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Synthesis and biological activity of new anti-inflammatory compounds containing the 1,4-benzodioxine and/or pyrrole system

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Abstract—A series of substituted derivatives containing the 1,4-benzodioxine or pyrrole nucleus are described. All the newly synthesized compounds were examined for their in vitro and in vivo anti-inflammatory activity. Several derivatives, including (*S*)-**2**, **14** and **17**, showed more anti-inflammatory activity in vivo in these assays (rat paw oedema induced by carrageenan) than the known classical anti-inflammatory agent ibuprofen, whereas other compounds like **1** were equipotent to ibuprofen. Compound **17** was the most outstanding derivative because of its remarkable in vivo anti-inflammatory activity. In this paper, we examine and discuss the structure–activity relationships and anti-inflammatory activities of these compounds.

1. Introduction

Non-steroid anti-inflammatory drugs (NSAIDs) are among the most widely utilized drugs worldwide, being the drugs of first choice in the treatment of rheumatoid disorders, osteoarthritis and also in other inflammatory diseases and injuries.[1] The anti-inflammatory activity is due to the ability to inhibit the cyclooxygenase (COX) activity of prostaglandin H synthase, an enzyme which mediates the production of prostanoids (including prostaglandins, protacyclins and thromboxanes) from arachidonic acid. Prostaglandins act as mediator in the process of inflammation. This mechanism of action was elicited by Vane.[2,3]

A large number of NSAIDs act as non-selective inhibitors of the COX, inhibiting both the cyclooxygenase-1 (COX-1) and the cyclooxygenase-2 (COX-2) isoenzymes. The inhibition of prostanoid biosynthesis is associated with side effects such as ulceration and nephrotoxicity.[3] These drugs were developed as an alternative to the corticosteroids and their analogues, which have many side effects.

The cyclooxygenase activity of the enzyme is the site of action of the non-steroid anti-inflammatory drugs (NSAIDs).[4,5] Selective COX-2 inhibitors have been developed and marketed based on the presumption that the postulated mechanism by which the non-selective NSAIDs cause gastrointestinal ulceration is the inhibition of the isoenzyme COX-1.[6] Recently, based on FDA data, COX-2 selective-inhibitors are associated with an increased incidence of serious adverse effects (cardiovascular disorders) compared to non-selective anti-inflammatory drugs.[7] The largest group of NSAIDs is represented by the class of arylalkanoic acids, as typified by their general chemical structure (Fig. 1).

The aromatic ring system appears to correlate with the double bond at the 5- and 8-positions of arachidonic

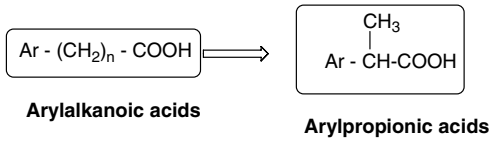

Figure 1. General structure of arylalkylcarboxylic acids.

Keywords: Anti-inflammatory activity; Carboxylic acids; 1,4-Benzodioxine; Pyrrole derivatives; AINEs.
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acid. A second area of lipophilicity that is generally not coplanar with the aromatic or heteroaromatic ring enhances activity. This second lipophilic area may correspond to the area of the double bond in the 11-position of arachidonic acid. The heterocyclic ring is believed to provide the necessary double bond geometry and the heterocyclic system itself may not be essential for the anti-inflammatory activity.[8]

The anti-inflammatory capacity of aryl acetic and aryl propionic acids, in which activity is maintained despite wide variations in the nature of the aryl group, has prompted recent research. Given the fast and effective anti-inflammatory activity and the therapeutic interest reported for certain aryl acetic and aryl propionic acids, we synthesized several acid compounds as anti-inflammatory agents containing heterocyclic nuclei.

Motivated by the aforementioned findings, and in continuation of our investigations in this field,[9,10] we aimed to synthesize a novel series of 1,4-benzodioxine derivatives and pyrrole-related compounds for a study of the structure activity relationships. The heterocyclic system (1,4-benzodioxine or pyrrole) was selected on the basis of previous studies realised by us,[9,10] and also keeping in mind the large work in the field of anti-inflammatory agents reported before.

2. Results and discussion

2.1. Chemistry

2,3-Dihydro-1,4-benzodioxines possessing substituents on the aryl ring system were conveniently prepared from commercially available 2,3-dihydro-1,4-benzodioxine (**19**) (Scheme 1).

The carboxylic acid **1** related to the 1,4-benzodioxine was synthesized from the 2,3-dihydro-1,4-benzodioxine (**19**) by acetylation with acetyl chloride followed by transformation of the resulting ketone to arylacetic acid under Willgerodt–Kindler reaction conditions.[9] We also prepared the carboxylic acids (*R*,*S*)-**2**, (*R*)-**2**, and (*S*)-**2** related to the 1,4-benzodioxine.[10]

The acylation of **19** using ethyl succinyl chloride yielded the desired ketoester, which was hydrolysed to the carboxylic acid **3**. Reduction of the ketoacid **3** with $NaBH_4$ in methanol afforded the lactone **4** in quantitative yield through reduction followed by cyclization. Reduction of **3** by $NaBH_3CN$ and trifluoroacetic acid or with a mixture of $Zn/HgCl_2$ in acidic media gave the substituted butyric acid **5**. The acid **5** was transformed into the desired cyclic ketone **22** with success, using CF_3COOH in dichloromethane. The oxime **6** was obtained by treatment of the ketone **22** with hydroxylamine in pyridine. The corresponding ketoacid **7** was synthesized from **22** by treatment with LDA followed by addition of ethyl chloroformate. Next, the intermediate ester was hydrolysed with 2 N NaOH. The ketone **22** was converted to the nitrile derivative **24** in 48% overall yield in a two-step sequence consisting of the cyanhydrine formation followed by treatment of the cyanhydrine with potassium bisulfate ($KHSO_4$) at 170 °C. The ester **24** was hydrolysed to the corresponding carboxylic acid **8** by treatment with 2 N NaOH. The ketone **22** was also converted to the carboxylic acid **9** by cyanation, followed by treatment with $KHSO_4$ at 100 °C (Scheme 1). Scheme 2 depicts a route to the synthesis of the carboxylic acid **10** from the tosylate **26**, previously prepared as described elsewhere.[11] The treatment of **26** with potassium cyanide followed by hydrolysis of the nitrile with 2 N NaOH provided the desired acid **10** in acceptable yield.

We failed to convert the acid **10** to the unsaturated analogue **11**, probably because of the easy degradation of the starting acid in the presence of oxidizing agents. Therefore, we developed an alternative route from the 2,3-dihydro-1,4-benzodioxine **19** (Scheme 3).

