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Original article

(*E*)-[2-(4-Methylsulphonylphenyl)-1-cyclopentenyl-1-methyliden]-(arylmethyloxy)amines. Methyleneaminoxymethyl (MAOM) analogues of diarylcyclopentenyl cyclooxygenase-2 inhibitors: synthesis and biological properties

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Abstract

The (*E*)-[2-(4-Methylsulphonylphenyl)-1-cyclopentenyl-1-methyliden](methyloxy)amine (**5**) and (arylmethyloxy)amines (**6**–**12**) were designed in order to verify the effects on the biological properties of the substitution of an aryl of selective diarylcyclopentenyl cyclooxygenase-2 (COX-2) inhibitors of type **3** with a methyleneaminoxymethyl moiety (MAOMM). Compounds **5**–**12** 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. The compound with the highest in vitro activity towards COX-2 (**9**) was also assayed in vivo for its antiinflammatory activity by means of the carrageenan-induced paw edema test in rats. Some of the new compounds showed an appreciable in vitro COX-2 inhibitory activity, with IC_{50} values in the μM (**6**,**7**,**9**,**10**,**11**) range. Compound **9** also exhibited an appreciable in vivo activity (29% inhibition at a dose of 30 mg kg^{-1}) when administered intraperitoneally. The structural parameters of **9** were determined by X-ray crystallographic analysis.

Keywords: Antiinflammatory drug; COX-2 inhibitor; Methyleneaminoxymethyl moiety; (*E*)-[2-(4-methylsulphonylphenyl)-1-cyclopentenyl-1-methylidene](arylmethyloxy)amines

1. Introduction

A great many of the non-steroideal antiinflammatory drugs (NSAIDs) most commonly used in the therapy of inflammatory conditions belong to the chemical class of arylacetic and arylpropionic acids. These compounds, as well as other types of NSAIDs, act through the inhibition of the cyclooxygenase (COX) enzyme [1,2]. This enzyme exists as two isoforms, a constitutive form COX-1, and an inducible form COX-2 [3,4]. The COX-1 isoform is constitutively expressed in most tissues and is involved in the physiological production of prostaglandins, which are responsible for gastric cytoprotection. In contrast, the COX-2 isoform is induced by inflammation mediators such as cytokines, mitogens and endotoxins [5–7]. The most common side-effects shown by the classic NSAIDs consist of ulceration, perforation and haemorrhage of the gastrointestinal tract, usually attributed to COX-1 inhibition [2,8]. On the basis of this knowledge, new inhibitors possessing an appreciable selectivity for COX-2 have been designed and synthesised, with the aim of obtaining drugs with reduced gastrolesive properties [9–11]. Among

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

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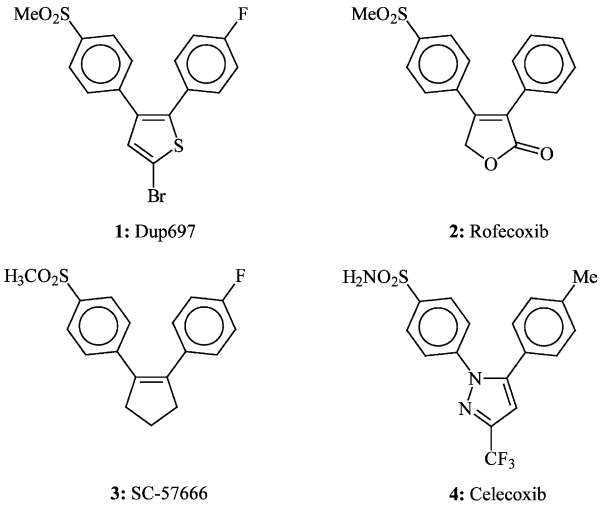


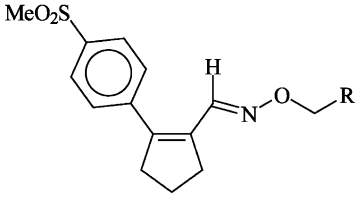
Fig. 1. Structures of some COX-2 selective antiinflammatory drugs characterised by a diaryl-substituted carbocyclic or heterocyclic 5-membered ring: Dup697 (**1**), rofecoxib (**2**), SC-57666 (**3**) and celecoxib (**4**).

these, the most widely studied class is that of the compounds (Fig. 1) in which the pharmacophore is characterised by two aromatic moieties attached to adjacent atoms in a bridging carbocyclic or heterocyclic 5-membered ring [12–17]. For optimal COX-2 inhibitory activity one of the two aromatic rings is substituted in the *para*-position by a methylsulphonyl (**1**–**3**) [12,14] or by a sulfonamide group (**4**) [13,15].

