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Original article

Enantiopure 3-(arylmethylidene)aminoxy-2-methylpropionic acids: synthesis and antiinflammatory properties☆

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Abstract – Some optically active 3-(arylmethylidene)aminoxy- (**3a**–**g**, **4a**–**g**) and fluorenylideneaminoxy-2-methylpropionic acids (**5**, **6**), were prepared as analogues of the antiinflammatory arylpropionic acids of type **B**, in which the aromatic group is substituted by an MAOM moiety. Some of the new compounds, tested in vivo for their antiinflammatory properties by means of the carrageenan-induced paw edema method in rats, exhibited activity indices similar to that shown in the same test by ibuprofen. Compounds **3a**,**b** and **4a**,**b**, for which at least one of the two enantiomers had shown an inhibition value higher than 40% in the in vivo test, were assayed for their in vitro enzymatic inhibitory activity, showing percentage inhibition values between 40 and 50% at a concentration of 10 μM against COX-2; at the same concentration, they appeared to be devoid of any activity towards COX-1. Compounds **3a**,**b** and **4a**,**b** also proved to possess a similar toxicity. The lack of enantioselectivity shown by compounds **3**–**6** was tentatively explained in terms of a conformational freedom of the enantiomers which allows their quasi-superimposition. © 2001 Éditions scientifiques et médicales Elsevier SAS

antiinflammatory drug / NSAID / 3-aminoxy-2-methylpropionic acid

1. Introduction

Prostaglandins are endogenous substances involved in different processes of a 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 H_2, is catalysed by cyclooxygenase (COX) [1, 2]. This enzyme exists as two isoforms, one constitutive (COX-1), and the other inducible (COX-2) [2, 3]. 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. The COX-2 isoform is induced by cytokines, mitogens and endotoxins [4–6] in inflammatory cells, and is responsible for the elevated production of prostaglandins during inflammation. Most of the non-steroidal antiinflammatory drugs (NSAIDs) act by reducing prostaglandin biosynthesis through the inhibition of the cyclooxygenase (COX) reaction [7, 8]. The NSAIDs more commonly used in the therapy of inflammatory conditions belong to the class of arylacetic (**A**) or arylpropionic acid (**B**) derivatives (*figure 1*) and lack selectivity towards the two types of COX

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

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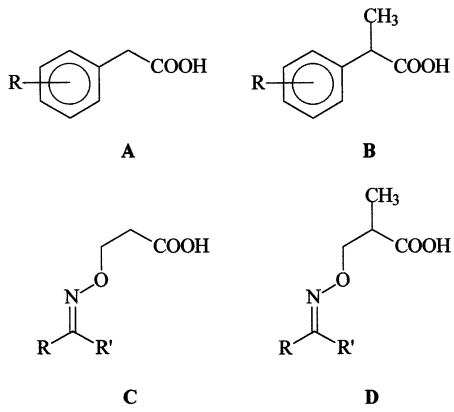
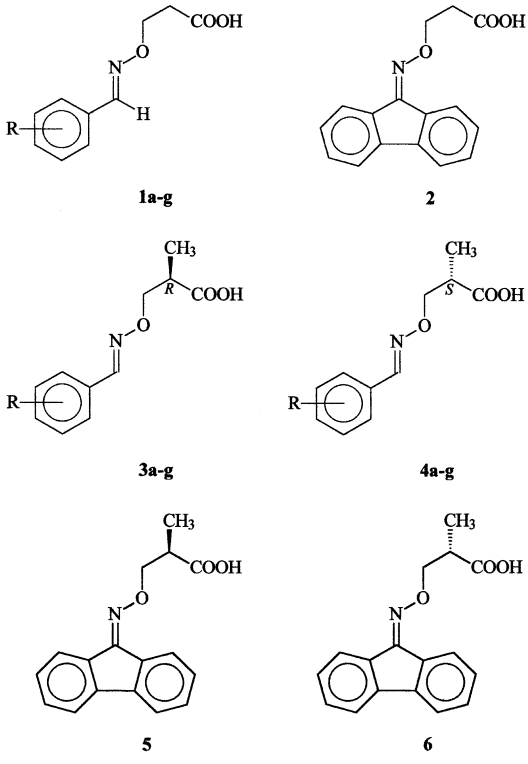


Figure 1. General structure of arylacetic (**A**) and arylpropionic (**B**) NSAIDs and their corresponding methyleneaminoxymethyl (MAOM) analogues **C** and **D**.



a: R=H; **b**: R=*m*-Cl; **c**: R=*m*-F; **d**: R=*m*-MeO; **e**: R=*p*-EtO; **f**: R=*p*-Me; **g**: R=*p*-CF_3

Figure 2. Structures of the previously described arylmethylidene (**1**) and fluorenylidene (**2**) derivatives and of the corresponding enantiopure α-methyl-substituted analogues **3**, **4** and **5**, **6**, showing their absolute configuration.

[9–11]. Furthermore, NSAIDs of type **B** usually exhibit a higher activity than the corresponding type **A** compounds [12, 13]; this has been attributed to the conformational differences in the common Ar–CH–COOH portion, due to the different steric interactions between the remaining hydrogen (for **A**) or the methyl (for **B**) with the adjacent aryl and the carboxyl group [13]. Compounds **B** exist in two enantiomeric forms, of which the *S* one is, in the majority of cases, more active than the *R* form, probably because it gives a better fit with the COX active site [12, 13].