The 2-bromo-1,4-benzodioxine **28** was prepared from the 2,3-dihydro-1,4-benzodioxine **19** following the conditions reported previously by Chapleo and co-workers[12] Alkylation of the 2-bromo-benzodioxine **28** by bromoacetic acid, in the presence of *t*-BuLi in THF at −78 °C, gave the acid **11** in acceptable yield. 2,3-Dihydro-1,4-benzodioxine **19** was converted to the carboxylic acid **12** by formylation of **29** followed by oxidation to the expected acid **12**.[13]

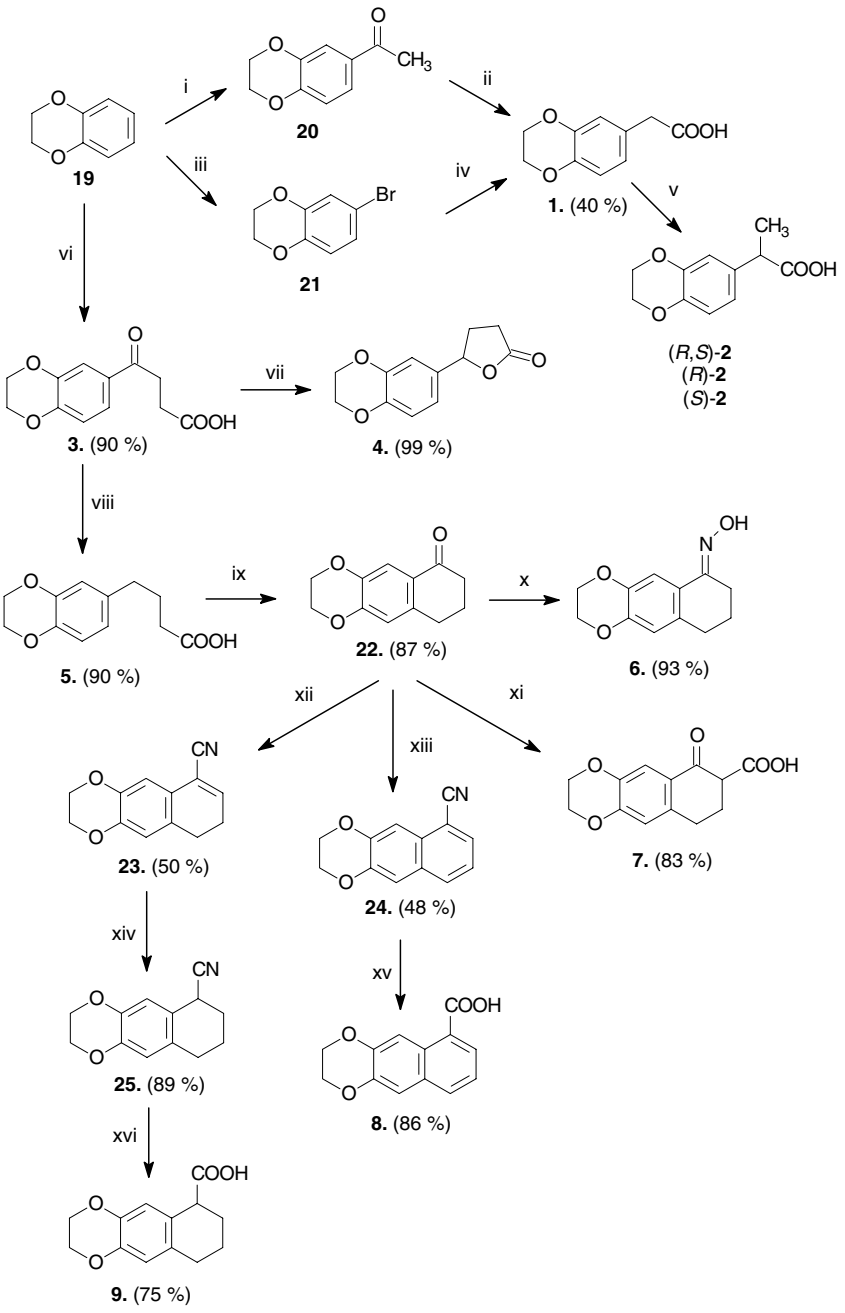
The introduction of a pyrrole ring is considered in Scheme 4. The commercially available aniline **30** was reacted with the 2,5-dimethoxytetrahydrofuran in acidic media giving the pyrrole derivative **31** in good yield.[14]

To prepare the carboxylic acid (**13**–**15**), the N-substituted pyrrole **31** was reacted with acetic anhydride in the presence of $BF_3{\cdot}(CH_3CH_2)_2O$, which gave a mixture of acetylated compounds (**32** and **33**) in acceptable yield. The separated isomers were treated with sulfur and morpholine under Willgerodt–Kindler reaction conditions,[15] yielding the corresponding intermediate thioamide, which was hydrolysed to the corresponding carboxylic acid **14** (34%) or **15** (71%). The modest yield of acid **14** was due to the formation of ketoacid **13** as a by-product (50%). The carboxylic acid **13** was obtained by hydrolysis of the intermediate ketothioamide formed under Willgerodt–Kindler conditions.

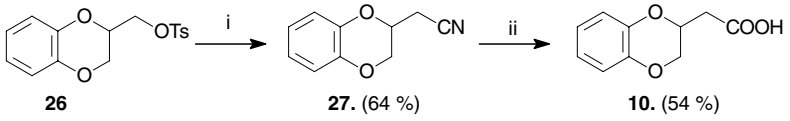
An alternative route to the preparation of **13** was the acylation of **31** with ClCOCOOEt followed by alkaline hydrolysis (Scheme 4). Other attempts were considered for the preparation of **14** and **15**, which lead to the expected carboxylic acids but with lower yield.

Scheme 5 depicts the synthesis of the carboxylic acids (**16**–**18**) that possess the fluorophenyl moiety in their structure. Compounds **16**–**18** were prepared from the 2-fluorophenylpyrrole **35**, as described elsewhere.[14]

All compounds were obtained as racemic mixtures. Only the aryl propionic acid **2** was resolved in the corresponding enantiomers.



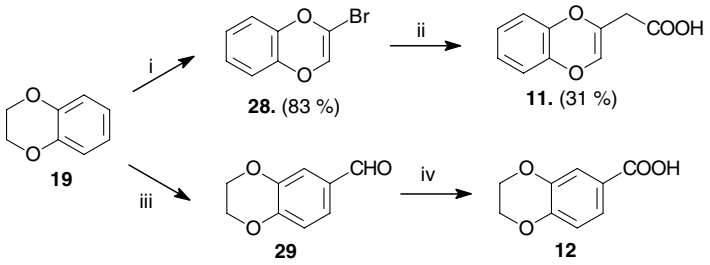
Scheme 1. Reagents and conditions: (i) $CH_3COCl/AlCl_3$; (ii) 1—S_8, morpholine, 2—KOH; (iii) NBS/MeOH; (iv) BuLi/$BrCH_2COOH$; (v) LDA/CH_3I/−78 °C; (vi) 1—$EtOCOCH_2CH_2COCl/TiCl_4$; 2—NaOH; (vii) 1—$NaBH_4$/MeOH/rt; 2—HCl; (viii) $NaCNBH_3/CF_3COOH$ (35% yield) or Zn/$HgCl_2$/HCl (84%); (ix) $(CF_3CO)_2O/CH_2Cl_2$/rt (87% yield); (x) NH_2OH/pyridine; (xi) 1—LDA/−78 °C/EtOCOCl, 2—$NaOH/H_3O^+$ (51% yield); (xii) $(CH_3CH_2)_2AlCN$/HCl; 2—$KHSO_4$/100 °C; (xiii) 1—$(CH_3CH_2)_2AlCN$; 2—$KHSO_4$/170 °C; (xiv) $NaBH_4$/MeOH; (xv) NaOH 2 N/reflux; (xvi) NaOH 2 N/reflux.



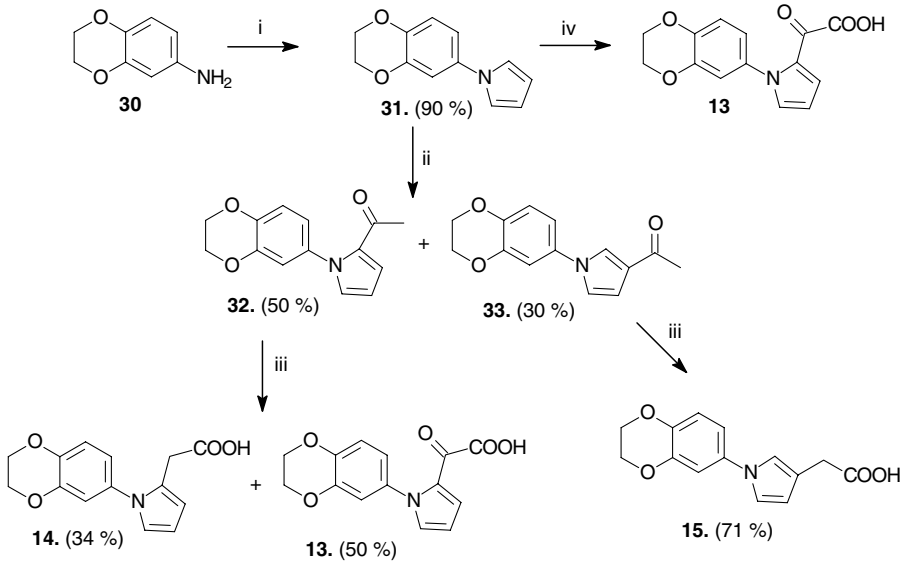
Scheme 2. Reagents: (i) KCN/DMSO; (ii) 1—KOH; 2—HCl.