For some time our research group has been interested in studying the effect of substituting selected aromatic rings with alternative non-aromatic moieties [18–24]. In particular, we have been interested in whether the biological activity can be preserved by isosteric replacement of a key aromatic ring with a non-aromatic group. In the present study, we sought to determine if the *para*-fluorophenyl ring of SC-57666 (**3**) could be replaced with a non-aromatic ring with maintenance of in vitro and in vivo activity. Specially, our plan called for the preparation of compounds **5**–**12**, and to determine the biological consequences of incorporation of the methyleneaminoxymethyl moiety (MAOMM). Previously, we showed that this aromatic replacement strategy could be successfully applied to other classes of drugs [18,21–23].

Using SC-57666 (**3**) as a template known to possess selective inhibitory activity against COX-2, our studies commenced with replacement of the *para*-fluorophenyl ring with the MAOMM (**5**). The MAOMM was then further substituted on the methyl carbon with an unsubstituted (**6**) or substituted aromatic moiety (**7**–**12**) (Table 1). Since the active site of COX-2 is known to possess a greater volume than COX-1 [25], the additional molecular size of the aryl substituted MAOMM derivatives **6**–**12** relative to **3** should contribute to decreased affinity towards COX-1 and thus greater selectivity for COX-2.

Table 1
Chemical and biological data of compounds **5**–**12**



5-12

Compound	R	m.p. (°C)	Formula [a]	In vitro inhibitory activity [b]	
				COX1	COX2
5	H	135–137	$C_{14}H_{17}NO_3S$	0	23%
6	C_6H_5	98–100	$C_{20}H_{21}NO_3S$	29%	1.9
7	4-Me-C_6H_4	146–148	$C_{21}H_{23}NO_3S$	33%	5.8
8	4-MeO-C_6H_4	114–116	$C_{21}H_{23}NO_4S$	45%	46%
9	3,4-OCH_2O-C_6H_3	130–132	$C_{21}H_{21}NO_5S$	27%	0.41
10	4-F-C_6H_4	136–138	$C_{20}H_{20}FNO_3S$	32%	3.1
11	4-Cl-C_6H_4	175–177	$C_{20}H_{20}ClNO_3S$	43%	1.8
12	2-Cl,6-F-C_6H_3	128–130	$C_{20}H_{19}ClFNO_3S$	33%	0
4 (celecoxib)				1.04	0.02

[a] All compounds were analysed for C, H and N.
[b] Data are indicated as IC_{50} (μM) or as percentage of inhibition at a concentration of 10 μM.

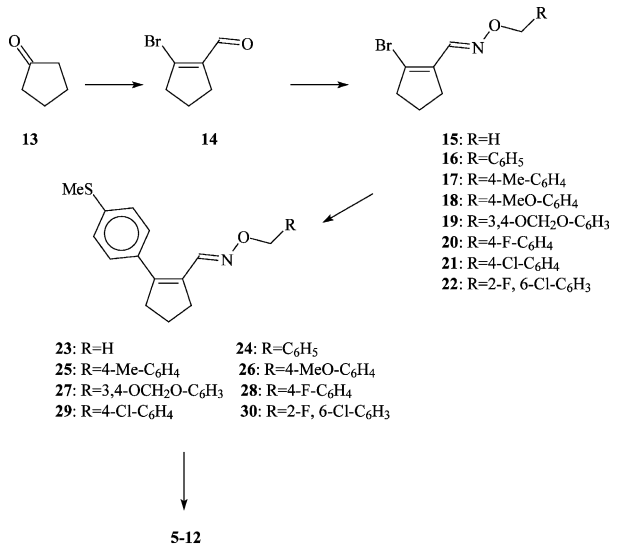


Fig. 2. Synthesis of methyl-(**5**) and arylmethyl-oximethers (**6**–**12**).

