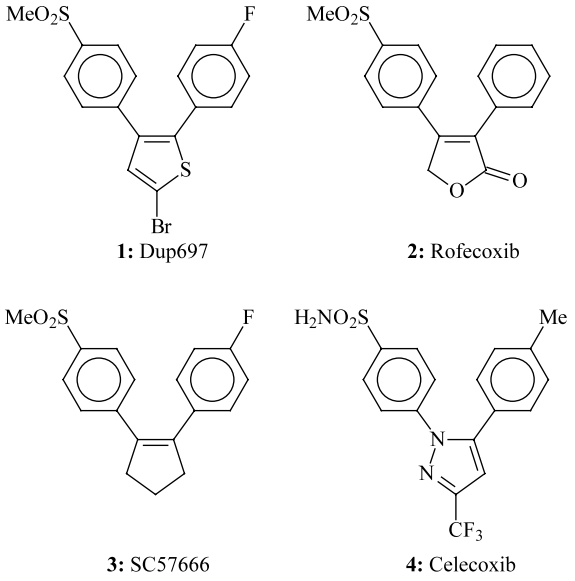


Fig. 1. Structures of some representative COX-2 inhibitors containing a 1,2-diaryl-substituted carbocyclic or heterocyclic five-membered ring.

group (**4**) [13–15] (Fig. 1). At present, compounds **2** (rofecoxib) and **4** (celecoxib) are marketed drugs.

In the course of a programme aiming to discover new molecules with a selective COX-2 inhibitory activity, we have described the synthesis and the COX-1 and COX-2 inhibitory activity of a series of compounds of type **5** (Fig. 2) which can be viewed as analogues of type **3** drugs, in which one of the two aryl groups is replaced by an aryl-substituted methyleneaminoxymethyl moiety (MAOMM) [16].

Some of the new compounds have been shown to possess an appreciable in vitro inhibitory activity, with IC_{50} values in the μM range, and in the case of the methylenedioxy derivative (**5a**), of 0.4, versus 0.02 μM of celecoxib (**4**) [16]. Furthermore compound **5a** showed a modest antiinflammatory activity in the in vivo test of carrageenan-induced paw edema in rats, only after intraperitoneal administration [16]. Since the sulfonamidic moiety, with respect to the methylsulfonic one, is known to provide better bioavailability in other COX-2 inhibitors structurally related to **4** [8,17], we decided to synthesise compounds **6a**,**b**, which are the sulfonamidic analogues of **5a**,**b**. In addition, also the corresponding sulfides (**7a**,**b**) and sulfoxides (**8a**,**b**) were prepared.

This paper also describes further chemical manipulations concerning the oximethereal substituent of some of these compounds, able to influence the conformational freedom of this side-chain. In compounds **9a** and **9b**, which represent superior homologues of **5a** and **5b**, the conformational situation of the oximethereal system should be modified, with respect to that of **5a** and **5b**, by the increase in the steric hindrance of the substituent linked to the oximic carbon, i.e. a methyl group instead of a hydrogen atom. In compounds **10** and **11**, which can be viewed as isomeric analogues of **8b** and **5b**, respectively, in which the atomic sequence $C{=}NOCH_2$ of the MAOMM is transformed into the formally inverted one $CH_2ON{=}C$, the oximethereal system should possess a higher conformational freedom with respect to **8b** and **5b**, as a result of the loss of conjugation between the oximic double bond and the unsaturated cyclopentenic portion.

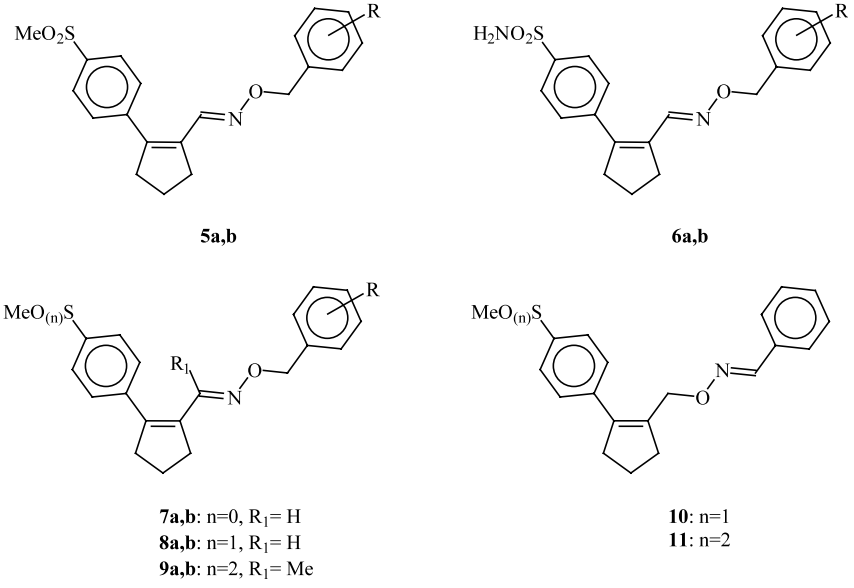
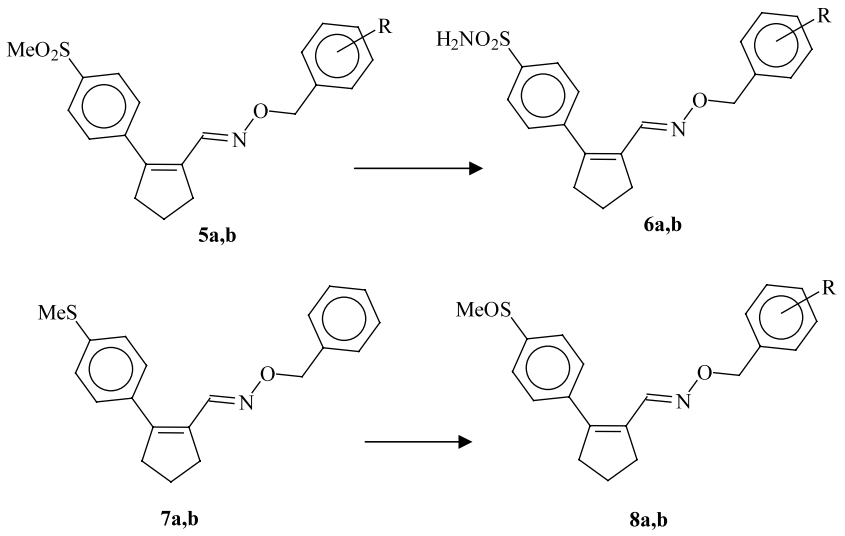


Fig. 2. Structures of oximethereal cyclopentene derivatives **5**–**11**.



a: R= 3,4-OCH_2O-; **b:** R=H

Fig. 3. Synthesis of compounds **6a**,**b** and **8a**,**b**.