2.2. Biological results and discussion

The potential therapeutic activity of these compounds was assessed both for their anti-inflammatory activity using the carrageenan rat paw oedema assay and for their inhibitory activity of the rat liver 3α-hydroxysteroid dehydrogenase (3α-HSD). The selected and promising compounds were also tested in vitro against human



Scheme 3. Reagents and conditions: (i) 1—NBS/CCl_4/hν; 2—K^+ *t*-BuO^-; (ii) *t*-BuLi/$BrCH_2COOH$/THF/−78 °C; (iii) 1—NBS/MeOH; 2—BuLi/−78 °C/DMF; (iv) CrO_3/*t*-BuOOH/CH_2Cl_2.



Scheme 4. Reagents: (i) 2,5-Dimethoxytetrahydrofuran/acetic acid; (ii) acetic anhydride/$BF_3{\cdot}(CH_3CH_2)_2O$; (iii) 1—S_8/morpholine; 2—NaOH/MeOH; (iv) 1—ClCOCOOEt/$BF_3{\cdot}(CH_3CH_2)_2O$; 2—NaOH 2 N.

COX-1 and COX-2 enzymes. Ibuprofen was used as reference compound for the in vivo and in vitro assays.

Mammalian 3α-hydroxysteroid dehydrogenases (3α-HSDs) are members of the aldo–keto reductase (AKR) superfamily,[16] which has manifold functions (regulation of steroid hormone levels and carcinogenicity) and thus includes potential drug targets.[17]

Moreover, rat liver 3α-HSD can be inhibited by major classes of non-steroidal anti-inflammatory drugs,[18] which has given rise to the rapid spectrophotometer assay developed by Penning[19] and used in this study.[20]

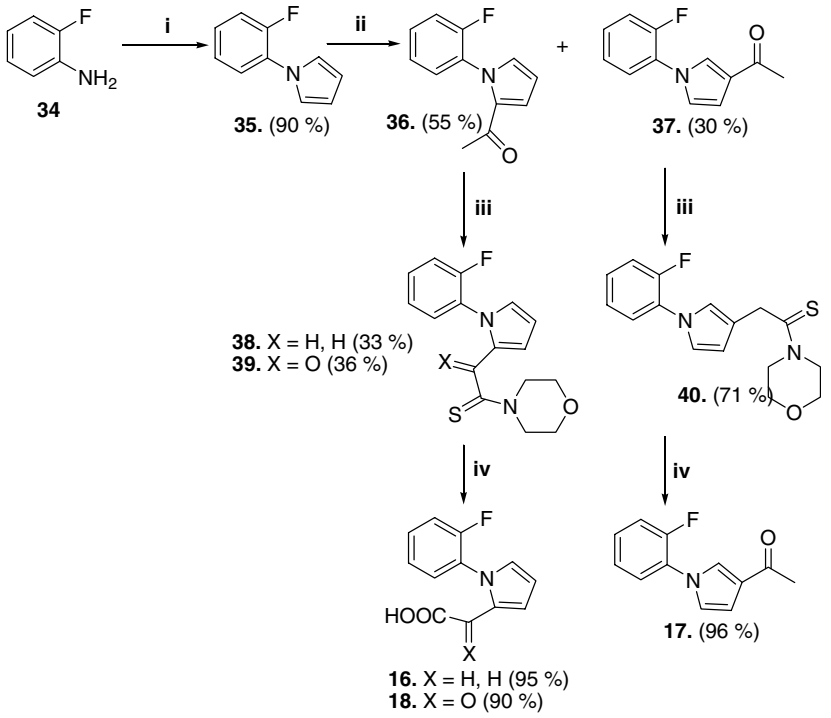
We calculated the percentage of inhibition at the screening concentration of 1000 μM and the IC_{50} of 3α-HSD enzymatic activity for all tested compounds (Table 1). Compounds **16**, **17** screened at 500 μM and compounds **8**, **18** at 250 μM, owing to the low solubility or excessive absorbance at 340 nm of their 1000 μM solutions.

Ibuprofen was also tested at 1000 μM for comparison and its IC_{50} was 100 μM. The anti-inflammatory activity of these synthesized compounds in vivo was evaluated using the carrageenan-induced rat paw oedema assay.[21] The potency and duration of action were compared with those of the reference compound ibuprofen.[22]

The highest enzyme inhibition was shown by compound **14**, whose IC_{50} was 5.8 μM, about 17 times higher than that of ibuprofen. Moreover, this compound had a stronger anti-inflammatory effect in vivo than ibuprofen. Likewise, compound **17**, with potent anti-3α-HSD enzymatic activity (IC_{50} = 34 μM), showed a higher inhibitory action for the carrageenan-induced oedema than ibuprofen. In addition, the in vivo anti-inflammatory activity was sustained after 4 h, whereas ibuprofen activity decreased quickly.

To analyse structure–activity relationships, four structural components were considered, the nature of the heterocycle nucleus, the position of the side chain on the heterocycle system, the length of the side chain (the distance between the heterocyclic nucleus and the present function) and the function of the side chain.

First, regarding the side chain function, the carboxylic acid confers greater activity both in vivo and in vitro than the oxime or lactone: while **1** showed % inh = 33 (3 h post-carrageenan), the lactone **4** presented % inh = 22.6 (3 h post-carrageenan) and the oxime **6** was



Scheme 5. Reagents and condition: (i) 2,5-Dimethoxytetrahydrofuran/acetic acid; (ii) acetic anhydride/$BF_3{\cdot}(CH_3CH_2)_2O$; (iii) S_8/morpholine/140 °C; (iv) NaOH 2 N.

inactive, on the other hand **1** showed IC_{50} (μM) = 218, **4** IC_{50} (μM) >1000 and **6** IC_{50} (μM) >500 in vitro. The most surprising result from our SAR analysis may be the complete lack of anti-inflammatory activity of the ketoacids **7**, **13** and **18**.

The influence of the distance between the aromatic heterocyclic system and the carboxylic function is marked and large differences between these compounds were observed especially in vivo.

The compounds **1**, **14** and **17** showed greater activity than ibuprofen according to the results of the carrageenan-induced oedema test. In a parallel examination, compounds **3**, **5**, **7**, **8** and **12**, which have more (**3** and **5**) or less (**7**, **8** and **12**) distance between the carboxylic acid and the aromatic heterocyclic ring, were the least active of the series, indicating that the acetic acid chain confers the highest anti-inflammatory activity.

Likewise, the modest or null anti-inflammatory activity of compounds **10** and **11**, which present the acetic acid chain linked on the non-aromatic heterocycle moiety, confirms that the influence of the arylacetic acid framework conditions the anti-inflammatory activity.