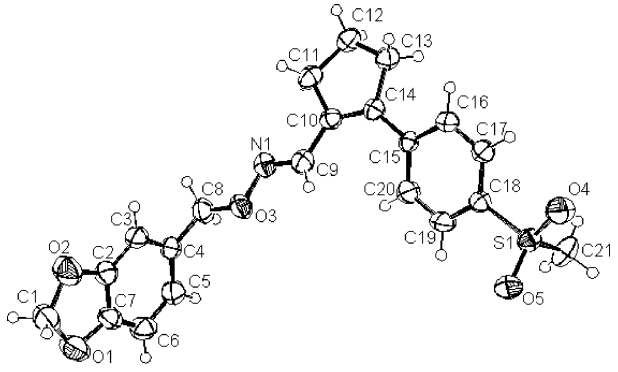


Fig. 3. ORTEP plot (50% probability level for thermal ellipsoids) of the X-ray structure of **9**, showing the crystallographic atom numbering scheme.

2. Chemistry

The methyl- (**5**) and the arylmethyl-oximethers (**6**–**12**) were prepared as described in Fig. 2, starting from cyclopentanone **13**, which was treated with PBr_3 in DMF to give the 1-bromo-2-formylcyclopentene **14** [26]. Reaction of bromoaldehyde **14** with the methoxime or the appropriate O-arylmethyloxyamine hydrochloride in a $CHCl_3$–H_2O mixture afforded exclusively the corresponding (2-bromo-1-cyclopentenyl-1-methylidene)methoxyamine (**15**) or (2-bromo-1-cyclopentenyl-1-methylidene)(arylmethyloxy)amines (**16**–**22**) with the *E* configuration around the oximic double bond. The cross-coupling reaction [27] of the bromo-oxime ethers (**15**–**22**) with *p*-methylthiophenylboronic acid in an EtOH–toluene mixture, in the presence of $Pd(PPh_3)_4$ and aqueous Na_2CO_3, yielded the corresponding methylsulphides (**23**–**30**) which, by oxidation with potassium peroxymonosulphate (oxone) in a THF–MeOH–H_2O mixture, afforded the final products with the *E* configuration **5**–**12**.

The structure and configuration of the intermediate compounds (**15**–**30**) and final products (**5**–**12**) were assigned on the basis of a comparison of the ^{1}H-NMR spectral data of all compounds, and by the crystallographic analysis of one of the final products (**9**). The X-ray structure of **9** is shown in Fig. 3, together with the crystallographic atom numbering scheme. Atomic coordinates are shown in Table 2. In compound **9** the configuration around the oximic double bond is *E*. The atoms of the cyclopentenic ring (C10–C11–C12–C13–C14) are practically coplanar, and the corresponding plane forms a dihedral angle of about 23° with the plane of the aromatic C15–C16–C17–C18–C19–C20 atoms. Also the MAOM portion atoms (C8–O3–N1–C9) are practically coplanar, and define a plane which forms an angle of 15° with the cyclopentenic one.

The configuration of the oxime **9** was determined to be (*E*) by X-ray crystallography. Based on the configuration of **9** it was possible to assign the same (*E*) configuration to compounds **5**–**8** and **10**–**12**. The (*E*) configuration assignment was made on the basis of the similar values of the chemical shift of the signal of the hydrogen atom linked to the oximic unsaturated carbon. During the synthetic sequence the configuration of

Table 2
Atomic coordinates of compound **9**

Atom	*X*/*a*	*Y*/*b*	*Z*/*c*
S1	0.69040 (4)	0.56575 (18)	−0.07185 (4)
O1	1.12537 (13)	0.21124 (56)	0.41839 (12)
O2	1.00480 (13)	0.28154 (56)	0.45399 (11)
O3	0.84128 (12)	−0.19910 (48)	0.26663 (10)
O4	0.62426 (11)	0.72804 (46)	−0.08816 (10)
O5	0.76328 (11)	0.69545 (47)	−0.06096 (10)
N1	0.76798 (14)	−0.30054 (57)	0.25231 (12)
C1	1.08334 (20)	0.35752 (83)	0.46140 (17)
C2	1.00109 (18)	0.09534 (73)	0.40773 (15)
C3	0.93836 (18)	−0.03822 (70)	0.38409 (14)
C4	0.94883 (17)	−0.22571 (70)	0.33767 (14)
C5	1.02075 (18)	−0.26931 (78)	0.31777 (16)
C6	1.08430 (19)	−0.12906 (76)	0.34189 (16)
C7	1.07222 (18)	0.05044 (78)	0.38710 (16)
C8	0.88042 (18)	−0.37472 (73)	0.31107 (14)
C9	0.73465 (20)	−0.16736 (80)	0.20881 (16)
C10	0.65614 (16)	−0.22731 (64)	0.18949 (14)
C11	0.61149 (17)	−0.41545 (72)	0.22857 (14)
C12	0.53239 (17)	−0.43211 (70)	0.19507 (13)
C13	0.53141 (17)	−0.22187 (67)	0.14413 (14)
C14	0.61331 (16)	−0.12965 (59)	0.14201 (13)
C15	0.63512 (15)	0.04662 (62)	0.09136 (12)
C16	0.57877 (18)	0.18861 (70)	0.05756 (14)
C17	0.59651 (17)	0.35113 (69)	0.00869 (14)
C18	0.67023 (16)	0.37413 (58)	−0.00709 (13)
C19	0.72709 (18)	0.23411 (76)	0.02459 (15)
C20	0.70963 (18)	0.06851 (77)	0.07316 (15)
C21	0.69972 (19)	0.32619 (68)	−0.12965 (14)
H9	0.76196 (155)	−0.02388 (617)	0.19318 (129)