2. Chemistry

The oximethereal sulfonamides **6a** and **6b** were prepared by a one-step procedure (Fig. 3), treating the corresponding methylsulfones **5a** and **5b** with lithium diisopropylamide (LDA) and triethylborane, and then with aminoxysulfonic acid in the presence of sodium acetate.

The sulfoxides **8a** and **8b** were synthesised by oxidation of the corresponding sulfides (**7a** and **7b**) [16] with 0.6 equiv. amount of potassium peroxymonosulfate (oxone) in a THF–MeOH–H_2O mixture.

The oxime-ethers substituted with a methyl group on the oximic carbon (**9a** and **9b**) were obtained as outlined in Fig. 4, starting from the cyclopentanone **12** [18], which by treatment with acetyl chloride in the presence of $AlCl_3$ afforded 1-acetyl-2-chlorocyclopentene (**13**). Reaction of **13** with the appropriate O-benzylhydroxylamine hydrochloride yielded the corresponding oxime-ethers with the *Z* (**14**) and *E* (**15**) configuration in a ratio of about 20:80 in the case of **14a** and **15a**, or 38:62 in the case of **14b** and **15b**; the separation was performed by column chromatography. The cross-coupling reaction [19] of the chloroderivatives **15a** or **15b** with *p*-methylthio-phenylboronic acid in anhydrous dioxane in the presence of P(*c*-$C_6H_{11})_3$, tris-(dibenzylideneacetone)-dipalladium and Cs_2CO_3, afforded the oxime-ether sulfides **16a** or **16b**, respectively. Oxidation of **16a** or **16b** with 1.2 equiv. of oxone gave the corresponding sulfones with the *E* configuration, **9a** or **9b**.

The sulfoxide (**10**) and sulfone (**11**) oxime-ethers were synthesised as indicated in Fig. 5. Reaction of the hydroxymethylvinylbromide derivative **17** with *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboximide in the presence of triphenylphosphine and diethylazodicarboxylate in THF yielded the imide-derivative **18**, which by hydrazinolysis with $H_2NNH_2{\cdot}H_2O$ in anhydrous EtOH, afforded the O-alkyl-hydroxylamine **19**. Treatment of **19** with benzaldehyde in a heterogeneous medium ($H_2O/CHCl_3$) gave the (*Z*)-(**20**) and (*E*)-bromooxime (**21**) in a ratio of about 20:80; the separation was performed by column chromatography. The cross-coupling reaction of the vinylbromide **21** with *p*-methylthiophenylboronic acid in toluene–EtOH solution in the presence of $Pd(PPh_3)_4$ and aqueous Na_2CO_3 afforded the sulfide **22**, which by oxidation with 1 or 2 equiv. of oxone in a THF–MeOH mixture gave the sulfoxide (**10**) or the sulfone (**11**) derivatives, respectively.

The *E*/*Z*-configuration of the oxime-ethers **14** and **15** (Fig. 4) was assigned bearing in mind that in the compounds with the *E* configuration (**15a**,**b**) the protons of the methyl linked to the oximic carbons resonate at field values lower (0.2 ppm) than in the isomers with the *Z* configuration (**14a**,**b**). The *E*-configuration of the intermediate sulfides **16a**,**b** and final products **9a**,**b**, was made because during the synthetic sequence the configuration around the oximic double bond should not change. Also the *E*/*Z*-configuration of the oxime-ethers **20** and **21** (Fig. 5) was assigned on the basis of the chemical shift values of the oximic proton signal, which resonates at lower fields (0.5 ppm) in the *E* isomer (**21**) with respect to the *Z* one (**20**). The *E*-configuration of the sulfide (**22**), sulfoxide (**10**) and sulfone (**11**) was made on the basis of the chemical shift values of their diagnostic oximic

hydrogens, which resulted very similar to that of the intermediate oxime-ether with the *E* configuration (**21**).

3. Biopharmacological results

The in vitro inhibitory activity towards COX-1 and COX-2 enzymes of compounds **6**–**11**, celecoxib (**4**), chosen as a reference compound, and previously studied methylsulfonyl compounds **5a**,**b** is reported in Table 1.

In regard to the COX-1 enzyme, as previously found for **5a** and **5b**, only the sulfide and the sulfoxide analogues of **5a**,**b** (**7a**,**b** and **8a**,**b**, respectively) proved to be slightly active, with percentage inhibition values ranging from 20% of **7a** to 29% of **5b** at a concentration of 10 μM or IC_{50} values of 23 and 32 μM for **8a** and **8b**, respectively. The other new compounds, i.e. the sulfonamidic analogues of **5a**,**b** (**6a**,**b**), the superior homologues of **5a**,**b** (**9a** and **9b**) in which the oximic carbon is substituted by a methyl group, and compounds **10** and **11**, in which the atomic sequence of the oximic side-chain is inverted with respect to **8b** and **5b**, proved to be practically inactive.