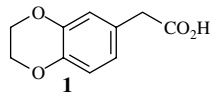
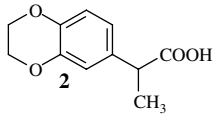
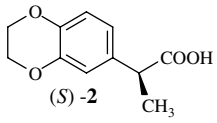
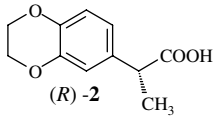
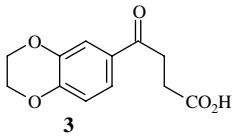
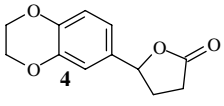
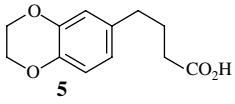
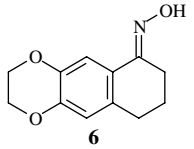
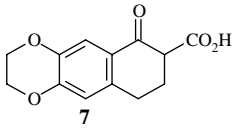
The tricyclic carboxylic acid **9**, considered a rigid analogue of **1**, showed modest anti-inflammatory properties both in vitro and in vivo. Moreover, the activity of **9** markedly depended on time, peaking at two hours post-carrageenan.

The inhibitory potency depends on the length of the side chain, on the position of the chain on the aromatic ring and also on the aromatic system itself. The compounds containing the pyrrole ring showed the highest activity both in the carrageenan-induced oedema test and in vitro (compounds **14** and **17**).

The greater anti-inflammatory activity of **17** in the rat paw assay and in vitro (% inh (3 h post-carrageenan) = 51.9, and IC_{50} (μM) = 34) in contrast to the activity shown by the position isomer **16** (% inh (3 h post-carrageenan) = 23.2), which was less potent than ibuprofen as an anti-inflammatory agent, indicated the contribution of the position of substituent on the pyrrole ring to the anti-inflammatory activity. We also noted the influence of the N-substituent on the pyrrole ring. Thus when the N-substituent was 2-fluorophenyl, the isomer substituted at the C-3 of the aromatic ring showed higher anti-inflammatory activity than the C-2 substituted isomer, but when the N-substituent was 2,3-dihydro-1,4-benzodioxin-6-yl, the compound substituted at the C-2 displayed more activity than the corresponding isomer substituted at the C-3 on the pyrrole ring. We would like to highlight the inversion of activities depending on the N-substituent and the position of the substitution on the aromatic ring (compounds **14**, **15** and compounds **16**, **17**).

The introduction of methyl in the side chain of **1** gave the aryl propionic acid **2**, which lowered the anti-inflammatory activity both in vitro and in vivo. The results in Table 1 suggest that the (*S*)-enantiomer is more potent than the corresponding (*R*)-enantiomer. Moreover, (*S*)-**2** exhibited a long duration of action, and after 5 h of oral administration (4 h post-carrageenan), it was 3.23 times more active than its enantiomer (*R*)-**2**. These

Table 1. In vivo and in vitro anti-inflammatory activity

Compound	Oedema induced by carrageenan (% oedema inh. relative to control)						Inh of 5β-dihydrocortisone reduction	
	2 h		3 h		4 h		%inh (1 mM)	IC_{50} (μM)
	Swelling	%inh	Swelling	%inh	Swelling	%inh		
 **1**			(a) 25.6 ± 3.7** (b) 38.2 ± 6.6	33.0	(a) 37.2 ± 7.4** (b) 49.9 ± 5.3	25.6	63.8 ± 5.1 (n = 8)	218
 **2**			(a) 21.6 ± 2.4* (b) 27.2 ± 3.6	21.3	(a) 21.6 ± 3.3# (b) 27.2 ± 6.8	20.6	42.2 ± 2.3 (n = 8)	>1000
 (*S*) -**2**			(a) 30.3 ± 5.6*** (b) 48.4 ± 2.3	37.4	(a) 31.7 ± 7.8*** (b) 54.5 ± 3.0	41.8	55 ± 4.2 (n = 6)	≅ 300
 (*R*) -**2**			(a) 31.6 ± 8.8* (b) 41.4 ± 2.5	23.7	(a) 33.5 ± 9.7# (b) 38.5 ± 3.7	13.0	43.7 ± 3.1 (n = 8)	>1000
 **3**			(a) 37.6 ± 5.5#	10.5	(a) 46.7 ± 6.3#	1.5	61.4 ± 3.9 (n = 6)	209
 **4**			(b) 42.0 ± 12.4 (a) 36.3 ± 5.9* (b) 46.9 ± 10.7	22.6	(b) 47.4 ± 10.5 (a) 44.9 ± 5.5* (b) 54.8 ± 8.6	18.1	46.2 ± 4.2 (n = 6)	>1000
 **5**			(a) 47.5 ± 9.9 (b) 42.0 ± 12.4	—	(a) 51.1 ± 7.4 (b) 47.4 ± 10.5	—	60.5 ± 3.2 (n = 6)	209
 **6**			—		—		NA	>500
 **7**			—		—		NA	>1000

(*continued on next page*)

Table 1 (*continued*)

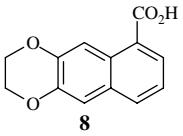
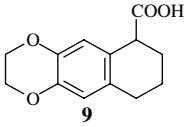
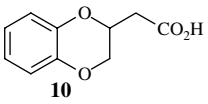
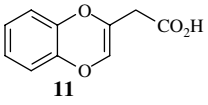
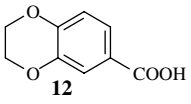
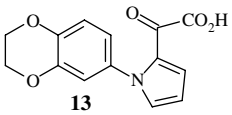
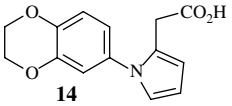
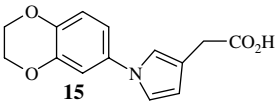
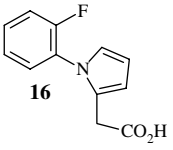
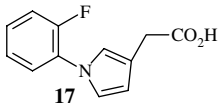
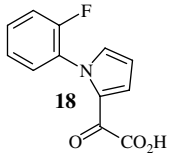
Compound	Oedema induced by carrageenan (% oedema inh. relative to control)						Inh of 5β-dihydrocortisone reduction	
	2 h		3 h		4 h		%inh (1 mM)	IC_{50} (μM)
	Swelling	%inh	Swelling	%inh	Swelling	%inh		
 **8**			(a) 47.4 ± 6.0 (b) 42.1 ± 6.4	—	(a) 48.6 ± 6.2 (b) 44.3 ± 5.9	—	67.1 ± 1.0 (n = 5)	100
 **9**	(a) 21.8 ± 5.4** (b) 29.0 ± 6.4	24.8	(a) 36.4 ± 8.0** (b) 42.1 ± 6.4	13.5	(a) 41.1 ± 7.4* (b) 44.3 ± 5.9	7.2	70.7 ± 2.7 (n = 5)	129
 **10**			(a) 35.8 ± 8.4** (b) 40.2 ± 4.7	10.9	(a) 34.4 ± 10.2* (b) 34.6 ± 2.3	0.5	25.4 ± 4.4 (n = 4)	>1000
 **11**			(a) 65.3 ± 5.3 (b) 57.2 ± 7.3	—	(a) 66.4 ± 7.4 (b) 54.4 ± 6.1	—	66.4 ± 4.3 (n = 6)	100
 **12**			(a) 42.8 ± 6.7# (b) 46.9 ± 10.7	8.7	(a) 60.8 ± 11.0 (b) 54.8 ± 8.6	—	54 ± 2.8 (n = 8)	630
 **13**			(a) 65.3 ± 6.7 (b) 57.2 ± 7.3	—	(a) 64.1 ± 6.5 (b) 54.4 ± 6.1	—	67** (n = 5)	70
 **14**			(a) 27.9 ± 3.2*** (b) 55.2 ± 8.7	49.4	(a) 38.1 ± 3.5** (b) 58.6 ± 7.9	34.9	75.4 ± 1.8 (n = 5)	5.8
 **15**	(1)		(a) 44.7 ± 6.8# (b) 50.8 ± 3.9	12.0	(a) 48.8 ± 6.1* (b) 56.2 ± 5.8	13.2	72.5 ± 1.9 (n = 5)	10
 **16**			(a) 42.4 ± 8.9* (b) 55.2 ± 8.7	23.2	(a) 44.9 ± 8.1** (b) 58.6 ± 7.9	23.4	71.3 ± 0.8 (n = 6)	18