Table 3
Antiinflammatory effect of compound **9** on carrageenan-induced paw edema in rats

Treatment	Dose (mg kg^{-1} i.p.)	Paw volume		% Inhibition
		Basal	3 h	
Control	–	0.79 ± 0.040	1.31 ± 0.023	–
Indomethacin	3 (po)	0.79 ± 0.036	1.12 ± 0.041*	36
	1	0.79 ± 0.042	1.25 ± 0.087	11
9	3	0.79 ± 0.026	1.25 ± 0.122	11
	10	0.79 ± 0.046	1.19 ± 0.087*	23
	30	0.79 ± 0.033	1.16 ± 0.140*	29

*, $p < 0.01$ vs. control, split plot test.

the oximic double bond should not change, therefore, it was possible to assign the (*E*) configuration to sulphides **23**–**30** and to the bromoderivatives **15**–**22**.

3. Biopharmacological results

The inhibitory activity of compounds **5**–**12** towards COX-1 and COX-2 was evaluated in vitro by measuring prostaglandin E2 (PGE2) production in U937 cell lines for COX-1 and activated J774.2 macrophages for COX-2. The results obtained are reported in Table 1, together with those obtained in the same tests for celecoxib (**4**), chosen as a reference compound between the tricyclic COX-2 selective inhibitors because of its current therapeutic interest. The compound substituted with a completely aliphatic aminoethereal chain (**5**) exhibited at a high concentration a very low inhibitory activity, directed only against COX-2. Among the O-arylmethyl oximethers, the most active compound at the COX-2 level proved to be the methylenedioxy-substituted derivative (**9**), which showed an IC_{50} value of 0.41 μM, versus IC_{50} value of celecoxib (**4**) of 0.02 μM. On the contrary, it appeared to possess a very low activity towards COX-1. In addition, **9** showed very low activity against COX-1; 27% at 10 μM. The 2,6-disubstituted analogue **12** showed very little or nor activity against COX-1 and COX-2, respectively. Other substitutions on the MAOMM aromatic ring afforded compounds that had some inhibitory activity against COX-1 at 10 μM, but considerably greater inhibitory activity against COX-2 (see Table 1).

For compound **9**, which showed the highest inhibitory activity towards COX-2 in the in vitro test, the in vivo activity was evaluated using the carrageenan-induced paw edema test in rats. In a first set of experiments, compound **9** was administered per os at increasing doses ranging from 1 to 30 mg kg^{-1}. No anti-inflammatory activity was observed with **9** over a wide dose range. The same test was, therefore, conducted again administering compound **9** intraperitoneally at the same increasing doses. In analogous experiments carried out injecting compound **9** intraperitoneally at a dose of 30 mg kg^{-1} in naïve animals, no signs of local irritation were observed. Results are shown in Table 3, together with the value obtained for indomethacin taken as reference drug and administered per os at a dose of 3 mg kg^{-1}. While at the lowest doses (1 and 3 mg kg^{-1}) compound **9** appeared to be practically inactive, at the highest doses (10 and 30 mg kg^{-1}), the compound showed percentage inhibitory indices of 23% and 29%, respectively; this last result is not much lower than that obtained for indomethacin at 3 mg kg^{-1} po.