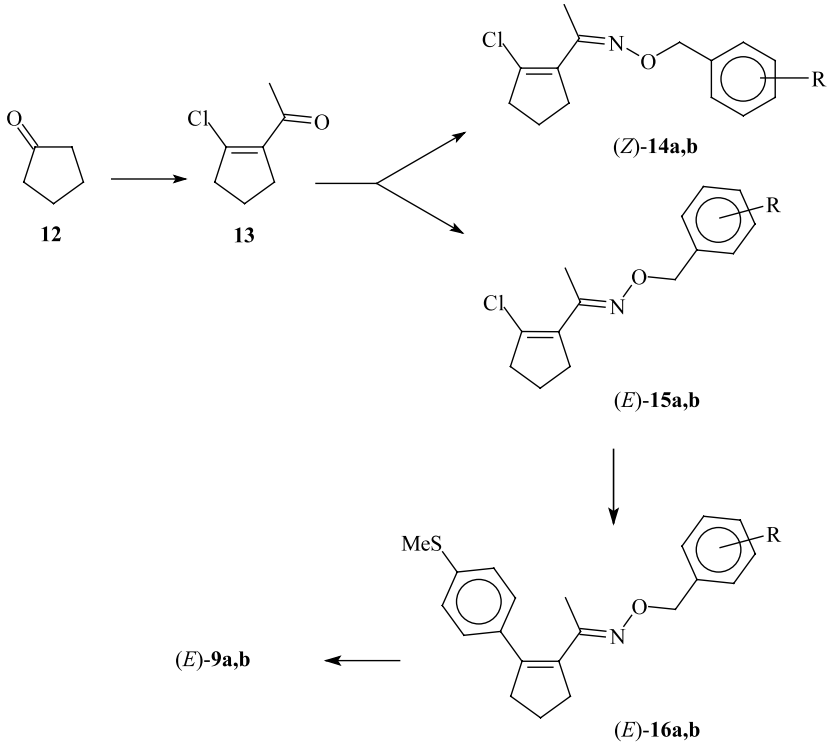
In regard to the inhibitory activity towards the COX-2 enzyme, while compound **11** was completely inactive, both sulfonamidic derivatives **6a**,**b** and compound **10** showed a very modest activity, with percentage inhibition values ranging from 12 to 20% at a concentration of 10 μM. The sulfides **7a**,**b** and the sulfoxide **8a** appeared to possess a higher activity, with an IC_{50} value in the micromolar range, and the sulfoxide **8b** proved to be the most active of the new compounds, with an activity index of 0.1 μM.

Among the superior homologues of **5a** and **5b** (**9a** and **9b**), only the unsubstituted (**9b**) proved to possess an appreciable activity, with an IC_{50} value of 1 μM, while the methylenedioxy-substituted one (**9a**) showed a very modest activity.

For compound **8b**, which showed the highest inhibitory activity towards COX-2 in the in vitro test, also the in vivo inhibitory activity was evaluated by the carrageenan-induced paw edema test in rats. In this experiment, compound **8b** was administered per os at increasing doses ranging from 1 to 30 mg kg^{-1}. No inhibitory effects were detected in any case.

4. Discussion and conclusions

The primary aim of this work was that of verifying the effects on the inhibitory activity towards COXs of the substitution of the methylsulfonyl moiety of type **5** compounds with different sulphur-containing moieties, such as the aminosulfonilic, methylsulfoxidic or methylsulfidic ones. A second aim was that of evaluating the



a: R= 3,4-OCH_2O-; **b:** R= H

Fig. 4. Synthesis of compounds **9a**,**b**.

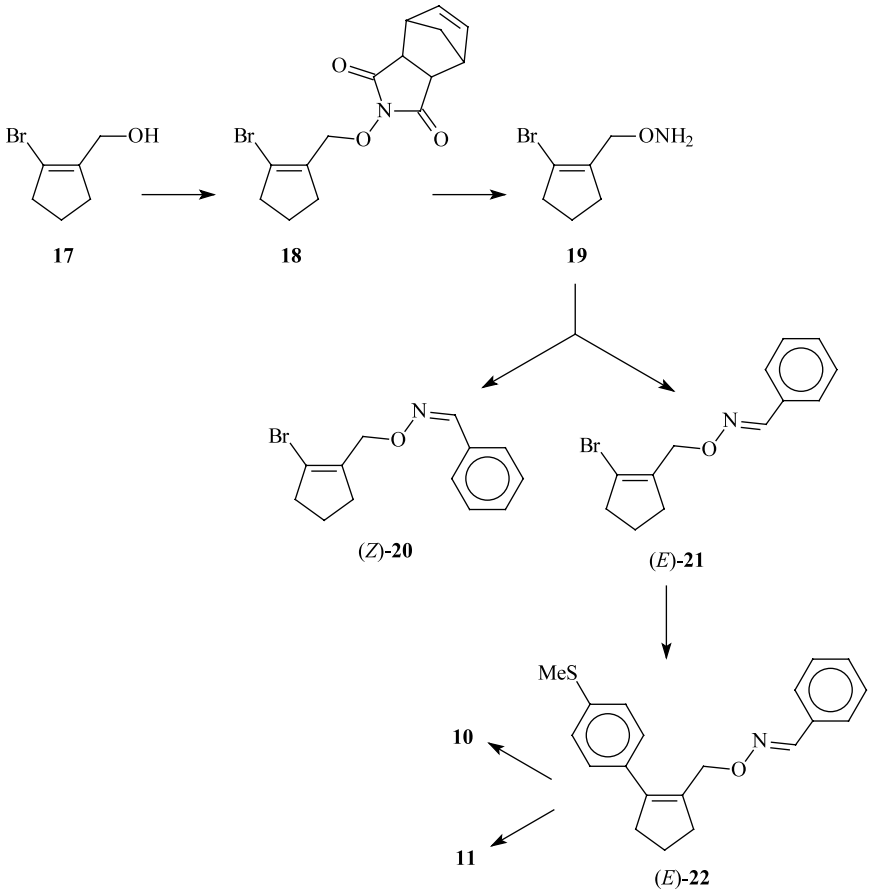


Fig. 5. Synthesis of compounds **10** and **11**.

effects on this type of activity of some modifications of the oxime-ether side-chain of compounds **5**, which presumably influence their conformational situation.