In previous papers of ours [14, 15], we have described the synthesis and the antiinflammatory properties, evaluated by the carrageenan-induced edema test in rats, of a series of β-aminoxypropionic acids of type **C**, some of which are shown in formulas **1** and **2**, designed as analogues of arylacetic type **A** NSAIDs, on the basis of the hypothesis that a methyleneaminoxymethyl moiety (C=NOCH$_2$, MAOMM) may act as a bioequivalent of aryl groups in this class of drugs.

Some of the new type **C** compounds, either completely aliphatic or substituted on the oximic carbon with an aryl, have been shown to possess an appreciable antiinflammatory activity, especially in the case of certain arylmethylidene derivatives, such as the unsubstituted (**1a**) and *m*-chloro-substituted (**1b**) ones [14, 15] (*figure 2*).

On the basis of these results, bearing in mind the positive effects on the pharmacological properties induced by the introduction of a methyl in the α-position with respect to the carboxylic group of the arylacetic drugs of type **A** [12, 13], leading to drugs of type **B**, it appeared to be of interest to study a series of *N*-substituted-3-aminoxy-2-methylpropionic acids of type **D** which are the α-methyl-substituted homologues of compounds of type **C**, with the aim of verifying whether this type of substitution may improve the level of activity also for the class of the aminoxypropionic acids. Furthermore, as the compounds of type **D** can be viewed as MAOM-substituted analogues of arylpropionic compounds of type **B** in which the type of chirality influences the trend of activity, we planned the preparation of both enantiomers of compounds of type **D** through an enantioselective synthesis starting from commercially available chiral compounds (*figure 1*).

Consequently, the arylmethylideneaminoxy-2-methylpropionic acids with the *R* (**3a**–**g**) and the *S*

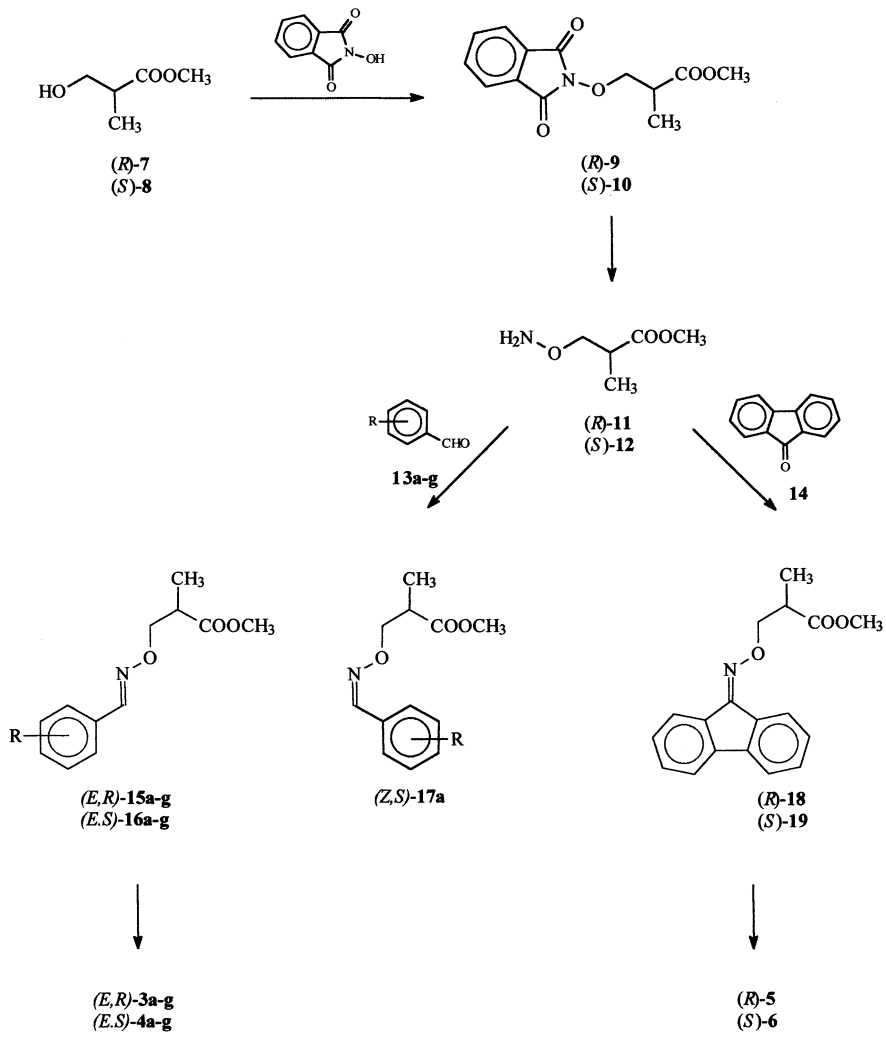


Figure 3. Synthesis of methylpropionic MAOM derivatives **3**–**6**.

(**4a**–**g**) (Fig. 2) configurations were prepared, in which the substituents on the phenyl ring correspond to those present in some of the corresponding type **C** compounds which in previous studies have been shown to possess different antiinflammatory activity levels [14, 15]. In addition, the fluorenylidene compounds **5** and **6** were prepared as the only oximethers of a ketone, whose corresponding analogue (**2**) proved to be completely inactive [14].