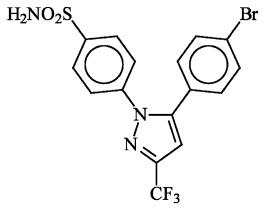
4. Discussion and Conclusions

This work was planned in order to verify the effects on the biological properties of the substitution with MAOMM of one of the two aryls of COX-inhibitor drugs such as the 1,2-diarylcyclopentenes (**3**). Some of the new compounds showed a more or less marked ability to interact with the two types of cyclooxygenases. With regard to activity towards COX-1, all the compounds in which the MAOM moiety was substituted by an aryl possessed similar inhibitory properties, with inhibition percentages at a concentration of 10 μM ranging from about 30% to 40%. On the contrary, the compound in which the MAOM was completely aliphatic, appeared to be inactive. In the same experimental conditions, celecoxib showed an IC_{50} value of about 1 μM. These data indicate that, on the whole, the new compounds weakly inhibit COX-1, regardless of the nature of the substituent present on the aromatic ring of **6**–**12**. The inactivity of compound **5**, in which the MAOM moiety is completely aliphatic, would seem to indicate that, in accordance with preceding findings [21,22], the presence of an aryl group on the methyl carbon of the MAOMM is essential for an appreciable interaction with the enzyme. An examination of the biological data reported in Table 1 shows that most of the new compounds substituted on the MAOMM with an aryl are able to inhibit the COX-2 enzyme, with IC_{50} values lower than 10 μM. Among these compounds, the 3,4-methylenedioxy-substituted analogue (**9**) exhibited an appreciable activity index in the sub-micromolar range (0.41 μM), whereas unsubstituted on the phenyl analogue (**6**) and the 4-chlorophenyl derivative (**11**) showed activity indices of about 2 μM. In contrast, the compounds with the completely aliphatic MAOM side-chain (**5**) or linked to an aryl in turn substituted by chlorine and fluorine atoms in the two *ortho*-positions (**12**) showed little or nor activity. These results suggest that an aryl group is required for COX-2 inhibitory activity in this series. Additional analogues will need to

be prepared to identify the optimal aromatic substitution pattern. From an examination of the data reported in Table 1 it may also be noted that all new MAOM-derivatives, with the exception of **8** and **12**, possess better activity against COX-2 than COX-1. However, the absolute values of their selectivity should be interpreted with caution, both because their extrapolability to the in vivo human condition has not been assessed, and because in our in vitro tests the reference compound celecoxib (**4**) showed a COX-2 selectivity significantly different from that determined by other experimental procedures [28].

The presence of an appreciable inhibitory activity in some of the new MAOM-derivatives indicate the existence of a bioisosteric relationship between aryls and the MAOMM also in the context of COX-2 inhibitors. In order to rationalise this finding in terms of conformational and steric analogies, it is possible to make a comparison of some structural parameters of compound **9** and of an analogue of celecoxib (i.e. SC 558, **31**, Fig. 4) [3], obtained from a drug-murine COX-2 complex. In this last compound, the two aromatic rings, one substituted by a sulphonamido group and the other one bromo-substituted are not coplanar with the pyrazole ring, making dihedral angles of 37° and 74°, respectively. This type of molecular geometry seems to be optimal for a positive drug-receptor interaction. In compound **9**, the plane of the *para*-methylsulphonylphenyl moiety and the plane of the MAOMM are not coplanar with the cyclopentenyl ring, even if they form dihedral angles of lesser amplitude (23° and 15°, respectively). From an observation of the structures of **9** and **31**, it may also be noted that the 3,4-methylenedioxyphenyl-substituted MAOMM of **9** presents a higher steric hindrance with respect to the aryl group of **31**; this makes it possible to hypothesise that the active site of the enzyme contains a lipophilic pocket able to receive groups of a larger size than that of a simple aryl group.

As regards the in vivo properties of the MAOMM derivatives studied, the results obtained for compound **9** administered intraperitoneally showed modest anti-inflammatory activity. The lack of activity of **9** after oral administration may be the result of limited gastrointestinal stability, poor absorption, and/or rapid first pass metabolism.



31: SC558

Fig. 4. Structure of the analogue (**31**) of celecoxib (**4**) previously utilised in a crystallographic study of drug-murine COX-2 complex.