The data in Table 1 indicate that the substitution of the methylsulfonyl group of **5a** and **5b** with the sulfonamidic one of **6a** and **6b** leads to compounds devoid of any in vitro inhibitory activity, discouraging their further in vivo studies. The substitution of the methylsulfonyl group of **5a** and **5b** with the methylsulfidic one as in **7a** and **7b**, does not have any appreciable effect on the activity towards COX-1, whereas it determines a decrease in their COX-2 activity of about 1.2 or 0.4 order of magnitude, respectively.

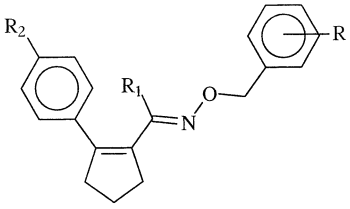
The substitution of the methylsulfonyl group of **5a** and **5b** with the methylsulfoxidic one of **8a** and **8b** leads to a slight increase in the inhibitory activity towards COX-1; on the contrary, the same type of substitution has an opposite effect towards COX-2, depending on the type of substituent on the aromatic ring of the oximic side-chain: it reduces the activity by about one order of magnitude in the case of the methylenedioxy compound **8a**, while it increases the same type of activity by more than one order of magnitude in the case of the unsubstituted compound **8b**.

Overall, the in vitro tests of compounds **5**–**8** indicate that among the sulfurated groups examined, only the sulfonamidic one of **6a**,**b** is completely unable to substitute the methylsulfonyl group effectively in the interaction with COX-2. A possible explanation of this observation is that some peculiar chemical and physical characteristics of **6a**,**b** may hinder their approach to the catalytic COX-2 site; an alternative hypothesis is that substantial differences may exist between the structure–activity relationship of oximether compounds, with respect to that of tricyclic drugs.

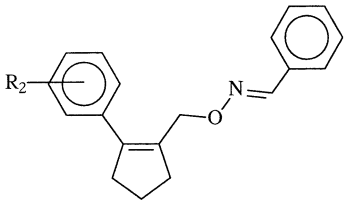
As for compounds in which the oximether side-chain has been modified (**9**–**11**), the results obtained with **9a**,**b** indicate that the substitution of the oximic hydrogen of **5a**,**b** with a methyl group leads to a complete loss of the activity towards COX-1; towards COX-2, the same modification, while has a significantly negative effect in the case of the methylenedioxy derivative **9a**, which is almost inactive, in the case of the unsubstituted compound **9b** approximately doubles the activity. For compounds **10** and **11**, the data in Table 1 show that the formal inversion of the atomic sequence of the MAOMM of **8b** and **5b** has a marked negative effect towards both types of COX, leading to the complete loss of activity.

In order to attempt a rationalisation of these results, a conformational study was carried out on compounds **5b**, **9b**, and **11**. Fig. 6 shows the superimposition of the most favourable conformations found for each of these compounds (**b**, **c**, and **d**), together with those obtained for SC57666 (**a**). A comparison of the conformational profile of **5b** (**b** in Fig. 6) with that of **9b** (**c** in Fig. 6) shows that their common portion (cyclopentene and

Table 1
Biological data of compounds **5a**,**b**–**9a**,**b** and **10**,**11**



5a,b-9a,b



10,11

Compound	R	R_1	R_2	In vitro inhibitory activity [a]	
				COX-1	COX-2
5a [b]	3,4-OCH_2O-	H	SO_2Me	27% [c]	0.4
5b [b]	H	H	SO_2Me	29% [c]	1.9
6a	3,4-OCH_2O-	H	SO_2NH_2	0	12% [c]
6b	H	H	SO_2NH_2	0	19% [c]
7a	3,4-OCH_2O-	H	SMe	20% [c]	8.0
7b	H	H	SMe	22% [c]	4.4
8a	3,4-OCH_2O-	H	SOMe	23	5.0
8b	H	H	SOMe	32	0.1
9a	3,4-OCH_2O-	Me	SO_2Me	0	20% [c]
9b	H	Me	SO_2Me	0	1
10	–	–	SOMe	0	20% [c]
11	–	–	SO_2Me	0	0
4 (Celecoxib)				1.04	0.02

[a] Data are indicated as IC_{50} (μM) or as the percentage of inhibition at a concentration of 10 μM.
[b] See Ref. [16].
[c] $IC_{50} > 1$ mM.

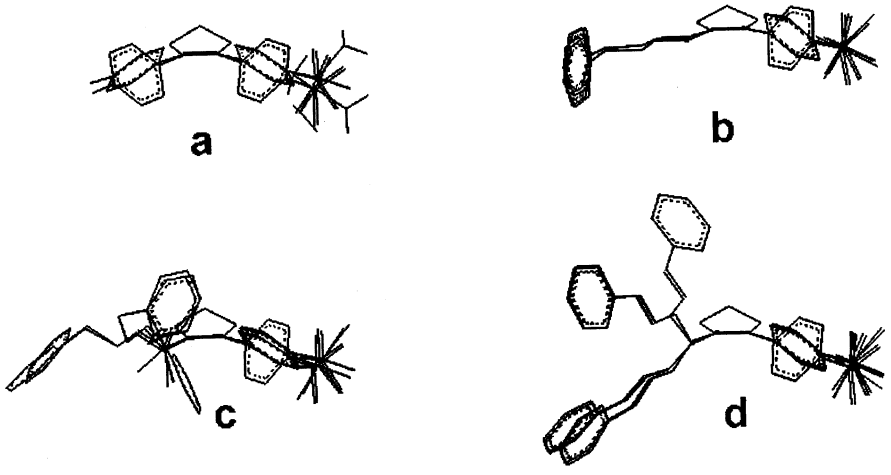


Fig. 6. Compounds SC57666 (**a**); **5b** (**b**); **9b** (**c**) and **11** (**d**). Conformations found through GMMX conformational searching techniques reported by superimposing the cyclopentene rings.

aryl substituent) present analogous conformational preferences; the oximether side-chain, on the other hand, while in **5b** always lies on the side opposite to the adjacent aryl, in compound **9b** may also be found on the same side as this aryl. The improvement in the activity of **9b** compared with **5b** might be attributed to these conformational differences, assuming that certain low-energy conformations which are preferred only for **9b** make a greater contribution to the activity thanks to a better ability to interact with the enzyme. The loss of activity on passing from **9b** to its methylenedioxy analogue **9a** may be tentatively attributed to this substituent, which in a structure possessing an additional methyl group, with respect to that of type **5**, may be of excessive hindrance for the access to the COX-2 enzyme.