2. Chemistry

The 3-aminoxy-2-methylpropionic acids **3**–**6**, were prepared as shown in *figure 3*. Mitsunobu reaction [16] of the appropriate optically active methyl-2-hydroxymethyl propionate **7** and **8** with the *R* or *S* configuration, respectively, in the presence of *N*-hydroxyphthalimide, diethylazodicarboxylate and triphenylphosphine in THF, yielded the corresponding *O*-phthalimido derivatives **9** and **10**, which by hydrazinolysis with $NH_2NH_2 \cdot H_2O$ afforded the hydroxylaminethers **11** and **12**. The reaction of **11** and **12** with the appropriate aldehydes **13a**–**g**, or fluorenone **14** in MeOH, gave the corresponding oximethers **15**–**19** which, in the cases in which an *E*/*Z* isomerism is possible, exclusively possessed the *E* configuration, with the only exception of the benzylidene derivative unsubstituted on the phenyl ring, which gave an 85:15 mixture of *E* **16a** and *Z* **17a**

isomers. The oximethers **15**, **16**, **18** and **19** were purified by column chromatography, and were then saponified with 1 N NaOH in THF–H_2O to yield the corresponding optically active arylmethylidene acids **3** and **4**, and fluorenylidene acids **5** and **6**. The acids were purified by crystallisation from the appropriate solvents (for **3a**–**c**,**f**,**g**, **4a**–**c**, **4f**,**g**, **5** and **6**) or by bulb-to-bulb distillation (for **3d**,**e** and **4d**,**e**).

The assignment of the type of configuration around the C=N double bond of the benzylidene-substituted esters with the *S* configuration (*E* **16a** and *Z* **17a**) was made unequivocally by means of ^{1}H-NMR spectrometry, seeing that in the oximethers with the *E* configuration, the oximic proton resonates at lower fields than the same hydrogen of the *Z* isomer.

Once the *E* configuration had been assigned to oximether **16a**, it was possible to attribute the same type of configuration also to esters **15a**–**g** and **16b**–**g** and to the corresponding acids **3** and **4**, on the basis of the observation that their proton linked to the oximic carbon resonates at chemical shift values practically identical or very close to those of the same proton of ester **16a**.

As regards the configurational situation of esters **15** and **16** and of the corresponding acids **3** and **4** at the level of their chiral carbon, the values of the specific rotatory power in the couples of enantiomers, practically equal in terms of their absolute value, but with the opposite sign, indicate that the single enantiomers might possess an analogous optical purity.

An ^{1}H-NMR analysis carried out on the couple of *m*-fluoro-substituted acids, **3c** and **4c**, using *R*(−)-cinconidine as the chiral resolving agent showed that both enantiomers possess an enantiomeric purity >96%. Assuming that the type of substituent on the phenyl ring of the remaining couples of acids does not affect the chiral stability of these compounds, an analogous optical purity was assigned also to the remaining acids of types **3** and **4**, and therefore also to the corresponding esters, **15** and **16**.

As regards the absolute configuration of the intermediate and final products, this derives from a knowledge of the structure of the starting hydroxymethylpropionic methylesters **7** and **8**.

3. Biopharmacological results and discussion

Compounds **3**–**6** were assayed for their antiinflammatory activity by means of the carrageenan-induced paw edema test in rats. The results obtained for **3**–**6** and for ibuprofen, taken as a reference drug, are shown in *table I*, together with those previously obtained in the same type of test with the corresponding arylmethylideneaminoxypropionic acids of type **C**, **1a**–**g** and **2**. All compounds (**3**–**6** and ibuprofen) were administered intraperitoneally at a dose of 50 mg kg^{-1}. The activity was expressed as percentage inhibition values.

Among the compounds with the *R* configuration (**3a**–**g**, **5**), those unsubstituted on the phenyl ring (**3a**) or *m*-halosubstituted (**3b** and **3c**) exhibited the highest activity, with percentage inhibition values ranging between 52% of **3a** and 43% of **3c**. A slightly lower activity was shown by the fluorenyl derivative **5**, while the other type **3** compounds (**3d**–**g**) appeared to possess a lower degree of activity (percentage inhibition values <20%).

As regards the compounds with the *S* configuration (**4a**–**g**, **6**), the *m*-fluorosubstituted compound **4c** and the fluorenyl derivative **6** showed the highest activity, with percentage inhibition values close to 40%; the other type **4** compounds, with the exception of **4e** which was found to be practically inactive, exhibited inhibition indices ranging from 28 to 22%.

For the compounds unsubstituted (**3a**, **4a**) or *m*-chlorosubstituted (**3b** and **4b**) on the phenyl ring, for which at least one of the two enantiomers had shown an inhibition value higher than 40% in the in vivo test, also the inhibition properties towards COX-1 and COX-2 were evaluated. The results obtained in vitro by measuring the prostaglandin (PGE_2) production in U937 cell lines for COX-1 and activated J774.2 macrophages for COX-2, at a concentration of 10 and 1 μM are reported in *table II*; this table reports the results obtained in the same test for celecoxib, one of the most selective NSAIDs recently introduced into clinical use, taken as a reference drug for this in vitro test. The four compounds **3a**,**b** and **4a**,**b** exhibited a similar trend of inhibitory activity; in particular, these compounds showed percentage inhibition values between 40 and 50% at a concentration of 10 μM towards COX-2, while at the same concentration, they appeared to be completely devoid of any activity towards COX-1. In the same experiment, celecoxib, tested at a concentration of 0.1 μM, exhibited a percentage inhibition value of about 50% towards COX-2 and a practically complete inactivity towards COX-1.