Table 1 (*continued*)

Compound	Oedema induced by carrageenan (% oedema inh. relative to control)						Inh of 5β-dihydrocortisone reduction	
	2 h		3 h		4 h		%inh (1 mM)	IC_{50} (μM)
	Swelling	%inh	Swelling	%inh	Swelling	%inh		
 **17**			(a) 24.4 ± 4.6*** (b) 50.8 ± 3.9	51.9	(a) 30.8 ± 6.5** (b) 56.2 ± 5.8	45.2	63.9 ± 4.0 (n = 4)	34
 **18**	(1)		NT		NT		27.6 ± 1.0 (n = 4)	>250
Ibuprofen	(a) 19.9 ± 4.1* (b) 24.2 ± 6.7	17.8	(a) 27.1 ± 3.6*** (b) 38.9 ± 6.0	30.3	(a) 34.3 ± 6.9** (b) 45.1 ± 5.0	23.9	70.5 ± 4.4 (n = 8)	100

(a) Swelling for the dose of 70 mg/kg. (b) Swelling control. (1) Low solubility. NT not tested. NA non-active. Values significantly differ from controls as indicated: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; # does not differ significantly according to unpaired one-tailed Student's t test.

results are consistent with published data, which show that the eutomer of the anti-inflammatory α-arylpropionic acids is the (*S*)-enantiomer. The (*S*)-**2** showed similar anti-inflammatory activity to the parent compound ibuprofen 3 h after carrageenan whereas administration, the anti-inflammatory activity of (*S*)-**2** after 4 h was 1.76 times higher than that of ibuprofen.

The preliminary in vitro results of enzymatic inhibition of COX-1 and COX-2 for (*S*)-**2** and **14** had shown non-preference for each one of the COX isoforms, while **16** (COX-2/COX-1 = 3.2) and **17** (COX-2/COX-1 = 4.4) showed moderate COX-2 selectivity (Table 2).

2.2.1. Conclusion. We have synthesized and evaluated a series of heterocyclic compounds as potential anti-inflammatory agents. Based on their structure, we conclude that the best aromatic nucleus is the pyrrole with an *N*-2-fluorophenyl substituent and an acetic acid subunit on the C-3 such as in compound **17**. In the 1,4-benzodioxine derivatives, the anti-inflammatory activity also depends on the arrangement of the acetic acid group at the heterocyclic system (compound **1** is more active than **10** or **11**).

Table 2. In vitro assays for inhibition of COX-1 and COX-2

Compound	COX-1 IC_{50}[a] (μM)	COX-2 IC_{50}[a] (μM)	Selectivity COX-1/COX-2
Ibuprofen	6.9	6.2	1.1
1	>100	>100	—
(*S*)-**2**	13.0	16.5	0.8
14	1.9	2.2	0.9
16	19.8	6.2	3.2
17	10.6	2.4	4.4

[a] The result is the average of at least two determinations.

Finally, the biological results of the two enantiomers of **2** showed that the (*S*)-enantiomer was more potent as anti-inflammatory compound than the (*R*)-enantiomer.

3. Experimental

3.1. General methods

Melting points were obtained on an MFB-595010 M Gallenkamp apparatus in open capillary tubes and are uncorrected. IR spectra were obtained using a FTIR Perkin-Elmer 1600 Infrared Spectrophotometer. Only noteworthy IR absorptions are listed (cm^{-1}). ^1H and ^{13}C NMR spectra were recorded on Varian Gemini-200 (200 and 50.3 MHz, respectively) or Varian Gemini-300 (300 and 75.5 MHz) Instruments using $CDCl_3$ as solvent with tetramethylsilane as internal standard or $(CD_3)_2CO$. Other ^1H NMR spectra and heterocorrelation ^1H–^{13}C (HMQC and HMBC) experiments were recorded on a Varian VXR-500 (500 MHz). Mass spectra were recorded on a Helwett–Packard 5988-A. Column chromatography was performed with silica gel (E. Merck, 70–230 mesh). Reactions were monitored by TLC using 0.25 mm silica gel F-254 (E. Merck). Microanalysis was determined on a Carlo Erba −1106 analyser. All reagents were of commercial quality or were purified before use. Organic solvents were of analytical grade or were previously purified by standard procedures.

3.2. 2-(2,3-Dihydro-1,4-benzodioxin-6-yl) acetic acid (1)

To a solution of the bromoderivative **21** (1 g, 3.2 mmol) in THF (4 mL) was added a solution of *n*-BuLi in hexane (2.2 mL, 3.52 mmol) at −78 °C under an argon atmosphere. The mixture was stirred at −78 °C for

1 h. Then, a solution of bromoacetic acid (486 mg, 3.52 mmol) in freshly distilled THF (2 mL) was added and the mixture was allowed to warm slowly to 20 °C, then a solution of saturated NH_4Cl was added, and the resulting mixture was stirred for 20 min. Finally it was extracted with ether (3× 20 mL) and the aqueous phase was acidified with 2 N HCl and extracted with CH_2Cl_2 (3× 20 mL). Organic layers were dried (Na_2SO_4), filtered and concentrated under vacuum, and the crude product was purified by silica gel column chromatography. Using a mixture of hexane/ethyl acetate (5:95) as eluent, the carboxylic acid was obtained as a white solid (40%, yield), mp: 71–73 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 2992 (COO–H); 1718 (C=O); 1253 (ArC–O–C); 1066 (C–O–C). ^1H NMR ($CDCl_3$, 500 MHz) δ (ppm): 3.51 (s, 2H, C–$2H_2$); 4.22 (s, 4H, CH_2–O–); 6.74 (dd, $J_1 = 8.5$, $J_2 = 2.0$ Hz, 1H, H-7′); 6.80 (d, $J = 2.0$ Hz, 1H, H-5′); 6.81 (d, $J = 8.5$ Hz, 1H, H-8′). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 40.2 (CH_2, C-2); 64.3 (CH_2, C-2′ y C-3′); 117.3 (CH, C-8′); 118.2 (CH, C-5′); 122.3 (CH, C-7′); 126.3 (C, C-6′); 142.8 (C, C-4′a); 143.4 (C, C-8′a); 177.9 (C, C_1 (COOH)). MS (EI), *m*/*z* (%): 121 (10%, $C_7H_5O_2^+$); 149 (100%, $C_9H_9O_2^+$); 180 (48%); 194 (19%, $C_{10}H_{10}O_4^+$). Anal. Calcd for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C. 61.72; H. 5.41.