A comparison of the conformational profile of **5b** (**b** in Fig. 6) and **11** (**d** in Fig. 6) reveals a marked difference in the oximic side-chain portion: unlike **5b**, in which this chain substantially assumes a single spatial orientation, in the inactive compound **11** (**d** in Fig. 6), it assumes various arrangements, which are all different from the preferential one of **5b**, which might potentially be the pharmacophoric one.

In regard to the lack of in vivo activity of compound **8b** in the carrageenan-paw edema test in rats after oral administration, this should be attributed to its limited gastrointestinal stability, poor absorption and/or rapid first pass metabolism.

5. Experimental

5.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken as paraffin oil mulls or as liquid films on a Unicam Mattson 1000 FTIR spectrometer. ^{1}H-NMR spectra of all compounds 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 chromatography was performed using 70–230 mesh silica gel. Mass spectra were detected with a Hewlett Packard 5988A spectrometer (EI, 70 eV). Evaporation was made in vacuo (rotating evaporator); the not commercially available O-arylmethyloxyamines used for the preparation of the chlorooximethers **14** and **15**, were synthesised as described in Ref. [20]. 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 the (E)-2-(4-aminosulfonylmethyl)-1-(arylmethyloxyiminomethyl)cyclopentene (6a,b)

A cooled (0 °C) and stirred solution of the appropriate methylsulfone **5a**,**b** [16] (1.70 mmol) in anhydrous THF (0.9 mL), was treated with LDA 2.0 M in hexane (1.1 mL, 2.2 mmol), and the resulting mixture was stirred at room temperature (r.t.) for 1 h. The mixture was cooled at 0 °C, and treated with a 1.0 M solution of triethylborane in THF (2.8 mL, 2.8 mmol). At the end of the addition, the reaction was stirred at r.t. for 1 h and then refluxed for 20 h. The mixture was cooled at 0 °C, treated with AcONa (0.93 g, 1.2 mmol), water (4 mL) and hydroxylamine-O-sulfonic acid (0.80 g, 7.1 mmol) and then stirred at r.t. for 24 h. The organic phase was diluted with AcOEt, washed with water and brine, dried and then evaporated to afford a crude residue, which was purified by column chromatography using as the eluent hexane–AcOEt (6:4). **6a** (10%): ^{1}H-NMR: δ 1.95–2.07 (m, 2H, $CH_2CH_2CH_2$), 2.77–2.89 (m, 4H, $CH_2CH_2CH_2$), 4.82 (s, 2H, NH_2), 5.00 (s, 2H, $ArCH_2O$), 5.94 (s, 2H, OCH_2O), 6.75–6.87 (m, 3H, Ar), 7.40 and 7.86 (AA^1BB^1 system, 4H, $J = 8.4$ Hz, $ArSO_2NH_2$), 8.01 ppm (s, 1H, CH=N). Anal. $C_{20}H_{20}N_2O_5S$ (C, H, N). **6b** (5%): ^{1}H-NMR: δ 1.92–2.10 (m, 2H, $CH_2CH_2CH_2$), 2.75–2.92 (m, 4H, $CH_2CH_2CH_2$), 4.82 (s, 2H, NH_2), 5.10 (s, 2H, $ArCH_2O$), 7.22–7.38 (m, 5H, $ArCH_2O$), 7.33 and 7.85 (AA^1BB^1 system, 4H, $J = 8.8$ Hz, $ArSO_2NH_2$), 8.00 ppm (s, 1H, CH=N). Anal. $C_{19}H_{20}N_2O_3S$ (C, H, N).

5.1.2. Preparation of the (E)-2-(4-methylsulfinylphenyl)-1-(arylmethyloxyiminomethyl)cyclopentene (8a,b)

A cooled (0 °C) and stirred solution of the appropriate methylsulfide **7a**,**b** [16] (1.47 mmol) in a 1:1 MeOH–THF mixture (8 mL) was treated dropwise with a solution of oxone (0.86 mmol) in water (4 mL). At the end of the addition, the reaction was stirred at r.t. for 12 h. The solvent was evaporated and the crude residue was taken up in AcOEt, washed with water and brine, dried and evaporated to afford the crude sulfoxide **8a** or **8b**, which was purified by crystallisation from $(i\text{-Pr})_2O$. **8a** (38%): ^{1}H-NMR: δ 1.96–2.04 (m, 2H, $CH_2CH_2CH_2$), 2.70 (s, 3H, $SOCH_3$), 2.82–2.93 (m, 4H, $CH_2CH_2CH_2$), 4.98 (s, 2H, $ArCH_2O$), 5.92 (s, 2H, OCH_2O), 6.75–6.85 (m, 3H, Ar), 7.33 and 7.58 (AA^1BB^1 system, 4H, $J = 8.4$ Hz, $ArSOCH_3$), 8.00 ppm (s, 1H, CH=N). Anal. $C_{21}H_{21}NO_4S$ (C, H, N). **8b** (76%): m.p. 128–130 °C; ^{1}H-NMR: δ 1.96–2.21 (m, 2H, $CH_2CH_2CH_2$), 2.70 (s, 3H, $SOCH_3$), 2.80–2.93 (m, 4H, $CH_2CH_2CH_2$), 5.09 (s, 2H, $ArCH_2O$), 7.27 (s, 5H, Ar), 7.31 and 7.58 (AA^1BB^1 system, 4H, $J = 8.0$ Hz, $ArSOCH_3$), 8.02 ppm (s, 1H, CH=N); MS m/e: 339 (M^+). Anal. $C_{20}H_{21}NO_2S$ (C, H, N).