In the case of compounds **3a**,**b** and **4a**,**b**, the approximate LD_{50} was also evaluated in the mouse by

i.v. administration. The values obtained ranged from 697 mg kg^{-1} of **3b** to 795 mg kg^{-1} of **3a**, thus indicating a similar toxicity.

Results obtained for compounds with the *R* configuration (**3a**–**f** and **5**), and for compounds with the *S* configuration (**4a**–**f** and **6**) in the in vivo test, shows that for these compounds in which the R substituent is hydrogen or *m*-chloro, the *R* isomer exhibits a better activity, while for the *m*-fluoro-substituted compounds, or for the 9-fluorenyl (**5** and **6**) derivatives, the activity shown by the two isomers is practically the same. Instead, for the compounds in which R is *m*-MeO, *p*-Me or *p*-CF_3, the isomers with the *S* configuration appear to be slightly more active than the *R* ones.

A comparison of the data obtained for the 2-methylpropionic acids **3**–**6** with those found for the analogues lacking the 2-methyl group (**1** and **2**) shows that in the case of the compounds unsubstituted, *m*-chloro- or *m*-fluoro-substituted on the phenyl ring, the presence of the methyl group does not appear to play an important role, with the exception of the *S* stereoisomers **4a** and **4b**, for which the activity decreased with respect to **1a** and **1b**. For the *p*-EtO-substituted compounds (**3e** and **4e**), the same methyl group leads to a marked decrease in the activity of both stereoisomers, compared with the corresponding type **1** analogues, while for the *m*-MeO- (**3d** and **4d**) and *p*-CF_3-substituted (**3g** and **4g**) compounds, the methyl does not contribute to any substantial modifi-

Table I. Chemical and pharmacological data of compounds **1**–**6**.

Compound	*R*	$[\alpha]_D^{22}$ (*c*)	m.p. or b.p. [mmHg] (°C)	Recrystallisation solvent [a]	Inhibition activity on carrageenan paw edema (50 mg kg^{-1} i.p.) [b] % inhibition at 4 h
(*R*)-**3a** [c]	H	−53.7 (1.38)	67–69	A	52** (10)
(*S*)-**4a** [c]	H	+53.6 (1.38)	67–69	A	28 (10)
1a [d]	H				66* (10)
(*R*)-**3b**	*m*-Cl	−45.6 (0.99)	64–66	A	46** (10)
(*S*)-**4b**	*m*-Cl	+45.2 (1.01)	63–65	A	22 (10)
1b [e]	*m*-Cl				53* (10)
(*R*)-**3c**	*m*-F	−48.9 (1.00)	57–59	A	43** (10)
(*S*)-**4c**	*m*-F	+48.7 (1.01)	57–59	A	40** (10)
1c [e]	*m*-F				39* (10)
(*R*)-**3d**	*m*-MeO	+44.8 (1.14)	250 (0.3)		16* (10)
(*S*)-**4d**	*m*-MeO	−43.3 (1.14)	250 (0.3)		26 (10)
1d [e]	*m*-MeO				10 (10)
(*R*)-**3e**	*p*-EtO	−42.9 (1.13)	230 (0.3)		16 (10)
(*S*)-**4e**	*p*-EtO	+43.3 (1.53)	230 (0.3)		9 (10)
1e [e]	*p*-EtO				52* (10)
(*R*)-**3f**	*p*-Me	−53.7 (1.18)	80–82	A	16 (10)
(*S*)-**4f**	*p*-Me	+52.2 (1.14)	75–78	A	28** (10)
1f [e]	*p*-Me				0 (10)
(*R*)-**3g**	*p*-CF_3	−39.5 (1.10)	32–34	B	12 (10)
(*S*)-**4g**	*p*-CF_3	+40.6 (2.11)	33–35	B	22 (10)
1g [e]	*p*-CF_3				15* (10)
(*R*)-**5**		−29.8 (1.09)	102–104	A	37** (10)
(*S*)-**6**		+30.1 (1.41)	103–105	A	37** (10)
2					0 (10)
(*R*,*S*)-Ibuprofen					42** (10)

Results marked with an asterisk or two asterisks are percentage reductions which are significant (*, $P<0.05$; **, $P<0.01$, respectively). The number of animals used per groups is reported in parentheses.

[a] A, hexane; B, AcOEt–hexane.

[b] A Student's *t*-test was carried out.

[c] See Ref. [17].

[d] See Ref. [14].

[e] See Ref. [15].

Table II. In vitro inhibitory activity of **3a**,**b** and **4a**,**b** towards COX-1 and COX-2.