3.3. 4-Oxo-4-(2,3-dihydro-1,4-benzodioxin-6-yl)butyric acid (3)

A solution of the corresponding intermediate ketoester (2 g, 8.46 mmol) in 2 N NaOH was heated under reflux for 2 h. The crude reaction mixture was warmed to room temperature and extracted with ether (3× 20 mL). The aqueous layer was acidified with 5 N HCl and extracted with ether (3× 20 mL). The organic layers were dried (Na_2SO_4), filtered off and concentrated under reduced pressure. The corresponding ketoacid was obtained as a white solid (90% yield), mp: 137–139 °C (hexane/ethyl acetate). IR (NaCl) ν (cm^{-1}): 3300 (OH); 1702 (COO–); 1682 (CO); 1288 (Ar–O); 1194 (C–O). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.78 (t, $J = 6.6$ Hz, 2H, CH_2–); 3.24 (t, $J = 6.6$ Hz, 2H, CH_2–); 4.31 (m, 4H, CH_2–O–); 6.91 (d, $J = 8.8$ Hz, 1H, H-8); 7.53 (m, 2H, H-5, H-7). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 29.6 (CH_2, CH_2–CO); 32.8 (CH_2, CH_2–CO); 64.1 and 64.6 (CH_2, CH_2–O–); 117.2* (CH, C-7); 117.6* (CH, C-5); 122.1 (CH, C-8); 130.2 (C, C-6); 143.3 (C, C-4a); 148.1 (C, C-8a); 178.6 (C, COO–); 196.3 (C, CO). * Interchangeable. Anal. Calcd for $C_{12}H_{12}O_5$: C, 61.01; H, 5.12. Found: C. 61.26; H. 5.44.

3.4. 6-(2-Oxo-tetrahydrofuran-5-yl)-2,3-dihydro-1,4-benzodioxine (4)

$NaBH_4$ (128 mg, 3.4 mmol) was added dropwise to a solution of the carboxylic acid **3** (200 mg, 0.85 mmol) in methanol (30 mL) under an argon atmosphere. The mixture was stirred at room temperature for 4 h. Methanol was removed and the residue was partitioned between CH_2Cl_2 and H_2O. The two layers were separated and the aqueous phase was extracted with dichloromethane after acidification with 5 N HCl. The organic layer was dried over Na_2SO_4, filtered off and evaporated to dryness. The purification on silica gel column chromatography (hexane/ethyl acetate 70:30 as eluent) of the crude product gives the lactone (99% yield) as a colourless oil. IR (NaCl) ν (cm^{-1}): 1778 (C=O); 1177 (C–O–); 1069 (C–O). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.15 (m, 1H, CH–); 2.63 (m, 3H, CH_2–CH–); 4.26 (s, 4H, CH_2–O–); 5.41 (m, 1H, CH–O); 6.86 (m, 3H, Ar). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 29.0 (CH_2, C-4′); 30.7 (CH_2, C-3′); 64.2 (CH_2, CH_2–O–); 81.1 (CH, C-5′); 114.6 (CH, C-5); 117.4* (CH, C-7); 118.5* (CH, C-8); 132.3 (C, C-6); 143.6 and 143.8 (C, C4a, C8a); 177.0 (C, C=O). *Interchangeable. MS (EI) *m*/*z* (relative intensity): 220 (M^+, 38).

3.5. 4-(2,3-Dihydro-1,4-benzodioxin-6-yl)butyric acid (5)

A mixture of Zn (1.8 g, 27.5 mmol), Hg_2Cl_2 (0.18 g, 0.66 mmol) and HCl concd (0.1 mL) in water (3 mL) was heated under reflux for 5 min. Then, the mixture was filtered and the solid residue was treated with H_2O (1 mL), HCl concd (2.6 mL), toluene (1.5 mL) and the ketoacid **3** (620 mg, 2.63 mmol). The reaction mixture was heated under reflux for 5 h. Then, the mixture was diluted with water (20 mL) and extracted with ether (3× 20 mL). The combined organic layers were washed with water (20 mL) and dried (Na_2SO_4). The drying agent was filtered off and the filtrate was evaporated to dryness to give yellow oil. The oil was purified by column chromatography on silica gel using a stepwise gradient starting with pure hexane up to 50% ethyl acetate to give a colourless oil (90%, yield). IR (NaCl) ν (cm^{-1}): 3200 (O–H); 1707 (C=O); 1287 (Ar–O); 1069 (C–O–). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 1.94 (q, $J = 7$ Hz, 2H, –CH_2–); 2.35 (t, $J = 7$ Hz, 2H, CH_2–); 2.59 (t, $J = 7$ Hz, 2H, –CH_2–); 4.22 (s, 4H, CH_2–O–); 6.86 (m, 2H, Ar); 6.80 (d, $J = 8$ Hz, 1H, H-8). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 26.2 (CH_2, CH_2–β); 33.18 (CH_2, CH_2–α); 34.11 (CH_2, CH_2–γ); 64.28 (CH_2, CH_2–O–); 116.9 (CH, C-5, C-7); 121.3 (CH, C-8); 134.4 (C, C-6); 141.7 (C, C-8a); 143.2 (C, C-4a); 180.0 (C, COOH). MS (EI), *m*/*z* (%): 222 (M^+, 21); 163 (89); 149 (52); 91 (100). Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.36. Found: C. 64.67; H. 6.53.

3.6. (2,3,6,7,8,9-Hexahydrobenzo[*g*][1,4]benzodioxin-6-yl)-ketoxime (6)

To a stirred solution of the ketone **22** (200 mg, 0.98 mmol) in 20 mL of dry pyridine was added 136 mg (1.96 mmol) of NH_2OH–HCl. The reaction mixture was heated under reflux and stirred for 3 h. After, the mixture was cooled and the solvent removed. The obtained residue was diluted with water and extracted with ether (3× 30 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness affording a crude product, which was purified by column chromatography (silica gel, hexane/ethyl acetate 70:30) to give the oxime as a white solid (199 mg, 0.091 mmol, 93% yield), mp: 188–190 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 3201 (O–H); 1573 (C=N); 1499 (C=C); 1036 (C–O). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 1.83 (q, $J = 6.4$ Hz, 2H, CH_2–); 2.65 (t, $J = 6.6$ Hz, 2H, CH_2–);

2.76 (t, J = 6.6 Hz, 2H, CH_2–); 4.25 (s, 4H, H-2 and H-3); 6.64 (s, 1H, H-10); 7.42 (s, 1H, H-5); 8.59 (s, 1H, OH, changeable with D_2O). ^{13}C NMR ($CDCl_3$+$(CD_3)_2CO$, 50.3 MHz) δ (ppm): 21.6 (CH_2, C-8); 23.6 (CH_2, C-9); 29.1 (CH2, C-7); 64.2 (CH_2, CH_2–O–); 64.6 (CH_2, CH_2–O–); 112.5 (CH, C-5); 116.3 (CH, C-10); 123.9 (C, C-5a); 133.6 (C, C-9a); 142.0 (C, C-4a); 144.4 (C, C-10a); 154.6 (C, C-6). Anal. Calcd for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39. Found: C. 63.45; H. 6.16; N, 6.61.

3.7. (6-Oxo-2,3,6,7,8,9-hexahydrobenzo[*g*][1,4]benzodioxin-7-yl) carboxylic acid (7)

To a cooled solution (−78 °C) of the ketone **22** (500 mg, 2.45 mmol) in anhydrous THF (3 mL) under an argon atmosphere, a 2 M solution of LDA in hexane (2.5 mL, 5 mmol) was added dropwise with stirring over a 15-min period. The mixture was slowly stirred at −78 °C for 3 h, and ethyl chloroformate (0.7 mL, 7.35 mmol) was slowly added with stirring. The solution was allowed to warm to room temperature (20 °C) overnight and then was neutralized with saturated aqueous ammonium chloride solution (5 mL). The THF layer was separated and the aqueous alkaline phase was acidified with 2 N HCl, and the crude of reaction was extracted with ether (3× 20 mL). The extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give brown oil, which was purified by silica gel chromatography using hexane/ethyl acetate (80:20) as eluent. The ester obtained was treated with 8% NaOH (20 mL) and stirred at room temperature for 24 h. Next, the mixture was acidified with 5 N HCl and extracted with CH_2Cl_2 (3× 30 mL). The extracts were dried (Na_2SO_4), filtered and evaporated under reduced pressure to give a white solid, which was purified by column chromatography (ethyl acetate) to give **7** (177 mg, 0.71 mmol, 83% yield) as a white solid, mp: 184–186 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 3300 (O–H); 1719 (C=O); 1698 (C=O); 1506 (C=C); 1299 (Ar–O); 1065 (C–O). ^1H NMR (CD_3OD, 200 MHz) δ (ppm): 2.00 (dt, J = 6 Hz, 2H, CH_2–); 2.93 (t, J = 6 Hz, 2H, CH_2–); 3.28 (t, J = 6 Hz, 1H, CH_2–); 4.24 and 4.25 (s, 4H, H-2 and H-3); 6.70 (s, 1H, H-10); 7.45 (s, 1H, H-5). ^{13}C NMR (CD_3OD, 50.3 MHz) δ (ppm): 33.3 (CH_2, C-8); 34.1 (CH_2, C-9); 54.2 (CH2, C-7); 66.6 (CH_2, CH_2–O–); 67.5 (CH_2, CH_2–O–); 121.9 (CH, C-5); 123.1 (CH, C-10); 124.6 (C, C-5a); 140.0 (C, C-9a); 144.3 (C, C-4a); 149.7 (C, C-10a); 172.3 (C, COOH). Anal. Calcd for $C_{13}H_{12}O_5$: C, 62.90; H, 4.87. Found: C. 63.18; H. 5.15.