*5.1.3. 2-Chloro-1-acetylcyclopentene (**13**)*

A cooled (0 °C) suspension of $AlCl_3$ (40.0 g, 0.30 mol) in CH_2Cl_2 (75 mL) was treated dropwise with acetylchloride (23.5 g, 0.30 mol) and then with cyclopentanone (12.7 g, 0.15 mol) [18]. After 2 h at r.t., the mixture was treated with ice and solid $NaHCO_3$. The aq. phase was extracted with $CHCl_3$ and the organic layer was washed with a saturated K_2CO_3 solution, filtered and evaporated to yield an oily residue which was purified by column chromatography using a 9:1 hexane–AcOEt mixture as the eluent. **13** (15%): ^{1}H-NMR: δ 1.87–1.95 (m, 2H, $CH_2CH_2CH_2$), 2.48 (s, 3H, CH_3CO), 2.63–2.85 ppm (br, 4H, $CH_2CH_2CH_2$); MS m/e: 144 (M^+). Anal. C_7H_9ClO (C, H).

*5.1.4. Preparation of the (E)- and (Z)-2-chloro-1-[1-(arylmethyloxyimino)ethyl]cyclopentenes (**14a**,**b** and **15a**,**b**)*

A mixture of **13** (1.0 g, 6.9 mmol) in $CHCl_3$ (45 mL) and the appropriate O-arylmethyloxyamine (13.8 mmol) in water (10 mL), was stirred at r.t. for 36 h. The organic phase was separated and the aq. solution was extracted twice with $CHCl_3$. Evaporation of the dried and filtered organic extracts afforded a residue consisting almost exclusively of the stereoisomeric mixture of the chlorooximes in a ratio of 80:20 for (*E*)-**14a** and (*Z*)-**15a**, and 60:40 for (*E*)-**14b** and (*Z*)-**15b**, which were separated by column chromatography. (*E*)-**14a** (38%): ^{1}H-NMR: δ 1.80–1.92 (m, 2H, $CH_2CH_2CH_2$), 2.20 (s, 3H, CH_3C=N), 2.62–2.71 (m, 4H, $CH_2CH_2CH_2$), 5.02 (s, 2H, $ArCH_2O$), 5.95 (s, 2H, OCH_2O), 6.75–6.88 ppm (m, 3H, Ar). Anal. $C_{15}H_{16}ClNO_3$ (C, H, N). (*Z*)-**15a** (9%): ^{1}H-NMR: δ 1.80–1.92 (m, 2H, $CH_2CH_2CH_2$), 2.02 (s, 3H, CH_3C=N), 2.62–2.71 (m, 4H, $CH_2CH_2CH_2$), 4.95 (s, 2H, $ArCH_2O$), 5.95 (s, 2H, OCH_2O), 6.75–6.88 ppm (m, 3H, Ar). Anal. $C_{15}H_{16}ClNO_3$ (C, H, N). (*E*)-**14b** (30%): ^{1}H-NMR: δ 1.80–1.93 (m, 2H, $CH_2CH_2CH_2$), 2.22 (s, 3H, CH_3C=N), 2.62–2.70 (m, 4H, $CH_2CH_2CH_2$), 5.13 (s, 2H, $ArCH_2O$), 7.31–7.37 ppm (m, 5H, Ar). Anal. $C_{14}H_{16}ClNO$ (C, H, N). (*Z*)-**15b**: ^{1}H-NMR: δ 1.80–1.92 (m, 2H, $CH_2CH_2CH_2$), 2.03 (s, 3H, CH_3C=N), 2.62–2.70 (m, 4H, $CH_2CH_2CH_2$), 5.08 (s, 2H, $ArCH_2O$), 7.31–7.37 ppm (m, 5H, Ar). Anal. $C_{14}H_{16}ClNO$ (C, H, N).

*5.1.5. Preparation of the (E)-2-(4-methylthiophenyl)-1-[1-(arylmethyloxyimino)ethyl]cyclopentene (**16a**,**b**)*

A mixture of $Pd_2(dba)_3$ (0.3 mmol), 4-methylthiophenylboronic acid (0.30 g, 1.8 mmol) and Cs_2CO_3 (0.67 g, 2.0 mmol) was treated with the appropriate 1,2-cyclopentenylidene derivative of *E* configuration **14a** or **14b** (1.7 mmol) in dioxane (1.4 mL) and then with a solution of $P(Cy)_3$ 10% in $C_6H_5CH_3$ (100 μL, 0.06 mmol) [19]. The resulting mixture was stirred under N_2 at 80 °C for 12 h. The mixture was diluted with Et_2O, filtered on celite and then evaporated to afford a crude residue, which was subjected to column chromatography to yield pure **16a** or **16b**. **16a** (40%): ^{1}H-NMR: δ 1.73 (s, 3H, CH_3C=N), 1.87–1.98 (m, 2H, $CH_2CH_2CH_2$), 2.48 (s, 3H, SCH_3), 2.75–2.82 (m, 4H, $CH_2CH_2CH_2$), 5.01 (s, 2H, $ArCH_2O$), 5.96 (s, 2H, OCH_2O), 6.75–6.87 (m, 3H, $ArCH_2O$), 7.15 ppm (s, 4H, $ArSCH_3$). MS m/e: 381 (M^+). Anal. $C_{22}H_{23}NO_3S$ (C, H, N). **16b** (28%): ^{1}H-NMR: δ 1.75 (s, 3H, CH_3C=N), 1.87–2.01 (m, 2H, $CH_2CH_2CH_2$), 2.48 (s, 3H, SCH_3), 2.74–2.81 (m, 4H, $CH_2CH_2CH_2$), 5.13 (s, 2H, $ArCH_2O$), 7.15–7.34 ppm (m, 9H, 2Ar). Anal. $C_{21}H_{23}NOS$ (C, H, N).