Compound	*R*	Cyclooxygenase inhibitory activity (% inhibition) [a]		
		Dose (μM)	COX-1	COX-2
(*R*)-**3a**	H	10	0	39
		1	0	23
(*S*)-**4a**	H	10	14	41
		1	0	30
(*R*)-**3b**	*m*-Cl	10	0	50
		1	0	24
(*S*)-**4b**	*m*-Cl	10	0	42
		1	0	5
Celecoxib		0.1	12	52

[a] S.E.M. was lower than 10% in all experiments.

cation of the antiinflammatory activity. Only in the case of the *p*-Me-substituted (**3f** and **4f**) compounds and the fluorenyl derivatives (**5**, **6**) for which the corresponding analogues lacking the methyl group (**1f** and **2**) are inactive, did the presence of this substituent in the 2-position with respect to the carboxyl group, determine the appearance of an antiinflammatory activity, especially in the case of both the fluorenyl enantiomers **5** and **6**.

As far as the in vitro activity of the compounds tested (**3a**,**b** and **4a**,**b**) is concerned, this appeared to be relatively modest, and directed exclusively towards COX-2; moreover, for the same compounds, this activity proved to be practically independent of their configuration and of the substituent (hydrogen or chlorine), on the aromatic ring.

4. Conclusions

An analysis of the pharmacological data reveals that, contrary to what might be expected on the basis of the positive effect of the introduction of a methyl group on the carbon adjacent to the carboxyl of compounds **A** leading to compounds **B**, in the majority of cases the same type of substitution in aminoxypropionic acids of type **C** does not affect the antiinflammatory activity of the corresponding methylpropionic analogues of type **D**. A possible explanation of these results may be sought in the fact that in compounds of type **D**, the additional methyl group gives rise to a lower degree of steric interactions with the adjacent groups compared with those found in compounds of type **B**, and should thus exert less influence on their conformational situation, and consequently on their ability to interact more effectively with the enzymic active site.

As regards the influence of the chirality on the activity, the pharmacological data do not show a homogeneous trend, seeing that the *R* enantiomers may appear to be more active (as in the case of compounds **3a**–**c**), or less active (as in the case of **3d**,**f**,**g**) than the corresponding *S* enantiomers (**4a**–**d**,**f**,**g**), or they may possess a very similar activity (as in the case of the remaining compounds), depending on the type of substituent on the oximic carbon.

The apparent absence of enantioselectivity of compounds **3**–**6** may be tentatively attributed to the high conformational freedom of the 3-methyleneaminoxy-2-methylpropionyl chain, which makes it possible to find low-energy conformations which allow a quasi-superimposition of the enantiomers. This kind of explanation has also been advanced to justify the lack of selectivity in the field of MAOM-type analogues of adrenergic β-blocking drugs [18, 19], which, however, possess a propanolaminic structure instead of the methylpropionic one of compounds **3**–**6**.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Boiling points refer to the air bath temperature of bulb-to-bulb distillation using a Büchi GKR-51 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 Bruker AC-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. GLC analyses were performed on a Perkin–Elmer model 8410 apparatus with a flame ionisation detector, using a 1.5 m×2.5 mm column packed with methylsilicone OV-1 3% on Supelcoport 80–100 mesh.

Evaporation was performed in vacuo (rotating evaporator). 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 ±0.4%.

*5.1.1. Synthesis of (R)-**9** and (S)-**10** methyl 2-methyl-3-(2-phthalimidoxy)propionates*

A solution of diethylazodicarboxylate (15 mL, 93 mmol) in anhydrous THF (20 mL) was added dropwise to a stirred solution of the commercially available (*R*)-**7** or (*S*)-**8** methyl 2-methyl-3-hydroxypropionate (10 g, 85 mmol), triphenylphosphine (22.4 g, 85 mmol) and *N*-hydroxyphthalimide (85 mmol). After 24 h at room temperature (r.t.) the solvent was evaporated to yield an oily residue which was triturated with Et_2O. The solid precipitate was filtered and the Et_2O solution was evaporated to give an oil which was chromatographed on a silica gel column eluting with a 9:1 C_6H_6–Et_2O mixture. Evaporation of the fractions containing the main product afforded practically pure **9** or **10**, which was crystallised from Et_2O–hexane: ^{1}H-NMR δ 1.36 (d, 3H, $J = 7.0$ Hz, CHCH_3), 3.03 (m, 1H, CHCH$_3$), 3.75 (s, 3H, CO_2CH_3), 4.41 ppm (m, 2H, OCH_2). **9** (44%): m.p. 44–46 °C; IR (Nujol) ν 1741 cm^{-1} (C=O); $[\alpha]_D^{22} = -9.86$ ($CHCl_3$, $c = 1.42$). $C_{13}H_{13}NO_5$ (C, H, N). **10** (48%): m.p. 44–46 °C; IR (Nujol) ν 1733 cm^{-1} (C=O); $[\alpha]_D^{22} = +10.0$ ($CHCl_3$, $c = 1.03$). $C_{13}H_{13}NO_5$ (C, H, N).