5. Experimental protocols

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 FT-IR spectrometer. ^{1}H-NMR spectra of all compounds were obtained with a Varian CFT 20 instrument or a Gemini 200 spectrometer operating at 80 or 200 MHz, respectively, in 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. Evaporation was performed in vacuo (rotating evaporator); the O-arylmethyloxyamines to be used for the preparation of the bromoximethers **15**–**22**, not commercially available, were prepared following the synthetic method described in ref [29]. 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. Synthesis of 2-bromo-1-cyclopenten-1-carboxyaldehyde (14)

Compound **14** was prepared following the reaction indicated in ref. [26]. A cooled (0 °C) and stirred mixture of DMF (11.6 mL, 0.15 mol) and anhydrous $CHCl_3$ (40 mL) was treated dropwise with PBr_3 (11.8 mL, 0.125 mol) and then with a solution of cyclopentanone (4.43 mL, 0.05 mol) in anhydrous $CHCl_3$ (20 mL). After 12 h at room temperature, the solvent was evaporated and the residue was treated with ice and solid $NaHCO_3$. The aqueous phase was extracted with Et_2O and the organic solvent was washed (5% K_2CO_3 and H_2O), dried and evaporated to yield an oily residue which by distillation at reduced pressure gave pure **14** (90%): b.p. 60–65 °C (0.3 mm Hg); ^{1}H-NMR δ 1.91–2.18 (m, 2H, $CH_2CH_2CH_2$), 2.41–2.63 (m, 2H, CH_2CCHO), 2.78–2.99 (m, 2H, CH_2CBr), 9.85 ppm (s, 1H, CHO); Lit. [26]: unreported data.

5.1.2. General procedure for the preparation of the (E)-(2-bromo-1-cyclopentenyl-1-methylidene)-(methyloxy)amine (15) and -(arylmethyloxy)amines (16–22)

A mixture of **14** (1.0 g, 5 mmol) in $CHCl_3$ (36 mL) and the appropriate O-methyl or O-arylmethyloxy-

lamine (5 mmol) in H_2O (5 mL), was stirred at room temperature for 24 h. The organic phase was separated and the aqueous solution was extracted twice with $CHCl_3$. Evaporation of the dried and filtered organic extracts afforded a residue consisting almost exclusively of the corresponding bromooximes **15**–**22**, which without further purification were used in the following reaction. ^{1}H-NMR data for the singlets attributable to *Me*O of **15** or Ar*CH*$_2$ of **16**–**22** and to their N=CH protons: **15** (86%) δ 3.88 (3H) and 7.97 ppm (1H); **16** (74%) δ 5.1 (2H), 8.04 ppm (1H); **17** (65%) δ 5.06 (2H), 8.02 ppm (1H); **18** (84%) δ 5.06 (2H), 8.06 ppm (1H); **19** (91%) δ 4.92 (2H), 7.94 ppm (1H); **20** (47%) δ 5.06 (2H), 8.02 ppm (1H); **21** (43%) δ 5.0 (2H), 8.0 ppm (1H); **22** (36%) δ 5.2 (2H), 7.98 ppm (1H).

5.1.3. General procedure for the preparation of the (E)-[2-(4-methylthiophenyl)-1-cyclopentenyl-1-methylidene](methyloxy)amine (23) and -(arylmethyloxy)amines (24–30)

A mixture of the appropriate 1,2-disubstituted cyclopentenylidene derivative (**15**–**22**) (3.18 mmol), Na_2CO_3 2 M (4 mL) and 4-methylthiophenylboronic acid (0.54 g, 3.19 mmol), in a 50% toluene–EtOH mixture (20 mL), was treated with $Pd(PPh_3)_4$ (0.1 g) and the resulting mixture was refluxed under N_2 with stirring for 12 h. Evaporation of the organic phase gave a residue which was taken up in AcOEt, washed with H_2O, dried and evaporated to afford a crude residue which was subjected to column chromatography, to yield pure **23**–**30**. ^{1}H-NMR data for the singlets attributable to *Me*O of **23** or Ar*CH*$_2$ of **24**–**30** and to their S*CH*$_3$ and N=CH protons: **23** (62%) δ 2.49 (3H, S*CH*$_3$), 3.88 (3H, *Me*O), 8.04 ppm (1H); **24** (51%) δ 2.46 (3H), 5.09 (2H), 8.08 ppm (1H); **25** (53%) δ 2.46 (3H), 5.04 (2H), 8.06 ppm (1H); **26** (29%) δ 2.46 (3H), 5.01 (2H), 8.05 ppm (1H); **27** (48%) δ 2.45 (3H), 5.0 (2H), 7.98 ppm (1H); **28** (55%) δ 2.47 (3H), 5.04 (2H), 8.06 ppm (1H); **29** (42%) δ 2.46 (3H), 5.04 (2H), 8.06 ppm (1H); **30** (70%) δ 2.45 (3H), 5.2 (2H), 8.02 ppm (1H).