*5.1.6. Preparation of the (E)-2-(4-methylsulfonylphenyl)-1-[1-(arylmethyloxyimino)ethyl]cyclopentene (**9a**,**b**)*

A solution of oxone (0.35 mmol) in water (1 mL) was added to a cooled (0 °C) solution of the appropriate methylsulfide **16a** or **16b** (0.29 mmol) in 1:1 MeOH–THF (2 mL). The resulting mixture was stirred at r.t. for 12 h. The solvent was then evaporated and the residue was dissolved in AcOEt. The organic phase was washed with water and brine, dried and evaporated to yield a crude residue, which was purified by crystallisation from EtOH. **9a** (58%): m.p. 134–135 °C; ^{1}H-NMR: δ 1.74 (s, 3H, CH_3C=N), 1.95–2.04 (m, 2H, $CH_2CH_2CH_2$), 2.78–2.85 (m, 4H, $CH_2CH_2CH_2$), 3.05 (s, 3H, SO_2CH_3), 4.99 (s, 2H, $ArCH_2O$), 5.98 (s, 2H, OCH_2O), 6.78–6.84 (m, 3H, $ArCH_2O$), 7.44 and 7.79 ppm (AA^1BB^1 system, 4H, $J = 8.4$ Hz, $ArSO_2$). Anal. $C_{22}H_{23}NO_5S$ (C, H, N). **9b** (82%): ^{1}H-NMR: δ 1.76 (s, 3H, CH_3C=N), 1.95–2.04 (m, 2H, $CH_2CH_2CH_2$), 2.78–2.85 (m, 4H, $CH_2CH_2CH_2$), 3.05 (s, 3H, SO_2CH_3), 5.11 (s, 2H, $ArCH_2O$), 7.33 (s, 5H, $ArCH_2O$), 7.44 and 7.82 ppm (AA^1BB^1 system, 4H, $J = 8.3$ Hz, $ArSO_2$). MS m/e: 368 (M^+). Anal. $C_{21}H_{23}NO_3S$ (C, H, N).

*5.1.7. 2-Bromo-1-(hydroxymethyl)cyclopentene (**17**)*

A solution of 2-bromo-1-cyclopentene-carbaldehyde (4.95 mmol) in EtOH (20 mL) was treated with $NaBH_4$ (11.3 mmol). The resulting mixture was stirred at r.t. for 24 h. The solvent was then evaporated and the residue was dissolved in CH_2Cl_2, washed with water, 10% aq. HCl, and brine, and then filtered and evaporated to give an oily residue consisting of practically pure **17** (54%): IR: ν 3320–3340 cm^{-1} (OH). ^{1}H-NMR: δ 1.92–2.06 (m, 2H, $CH_2CH_2CH_2$), 2.38–2.61 (m, 4H, $CH_2CH_2CH_2$) and 4.23 ppm (s, 2H, CH_2OH). MS m/e: 176 (M^+).

*5.1.8. endo-N-(2-Bromo-1-cyclopentenylmethyloxy)-5-norbornene-2,3-dicarboximide (**18**)*

A solution of 2-bromo-1-(hydroxymethyl)cyclopentene (**17**) (1.0 g, 5.6 mmol) in anhydrous THF (23 mL) was treated with *endo-N*-hydroxy-5-norbornene-2,3-di-

carboximide (1.0 g, 5.6 mmol), triphenylphosphine (1.47 g, 5.6 mmol) and diethyl azodicarboxylate (1.11 g, 6.17 mmol) and the resulting mixture was stirred at r.t. for 12 h. The solvent was then evaporated and the crude residue was dissolved in Et_2O. The insoluble salts were filtered and the organic phase was evaporated to yield a solid residue which was subjected to chromatography eluting with 6:3:1 hexane–AcOEt–MeOH mixture to give pure **18** (84%): ^{1}H-NMR: δ 1.35–2.20 (br m, 4H, $CH_2CH_2CH_2$ and CH_2 norbornene), 2.53–2.71 (m, 4H, $CH_2CH_2CH_2$), 3.12–3.38 (m, 4H, H1–H4 norbornene), 4.56 (s, 2H, CH_2O), 6.13 (s, 2H, H5, H6 norbornene). Anal. $C_{15}H_{16}BrNO_3$ (C, H, N).

5.1.9. O-(2-Bromo-1-cyclopentenylmethyl)hydroxylamine hydrochloride (19·HCl)

A solution of hydrazine monohydrate (0.22 g, 4.4 mmol) was added dropwise to a suspension of **18** (1.5 g, 4.4 mmol) in anhydrous EtOH (10 mL) and the mixture was refluxed for 8 h and then evaporated. The crude residue was taken up in Et_2O and the organic layer was filtered, cooled (0 °C) and treated with Et_2O·HCl. The solid precipitate was filtered and crystallised from MeOH. **19**·HCl (62%): ^{1}H-NMR: δ 1.92–2.06 (m, 2H, $CH_2CH_2CH_2$), 2.48–2.59 (m, 4H, $CH_2CH_2CH_2$), 4.27 (s, 2H, CH_2O), 5.35 ppm (s, 2H, NH_2).