*5.1.2. Preparation of (R)-**11** and (S)-**12** methyl 2-methyl-3-aminoxypropionate*

A solution of 98% $H_2NNH_2 \cdot H_2O$ (19 mmol) in anhydrous EtOH (5 mL) was added to a solution of the phthalimido derivatives **9** or **10** (5.0 g, 19 mmol) in anhydrous EtOH (35 mL). After 1 h at the reflux temperature, the mixture was cooled at r.t. and then filtered. The organic solution was evaporated and the crude residue was dissolved in $CHCl_3$, washed (3% aq. K_2CO_3 and H_2O), filtered and evaporated to dryness to yield an oily residue consisting almost exclusively in the aminoxyester **11** or **12**. The crude product was dissolved in anhydrous Et_2O and the resulting solution was added to a 7:3 Et_2O–MeOH solution of oxalic acid. The solid precipitate was collected by filtration and then crystallised from EtOH to give the pure oxalate salt of **11** or **12**: ^{1}H-NMR (D_2O) δ 1.20 (d, 3H, $J = 7.0$ Hz, CHCH_3), 2.99 (m, 1H, CHCH$_3$), 3.75 (s, 3H, CO_2CH_3), 4.20 ppm (m, 2H, OCH_2); (*R*)-**11**·$H_2C_2O_4$ (58%): m.p. 92–94 °C; $C_7H_{13}NO_7$ (C, H, N). (*S*)-**12**·$H_2C_2O_4$ (64%): m.p. 92–94 °C; $C_7H_{13}NO_7$ (C, H, N).

An analytical sample of (*R*)-**11** or (*S*)-**12** as a free base was obtained by dissolving 50 mg of the corresponding oxalate salt in H_2O (2 mL), adding solid K_2CO_3 (100 mg), extracting with $CHCl_3$, and then evaporating the filtered organic extracts: ^{1}H-NMR δ 1.17 (d, 3H, $J = 7.0$ Hz, CHCH_3), 2.90 (m, 1H, CHCH$_3$), 3.75 ppm (s, 3H, CO_2CH_3).

*5.1.3. General procedure for the synthesis of (E,R)-**15a–g**, (E,S)-**16a–g**, (Z,S)-**17a**, methyl 3-aryl-methylideneaminoxy-2-methylpropionates and (R)-**18** and (S)-**19** methyl fluorenylideneaminoxy-2-methylpropionates*

A solution of the appropriate aromatic aldehyde **13a–g** or fluorenone **14** (26 mmol) and of the aminoxyester (*R*)-**11** or (*S*)-**12** (3.06 g, 26 mmol) in MeOH (60 mL) was stirred at r.t. until the disappearance of the starting carbonyl compound (TLC). The solvent was then evaporated and the residue was taken up with $CHCl_3$ and washed with 10% H_3PO_4 and H_2O. Evaporation of the filtered organic layers afforded an oily residue which was purified by chromatography on a silica gel column eluting with a 7:1:2 toluene–hexane–CH_2Cl_2 mixture. Evaporation of the fractions containing the faster moving significant compound yielded the corresponding practically pure (GLC) aldoximethers (*E*,*R*)-**15a–g** or (*E*,*S*)-**16a–g** or fluorenonoximethers (*R*)-**18** or (*S*)-**19**. Only in the case of the chromatography of the crude reaction mixture of aminoxyester (*R*)-**11** with benzaldehyde **13a**, was a second set of fractions obtained yielding a small quantity of practically pure (*Z*,*S*) oximether **17a**. (*E*,*R*)-**15a** (63%); $[\alpha]_D^{22} = -49.1$ ($CHCl_3$, $c = 1.16$). (*E*,*R*)-**15b** (41%); $[\alpha]_D^{22} = -51.8$ ($CHCl_3$, $c = 1.0$). (*E*,*R*)-**15c** (55%); $[\alpha]_D^{22} = -49.4$ ($CHCl_3$, $c = 1.10$). (*E*,*R*)-**15d** (46%); $[\alpha]_D^{22} = -47.1$ ($CHCl_3$, $c = 1.61$). (*E*,*R*)-**15e** (49%); $[\alpha]_D^{22} = -46.9$ ($CHCl_3$, $c = 1.13$). (*E*,*R*)-**15f** (51%); $[\alpha]_D^{22} = -51.8$ ($CHCl_3$, $c = 1.30$). (*E*,*R*)-**15g** (48%); $[\alpha]_D^{22} = -41.5$ ($CHCl_3$, $c = 2.06$). (*E*,*S*)-**16a** (58%): $[\alpha]_D^{22} = +51.4$ ($CHCl_3$, $c = 1.05$). (*E*,*S*)-**16b** (54%); $[\alpha]_D^{22} = +50.8$ ($CHCl_3$, $c = 0.99$). (*E*,*S*)-**16c** (50%); $[\alpha]_D^{22} = +49.9$ ($CHCl_3$, $c = 1.84$). (*E*,*S*)-**16d** (44%); $[\alpha]_D^{22} = +46.6$ ($CHCl_3$, $c = 1.16$). (*E*,*S*)-**16e** (43%); $[\alpha]_D^{22} = +47.4$ ($CHCl_3$, $c = 1.13$). (*E*,*S*)-**16f** (56%); $[\alpha]_D^{22} = +52.0$ ($CHCl_3$, $c = 1.09$). (*E*,*S*)-**16g** (34%); $[\alpha]_D^{22} = +42.0$ ($CHCl_3$, $c = 1.63$). (*Z*,*S*)-**17a**: IR (Nujol) ν 1749 cm^{-1} (C=O); $[\alpha]_D^{22} = +51.4$ ($CHCl_3$, $c = 1.05$). (*R*)-**18** (23%); $[\alpha]_D^{22} = -19.8$ ($CHCl_3$, $c = 1.09$). (*S*)-**19** (20%); $[\alpha]_D^{22} = +20.3$ ($CHCl_3$, $c = 1.10$). IR (Nujol) and ^{1}H-NMR. (*E*,*R*)-**15a** and (*E*,*S*)-**16a**: ν 1741 cm^{-1} (C=O); δ 1.20 (d, 3H, $J = 7.0$ Hz, CHCH_3), 2.97 (m, 1H,