3.8. 2,3-Dihydrobenzo[*g*][1,4]benzodioxin-6-yl carboxylic acid (8)

A solution of the nitrile **24** (150 mg, 0.71 mmol) in 50 mL of 2 N NaOH was heated under reflux for 16 h. The solution was cooled (at 0 °C) and acidified with 2 N HCl, and the mixture was extracted with ether (3× 20 mL). The organic layer was dried (Na_2SO_4), filtered off and evaporated to dryness. The residue was purified by column chromatography on silica gel using hexane/ethyl acetate 60:40 as eluent to give the desired carboxylic acid as a yellow solid (86% yield), mp: 230–232 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 3477 (COO–H); 1668 (C=O); 1468 (C=C); 1288 (Ar–O); 1072 (C–O). ^1H NMR ($(CD_3)_2CO$, 200 MHz) δ (ppm): 4.39 (s, 4H, CH_2–O–); 7.40 (t, J = 8.4 Hz, 1H, H-8); 7.41 (s, 1H, H-10); 7.93 (d, J = 8.4 Hz, 1H, H-9); 8.20 (d, J = 8.4 Hz, 1H, H-7); 8.60 (s, 1H, H-5). ^{13}C NMR ($(CD_3)_2CO$, 50.3 MHz) δ (ppm): 61.9 and 61.0 (CH_2, CH_2–O–); 108.9 (CH, C-10); 113.3 (CH, C-5); 121.4 (C, C-9a); 122.2 (CH, C-8); 125.2 (C, C-5a); 126.6 (CH, C-7); 127.9 (C, C-6); 129.4 (CH, C-9); 141.8 (C, C-4a); 143.2 (C, C-10a); 165.5 (C, COOH). Anal. Calcd for $C_{13}H_{10}O_4$: C, 67.82; H, 4.38. Found: C. 67.94; H. 4.56.

3.9. 2,3,6,7,8,9-Hexahydrobenzo[*g*][1,4]benzodioxin-6-yl carboxylic acid (9)

A solution of the nitrile **25** (200 mg, 0.928 mmol) in 2 N NaOH (40 mL) was heated at reflux temperature for 14 h. The solution was cooled to 25 °C, acidified with 5 N hydrochloric acid and extracted with ether (80 mL). The organic phase was dried, filtered off and evaporated under reduced pressure to give a reddish solid which was purified by silica gel column chromatography, eluting with hexane/ethyl acetate 30:70. The acid **9** was obtained as a white solid (163 mg, 0.69 mmol) in 75% yield, mp 137–139 °C (hexane /ethyl acetate). IR (KBr) ν (cm^{-1}): 3290 (O–H); 1704 (C=O); 1507 (C=C); 1251 (Ar–O); 1065 (C–O). ^1H NMR ($(CD_3)_2CO$, 200 MHz) δ (ppm): 1.61 (m, 2H, CH_2–); 1.71 (m, 2H, CH_2–); 1.82 (m, 2H, CH_2–); 3.48 (t, J = 6 Hz, CH–); 6.40 (s, 1H, Ar); 6.58 (s, 1H, Ar). ^{13}C NMR ($(CD_3)_2CO$, 50.3 MHz) δ (ppm): 18.1 (CH_2, C-8); 24.0 (CH_2, C-7); 26.5 (CH_2, C-9); 41.1 (CH, C-6); 61.7 and 61.8 (CH_2, CH_2–O–); 114.1 (C, C-5); 114.7 (CH, C-10); 123.7 (C, C-5a); 127.3 (C, C-9a); 139.1 (C, C-4a and C-10a); 172.7 (C, C=O). Anal. Calcd for $C_{13}H_{13}O_4$: C, 66.66; H, 6.02. Found: C. 66.47; H. 6.21.

3.10. 2-(2,3-Dihydro-1,4-benzodioxin-2-yl) acetic acid (10)

A solution of the nitrile **27** (400 mg, 2.3 mmol) in 2N NaOH (50 mL) was heated under reflux temperature for 12 h. The solution was cooled to 25 °C, was acidified with 5 N hydrochloric acid and extracted with ether (3× 20 mL). The organic phase was dried, filtered off and evaporated under reduced pressure to give reddish oil. Treatment of this oil with hexane/ethyl acetate gave the white solid **10** (240 mg, 1.24 mmol) in 54%, mp: 89–91 °C. IR (KBr) ν (cm^{-1}): 3358 (COO–H); 1705 (C=O); 1203 (Ar–O); 1073 (C–O). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2,71 (dd, J_1 = 16.0, J_2 = 6 Hz, 1H, H-2); 2.85 (dd, J_1 = 16.0, J_2 = 6.8 Hz, 1H, H-2); 4.02 (dd, J_1 = 11.3, J_2 = 6.6 Hz, 1H, H – $3'_{ax}$); 4.32 (dd, J_1 = 11.3, J_2 = 2.2 Hz, 1H, H – $3'_{ec}$); 4.62 (m, 1H, H-2′); 6.87 (m, 4H, Ar and COOH). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 35.9 (CH_2, C-2); 66.8 (CH_2, C-3′); 69.1 (CH, CH-2′); 117.2 (CH, C-5′); 117.5 (CH, C-8′); 121.6 (CH, C-6′) y 121.8 (CH, C-7′); 142.7 (C, C4′a); 142.8 (C, C-8′a); 175.9 (C, C_1–COOH). Anal. Calcd

for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 61.72; H, 5.38.

3.11. 2-(1,4-Benzodioxin-2-yl) acetic acid (11)

A 1.6 M solution of butyl lithium in hexane (1.76 mL, 2.90 mmol) under argon atmosphere was added to a solution of 2-bromo-1,4-benzodioxine (500 mg, 2.35 mmol) in anhydrous THF (4 mL) cooled to −78 °C. The mixture was stirred for 1 h at −78 °C. Then a solution of 2-bromoacetic acid (486 mg, 3.52 mmol) in THF (2 mL) was added at −78 °C and stirred for another half hour. The resulting mixture was allowed to warm slowly to room temperature. The reaction was quenched with saturated NH_4Cl solution (2 mL) and the obtained carboxylic acid was extracted with ether (3× 20 ml). The aqueous layer was acidified with 2 N HCl (5 mL) and extracted with CH_2Cl_2 (3× 20 mL). The last organic layer was dried (Na_2SO_4), filtered off and evaporated to dryness. A purification on silica gel column chromatography (hexane/ethyl acetate 5:95 as eluent) gave the 2-(1,4-benzodioxin-2-yl) acetic acid as a white solid (38%, yield), mp: 129–130 °C (hexane/ethyl acetate). ^{1}H NMR (CD_3COCD_3, 200 MHz) δ (ppm): 2.94 (s, 2H, CH_2–); 5.98 (s, 1H, H-3); 6,57 (m, 2H, H-5, H-8); 6,73 (m, 2H, H-6 and H-7). ^{13}C NMR (CD_3COCD_3, 50.3 MHz) δ (ppm): 35.5 (CH_2, CH_2–COOH); 117.1* (CH, C-5); 117.3* (CH, C-8); 125.4# (CH, C-3); 125.5# (CH, C-6); 125.6# (CH, C-7); 133.9 (C, C-2); 143.2 and 143.8 (C, C-4a and C-8a); 170.8 (C, C (COOH)). MS (EI), *m*/*z* (%): 192 (M, 3); 147 (M-45, 38); 69 (M-123, 100). Anal. Calcd for $C_{10}H_8O_4$: C, 62.50; H, 4.20. Found: C, 62.46; H, 4.34. * and# Interchangeables.