5.1.4. General procedure for the preparation of the (E)-[2-(4-methylsulphonylphenyl)-1-cyclopentenyl-1-methylidene]-(methyloxy)amine (5) and -(arylmethyloxy)amines (6–12)

A cooled (0 °C) and stirred solution of the appropriate methylsulphide derivative **23**–**30** in 1:1 MeOH–THF mixture (15 mL), was treated dropwise with a solution of oxone ($2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$) (1.35 mmol) in H_2O (7 mL). At the end of the addition, the reaction was stirred at room temperature for 12 h. The solvent was evaporated and the crude residue was taken up in AcOEt, washed with a saturated solution of NaCl, dried and evaporated to give the corresponding crude sulphone (**5**–**12**), which was purified by crystallisation from EtOH. ^{1}H-NMR data for SO_2CH_3, for the singlets attributable to *Me*O of **5** or Ar*CH*$_2$ of **6**–**12** and to their N=CH protons: **5** (80%) δ 3.08 (3H, SO_2CH_3), 3.92 (3H, *Me*O), 7.98 ppm (1H); **6** (50%) δ 3.03 (3H), 5.1 (2H), 8.0 ppm (1H); **7** (35%) δ 3.02 (3H), 5.05 (2H), 7.98 ppm (1H); **8** (82%) δ 3.02 (3H), 5.02 (2H), 7.97 ppm (1H); **9** (50%) δ 3.03 (3H), 4.99 (2H), 7.97 ppm (1H); **10** (24%) δ 3.03 (3H), 5.05 (2H), 7.97 ppm (1H); **11** (37%) δ 3.04 (3H), 5.06 (2H), 7.98 ppm (1H); **12** (43%) δ 3.02 (3H), 5.2 (2H), 7.94 ppm (1H). MS (*m*/*e*): **5**, [M$^+$] 279; **6**, [M$^+$] 355; **7**, [M$^+$] 369; **8**, [M$^+$] 385; **9**, [M$^+$] 399; **10**, [M$^+$] 373; **11**, [M$^+$] 389; **12**, [M$^+$] 407. For the analytical and physical data of **5**–**12** see Table 1.

5.2. Crystal-structure determination

Crystals of the *E* oximether **9**, suitable for X-ray analysis, mp 130–131 °C, were obtained by slow crystallisation from EtOH: $C_{21}H_{21}NO_5S$, $M = 399.461$, monoclinic, space group $P_{21/c}$, $a = 17.522$ (3) A, $b = 4.947$ (5) A, $c = 21.997$ (2) A, $\beta = 93.40$ (3), $V = 1903.4$ (2.0) A^3, $Z = 4$, $D_{calc} = 1.394$ g cm^{-3}. A Siemens AED diffractometer, equipped with an Mo Kα source (using the $\vartheta/2\vartheta$ scan mode, scan speed 3/12 deg min^{-1}, scan width $1.20 + 0.34 \tan \vartheta$, ϑ in the range 3–30°) was used to obtain intensity data. The structure was solved by direct methods using SIR97 [30] and refined using SHELX96 [31] by full matrix least squares on Fo with anisotropic thermal parameters, isotropic for the hydrogens. The final *R* indexes were, respectively: $R_1 = 0.0457$ for 1591 reflections with $F_o > 4\sigma$ (F_o), $R_2 = 0.1434$ for all the 4161 reflections $wR_2 = 0.1282$.

5.3. Biopharmacological methods

5.3.1. Enzyme assays

All the compounds were tested in intact cell assays to verify their capacity to inhibit 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 are 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. [32] with minor modifications as suggested by Grossman et al. [33]. Murine J774.2 cells were pretreated for 1 h with 300 μM aspirin to inactivate endogenous constitutive COX-1 and 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 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. S.E.M. were lower than 10% in all experiments.

5.3.2. Carrageenan-induced paw edema

A modified version of the method of Winter et al. was used [34]. 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 aqueous 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.

6. Supplementary material

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited at the Cambridge Crystallographic Data Centre, as supplementary publication. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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