CHCH_3), 3.63 (s, 3H, CO_2CH_3), 4.22 (m, 2H, OCH_2), 8.00 ppm (s, 1H, CH=N); (*E*,*R*)-**15b** and (*E*,*S*)-**16b** *v* 1741 cm^{-1} (C=O);*δ* 1.23 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 2,98 (m, 1H, *CH*CH_3), 3.70 (s, 3H, CO_2CH_3), 4.30 (m, 2H, OCH_2), 8.10 ppm (s, 1H, CH=N); (*E*,*R*)-**15c** and (*E*,*S*)-**16c**: *v* 1741 cm^{-1} (C=O); *δ* 1.23 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 3.08 (m, 1H, *CH*CH_3), 3.70 (s, 3H, CO_2CH_3), 4.30 (m, 2H, OCH_2), 8.10 ppm (s, 1H, CH=N); (*E*,*R*)-**15d** and (*E*,*S*)-**16d**: *v* 1741 cm^{-1} (C=O); *δ* 1.20 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 2.88 (m, 1H, *CH*CH_3), 3.67 (s, 3H, CO_2CH_3), 3.80 (s, 3H, OCH_3), 4.23 (m, 2H, OCH_2), 7.98 ppm (s, 1H, CH=N); (*E*,*R*)-**15e** and (*E*,*S*)-**16e**: *v* 1741 cm^{-1} (C=O); *δ* 1.20 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 1.40 (t, 3H, *J* = 7.0 Hz, OCH_2*CH*$_3$), 2.87 (m, 1H, *CH*CH_3), 3.67 (s, 3H, CO_2CH_3), 4.0 (q, 2H, *J* = 7.0 Hz, O*CH*$_2$$CH_3$), 4.20 (m, 2H, OCH_2), 7.95 ppm (s, 1H, CH=N); (*E*,*R*)-**15f** and (*E*,*S*)-**16f**: *v* 1741 cm^{-1} (C=O); *δ* 1.25 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 2.40 (s, 3H, CH_3), 2.96 (m, 1H, *CH*CH_3), 3.76 (s, 3H, CO_2CH_3), 4.30 (m, 2H, OCH_2), 8.12 ppm (s, 1H, CH=N); (*E*,*R*)-**15g** and (*E*,*S*)-**16g**: *v* 1741 cm^{-1} (C=O); *δ* 1.26 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 2.96 (m, 1H, *CH*CH_3), 3.73 (s, 3H, CO_2CH_3), 4.45 (m, 2H, OCH_2), 8.13 ppm (s, 1H, CH=N); (*Z*,*S*)-**17a**: *δ* 1.20 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 3.03 (m, 1H, *CH*CH_3), 3.70 (s, 3H, CO_2CH_3), 4.37 (m, 2H, OCH_2), 7.93 ppm (s, 1H, CH=N); (*R*)-**18** and (*S*)-**19**: *v* 1741 cm^{-1} (C=O); *δ* 1.28 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 3.08 (m, 1H, *CH*CH_3), 3.72 (s, 3H, CO_2CH_3), 4.57 ppm (m, 2H, OCH_2).

5.1.4. General procedure for the synthesis of (E,R)-3a–g, (E,S)-4a–g, 3-arylmethylideneaminoxy-2-methylpropionic acids and (R)-5 and (S)-6 fluorenylideneaminoxy-2-methylpropionic acids