5.1.10. (Z)-(20) and (E)-1-(Benzylideneiminoxymethyl)-2-bromocyclopentene (21)

A solution of **19**·HCl (0.63 g, 2.8 mmol) in water (5 mL) was added to a stirred solution of benzaldehyde (0.30 g, 2.8 mmol) in $CHCl_3$ (15 mL). The mixture was vigorously stirred at r.t. for 24 h. The organic phase was then separated, the aq. solution was extracted twice with $CHCl_3$ and the collected organic layers were then filtered and evaporated to dryness. The crude residue consisting of **20** and **21** in a ratio of about 20:80 was submitted to column chromatography using 6:4 hexane–$CHCl_3$ as the eluent to yield the pure configurational isomers **20** and **21**. **20** (2%). ^{1}H-NMR: δ 1.74–2.13 (m, 2H, $CH_2CH_2CH_2$), 2.38–2.63 (m, 4H, $CH_2CH_2CH_2$), 4.77 (s, 2H, CH_2O), 7.35–7.89 ppm (m, 6H, Ar and CH=N). **21** (52%): ^{1}H-NMR: δ 1.91–2.10 (m, 2H, $CH_2CH_2CH_2$), 2.39–2.67 (m, 4H, $CH_2CH_2CH_2$), 4.77 (s, 2H, CH_2O), 7.22–7.59 (m, 5H, Ar), 8.04 ppm (s, 1H, CH=N). Anal. $C_{13}H_{14}BrNO$ (C, H, N).

5.1.11. (E)-1-(Benzylideneiminoxymethyl)-2-(4-methylthiophenyl)cyclopentene (22)

A solution of **21** (0.40 g, 1.4 mmol) and methylthiophenylboronic acid (0.24 g, 1.4 mmol) in 1:1 $C_6H_5CH_3$–EtOH mixture (30 mL), was treated with 2.0 M aq. Na_2CO_3 (3 mL) and $Pd(PPh_3)_4$ (40 mg). The stirred mixture was refluxed under N_2 for 12 h and then evaporated. The crude residue was dissolved in AcOEt, washed with water and brine, dried and evaporated to yield a crude residue which was subjected to column chromatography eluting with a 9.5:0.5 hexane–AcOEt mixture to give pure **22** as a solid. **22** (49%): ^{1}H-NMR: δ 1.85–2.03 (m, 2H, $CH_2CH_2CH_2$), 2.47 (s, 3H, SCH_3), 2.65–2.82 (m, 4H, $CH_2CH_2CH_2$), 4.80 (s, 2H, CH_2O), 7.23–7.58 (m, 9H, 2Ar) and 8.06 ppm (s, 1H, CH=N).

5.1.12. (E)-1-(Benzylideneiminoxymethyl)-2-(4-methylsulfinylphenyl)cyclopentene (10) and (E)-1-(benzylideneiminoxymethyl)-2-(4-methylsulfonylphenyl)cyclopentene (11)

A cooled (0 °C) and stirred solution of **22** (0.22 g, 0.69 mmol) in a 1:1 THF–MeOH mixture (8 mL) was treated dropwise with a solution of oxone (0.71 mmol) in water (3 mL). At the end of the addition, the reaction mixture was stirred at r.t. for 12 h and then evaporated. The residue was taken up in AcOEt, washed with brine, dried and evaporated to give the crude product consisting of sulfoxide **10** and sulfone **11** which were separated by column chromatography using AcOEt as the eluent. **10** (36%): m.p. 126–127 °C (*i*-PrOH); ^{1}H-NMR: δ 1.85–2.10 (m, 2H, $CH_2CH_2CH_2$), 2.72–2.85 (br m, 7H, $SOCH_3$ and $CH_2CH_2CH_2$), 4.79 (s, 2H, CH_2O), 7.22–7.55 (m, 9H, 2Ar) and 8.06 ppm (s, 1H, CH=N). Anal. $C_{20}H_{21}NO_2S$ (C, H, N). **11** (40%): m.p. 135–137 °C (EtOH); ^{1}H-NMR: δ 1.85–2.16 (m, 2H, $CH_2CH_2CH_2$), 2.72–2.86 (br m, 4H, $CH_2CH_2CH_2$), 3.05 (s, 3H, SO_2CH_3), 4.78 (s, 2H, CH_2O), 7.33–7.84 (m, 9H, 2Ar) and 8.06 ppm (s, 1H, CH=N). Anal. $C_{20}H_{21}NO_3S$ (C, H, N).

5.2. Conformational studies

A conformational analysis on compounds SC57666, **5b**, **9b** and **11** was performed through the Global-MMX method (GMMX) which is part of the PCMODEL computer program [21]. GMMX uses a conformational searching technique, which randomly moves torsion angles selected by the user and cartesian coordinates of all heavy atoms and then performs an energy minimisation. In our case the bonds selected to be moved were those which connect the phenyl rings and the cyclopentene ring to the rest of the molecular structure, the forcefield was MMFF94, an energy window of 1 kcal mol^{-1} was used and a total of 5000 starting conformations were taken into account. The conformational searching found 8 conformations for SC57666 16 for **5b**, 17 for **9b** and 27 for **11**. In Fig. 6 all these conformations were reported.

5.3. Biopharmacological methods

5.3.1. Enzyme assays

All compounds **6**–**11** were tested following the procedure previously described [16] in intact cell assays to verify their capacity to inhibit prostaglandin E2 (PGE2)

production, considered as an index of activity on COX-1 and COX-2 enzymes. For the COX-1 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 PGE2 content in the supernatant was measured by a commercial immunoenzymatic assay (Amersham). The COX-2 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 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 as described above. All compounds were tested in duplicate. For each product a stock solution was prepared in DMSO at a concentration of 100 mM. Linear regression was used to calculate IC_{50} values. SEM were lower than 10% in all experiments.

5.3.2. Carrageenan-induced paw edema

Compound **8b** was tested after oral administration following a modified version of the method of Winter et al. [24] previously reported [16]. Paw edema was induced by injecting 0.05 ml of a 1% carrageenan suspension, prepared in sterile saline, into the subcutaneous plantar tissue of the right hind paw. Paw volume was measured using a plethysmometer (Ugo Basile, Italy) before (basal value) and 3 h after carrageenan injection. Molecules were suspended in a 0.5% methylcellulose aq. solution and orally or intraperitoneally administered (10 ml kg^{-1} body weight) 30 min before irritant injection. Indomethacin, used as the standard, was administered at 3 mg kg^{-1} po. Control rats received only the vehicle. Statistical analysis was performed by the split plot test followed by Tukey's test for individual comparisons.

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