A solution of 1 N NaOH (5.5 mL) was added dropwise to a cooled (0 °C) solution of the appropriate methylester (**15a**–**g**, **16a**–**g**, **18** or **19**) (5.0 mmol) in THF (25 mL) and the resulting solution was stirred at the same temperature until the disappearance of the starting ester (TLC). The solution was concentrated and the aqueous residue was then washed with Et_2O, acidified to pH 3 with 10% aq. HCl and extracted with $CHCl_3$. Evaporation of the filtered organic layer afforded a residue which was crystallised from the appropriate solvent, in the case of the solids, or purified by distillation at reduced pressure in the case of the liquids. (*E*,*R*)-**3a** (27%); $C_{11}H_{13}NO_3$ (C, H, N). (*E*,*R*)-**3b** (34%); $C_{11}H_{12}ClNO_3$ (C, H, N). (*E*,*R*)-**3c** (55%); $C_{11}H_{12}FNO_3$ (C, H, N). (*E*,*R*)-**3d** (36%); $C_{12}H_{15}NO_4$ (C, H, N). (*E*,*R*)-**3e** (46%); $C_{13}H_{17}NO_4$ (C, H, N). (*E*,*R*)-**3f** (51%); $C_{12}H_{15}NO_3$ (C, H, N). (*E*,*R*)-**3g** (44%); $C_{12}H_{12}F_3NO_3$ (C, H, N). (*E*,*S*)-**4a** (43%); $C_{11}H_{13}NO_3$ (C, H, N). (*E*,*S*)-**4b** (32%); $C_{11}H_{12}ClNO_3$ (C, H, N). (*E*,*S*)-**4c** (36%); $C_{11}H_{12}FNO_3$ (C, H, N). (*E*,*S*)-**4d** (21%); $C_{12}H_{15}NO_4$ (C, H, N). (*E*,*S*)-**4e** (40%); $C_{13}H_{17}NO_4$ (C, H, N). (*E*,*S*)-**4f** (46%); $C_{12}H_{15}NO_3$ (C, H, N). (*E*,*S*)-**4g** (25%); $C_{12}H_{12}F_3NO_3$ (C, H, N). (*R*)-**5** (20%); $C_{17}H_{15}NO_3$ (C, H, N). (*S*)-**6** (57%); $C_{17}H_{15}NO_3$ (C, H, N). IR (Nujol) and ^{1}H-NMR. (*E*,*R*)-**3a** and (*E*,*S*)-**4a**: *v* 1703 cm^{-1} (C=O); *δ* 1.28 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 3.10 (m, 1H, *CH*CH_3), 4.30 (m, 2H, OCH_2), 8.15 ppm (s, 1H, CH=N); (*E*,*R*)-**3b** and (*E*,*S*)-**4b**: *v* 1703 cm^{-1} (C=O); *δ* 1.27 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 2.68 (m, 1H, *CH*CH_3), 4.42 (m, 2H, OCH_2), 8.07 ppm (s, 1H, CH=N); (*E*,*R*)-**3c** and (*E*,*S*)-**4c**: *v* 1703 cm^{-1} (C=O); *δ* 1.32 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 3.03 (m, 1H, *CH*CH_3), 4.40 (m, 2H, OCH_2), 8.03 ppm (s, 1H, CH=N); (*E*,*R*)-**3d** and (*E*,*S*)-**4d** *v* 1718 cm^{-1} (C=O) (liquid film); *δ* 1.27 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 2,95 (m, 1H, *CH*CH_3), 3.82 (s, 3H, OCH_3), 4.33 (m, 2H, OCH_2), 8.03 ppm (s, 1H, CH=N); (*E*,*R*)-**3e** and (*E*,*S*)-**4e**: *v* 1718 cm^{-1} (C=O) (liquid film); *δ* 1.27 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 1.38 (t, 3H, *J* = 7.0 Hz, OCH_2*CH*$_3$), 3.06 (m, 1H, *CH*CH_3), 4.0 (q, 2H, *J* = 7.0 Hz, O*CH*$_2$$CH_3$), 4.28 (m, 2H, OCH_2), 7.99 ppm (s, 1H, CH=N); (*E*,*R*)-**3f** and (*E*,*S*)-**4f**: *v* 1726 cm^{-1} (C=O); *δ* 1.27 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 2.38 (s, 3H, CH_3), 2.96 (m, 1H, *CH*CH_3), 4.33 (m, 2H, OCH_2), 8.10 ppm (s, 1H, CH=N); (*E*,*R*)-**3g** and (*E*,*S*)-**4g**: *v* 1719 cm^{-1} (C=O); *δ* 1.27 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 2.98 (m, 1H, *CH*CH_3), 4.33 (m, 2H, OCH_2), 8.17 ppm (s, 1H, CH=N); (*R*)-**5** and (*S*)-**6**: *v* 1719 cm^{-1} (C=O); *δ* 1.37 (d, 3H, *J* = 7.0 Hz, CH*CH*$_3$), 3.17 (m, 1H, *CH*CH_3), 4.55 ppm (m, 2H, OCH_2).

An equimolar mixture of racemic *m*-F-substituted-3-benzylideneaminoxy-2-methyl-propionic acids **3c** and **4c**, with (*R*)-cinconidine in $CDCl_3$ displayed in its ^{1}H-NMR spectrum two doublets at 1.24 and 1.22 ppm attributable to 2-methyl protons of *E*,*R*-(**3c**) and *E*,*S*-(**4c**) stereoisomers, respectively.

5.2. Biopharmacological methods

5.2.1. Carrageenan-induced paw edema

The procedure was the same already followed in previous papers [14, 15]. Ten or 20 female Wistar rats (200–250 g body weight) were used for each group. Hind paw volumes were measured using a water plethysmometer (Basile) in accordance with the method de-

scribed by Winter et al. [20]. Then the compounds to be tested were administered i.p. at a constant dose of 50 mg kg^{-1}, using a solution in aq. $NaHCO_3$ (pH 7.4) at a concentration of 10 mg mL^{-1}. Control rats received the same volume of the vehicle. Thirty min later, 0.05 mL of a 1% solution of carrageenan (Sigma) was subcutaneously injected into the plantar surface of the right hind paw. The increase in paw volume 4 h after the injection of the phlogistic agent was adopted as a measure of the effect, and results were expressed as a percentage reduction of control edema. Student's *t*-test for grouped data was used for statistical evaluation. Differences were considered to be statistically significant when P was ≤ 0.05.

5.2.2. Approximate LD_{50}

The compounds to be tested (**3a**,**b** and **4a**,**b**) were administered i.v. in aq. $NaHCO_3$ solution (pH 7.5) at increasing doses, to male albino Swiss mice from Nossan Farm. Approximate LD_{50} values were determined in accordance with the method of Litchfield et al. [21].

5.2.3. Enzyme assays

Compounds **3a**,**b** and **4a**,**b** were tested in intact cell assays to verify their capacity to inhibit PGE_2 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 PGE_2 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 PGE_2 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.

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