3.12. (2,3-Dihydro-1,4-benzodioxin-6-yl) carboxylic acid (12)

To a solution of aldehyde **29** (1 g, 6.1 mmol) in CH_2Cl_2 (25 mL) were added under hard stirring CrO_3 (800 mg, 8 mmol) and *t*-BuOOH (721 mg, 8 mmol), and the resulting mixture was stirred at room temperature for 24 h. The crude residue was treated with concentrated NH_4OH. The aqueous phase was separated, treated with 2 N HCl and extracted with ether (3× 20 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness to give the carboxylic acid **12** as a colourless oil (920 mg, 5.1 mmol, 84% yield). IR (KBr) ν (cm^{-1}): 3303 (COO–H); 1712 (C=O); 1235 (Ar–O). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 4.30 (m, 4H, CH_2–O); 6.91 (d, J = 9.2 Hz, 1H, H-8); 7.63 (m, 2H, H-5, H-7). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 64.0 (CH_2, CH_2–O); 64.6 (CH_2, CH_2–O); 117.2 (CH, C-5); 119.6 (CH, C-7); 122.4 (CH, C-6); 124.1 (CH, C-8); 143.1 (C, C-4a); 148.4 (C, C-8a); 171.3 (C, CO).

3.13. Hydrolysis of the thioacetamides: general procedure

A mixture of the intermediate thioamide (0.30 mmol) in 60 mL of a mixture (1:1) of aqueous 8% NaOH solution and methanol was stirred under reflux for 4 h. The cooled mixture was acidified with diluted HCl solution and the solvent was removed under vacuum. The residue obtained was diluted with ice-water and the solution was extracted with ether (3× 30 mL). The organic layers were dried (Na_2SO_4), filtered off and concentrated under reduced pressure to give the corresponding arylacetic acid.

3.14. 2-[*N*-(2,3-Dihydro-1,4-benzodioxin-6-yl)pyrrol-2-yl]-2-oxoacetic acid (13). 2-[*N*-(2-Fluorophenyl) pyrrol-2-yl]-2-oxoacetic acid (18)

The carboxylic acids **13** and **18** were obtained by hydrolysis of the corresponding ketothioamide, by-product formed on the Willgerodt reaction from the methylketone **32** and **36**, respectively. From the methylketone **32** and following the procedure for the hydrolysis described above was obtained **13** (130 mg, 0.47 mmol, 94% yield) as a white solid, mp: 162–164 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 3245 (OH); 1769 (C=O); 1645 (C=O); 1345 (Ar–O); 1063 (C–O). ^{1}H NMR ($(CD_3)_2CO$, 200 MHz) δ (ppm): 4.17 (s, 2H, CH_2–O); 6.26 (s, 1H, H-4 pyrrole); 6.70 (m, 3H, H-7 and H-8 pyrrole); 7.20 (m, 1H, H-3 pyrrole); 7.41 (m, 1H, H-5 pyrrole). ^{13}C NMR ($(CD_3)_2CO$, 50.3 MHz) δ (ppm): 65.6 (CH_2, CH_2–O); 111.3 (CH, C-3′ pyrrole); 111.7 (CH, C-4′ pyrrole); 118.1 (CH, C-5); 120.2 (CH, C-7); 126.7 (CH, C-8); 128.1 (C, C-2′ pyrrole); 133.2 (C, C-6); 135.8 (CH, C-5 pyrrole); 142.5 (C, C-4a); 142.9 (C, C-8a); 164.2 (C, COOH); 176.1 (C, C=O). Anal. Calcd for $C_{14}H_{11}NO_5$: C, 61.54; H, 4.06; N, 5.13. Found: C, 61.69; H, 4.36; N, 5.29.

Compound **18** was obtained by hydrolysis of the intermediate ketothioamide following the general method described as a white solid (140 mg, 0.60 mmol, 90% yield), mp: 129–131 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 2923 (OH); 1735 (C=O); 1620 (C=O); 1504 (C=C); 1047 (C–O). ^{1}H NMR ($(CD_3)_2CO$, 200 MHz) δ (ppm): 6.51 (s, 1H, H-4 pyrrole); 7.19 (m, 1H, H-5 pyrrole); 7.30 (m, 4H, Ar); 8.16 (m, 1H, H-3 pyrrole). 8.92 (br s, 1H, COOH, exchanges with D_2O). ^{13}C NMR ($(CD_3)_2CO$, 50.3 MHz) δ (ppm): 112.5 (CH, C-3 pyrrole); 117.1 (CH, J = 20 Hz, C-3′); 125.8 (CH, C-5′); 126.6 (CH, C-4 pyrrole); 129.1 (C, C-2 pyrrole); 129.6 (CH, C-5 pyrrole); 129.7 (C, C-1′); 131.3 (CH, J = 8.2 Hz, C-4′); 135.5 (CH, C-6′); 158.6 (C, J = 249 Hz, C-2′); 164.4 (C, COOH); 175.0 (C, C=O). Anal. Calcd for $C_{12}H_8NO_3$: C, 61.81; H, 3.46; N, 6.01. Found: C, 61.57; H, 3.58; N, 6.15.

3.15. 2-[*N*-(2,3-Dihydro-1,4-benzodioxin-6-yl)-pyrrol-2-yl] acetic acid (14)

Following the general procedure and starting from the corresponding thioamide (100 mg, 0.3 mmol) derivative the acetic acid **14** was obtained as a white solid (90% yield), mp: 97–99 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 3230 (OH); 1712 (C=O); 1250 (Ar–O); 1163 (C–O). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 3.60 (s, 2H, CH_2–Ar); 4.27 (s, 4H, CH_2–O–); 6.21 (m, 2H, H-3 and H-4 pyrrole); 6.74 (m, 1H, H-5 pyrrole); 6.82 (m, 3H, Ar). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 32.3 (CH_2, CH_2–Ar); 64.3 (CH_2, CH_2–O–); 108.2 (CH, C-3 pyrrole); 109.5 (CH, C-4 pyrrole); 115.2 (CH, C-5′); 117.4 (CH, C-7′); 119.5 (CH, C-8′); 122.9 (CH, C-5

pyrrole); 124.9 (C, C-2 pyrrole); 133.0 (C, C-6′); 143.1 (C, C-8′a); 143.5 (C, C-4′a); 176.6 (C, COOH). Anal. Calcd for $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40. Found: C, 64.71; H. 5.26; N, 5.57.

3.16. 2-(*N*-(2,3-Dihydro-1,4-benzodioxin-6-yl)pyrrol-3-yl) acetic acid (15)

The preparation of the acetic acid **15** (89%) was carried out from the corresponding thioamide (300 mg, 0.87 mmol) following the general procedure described above. The acid **15** was obtained as a white solid, mp: 117–119 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 3217 (OH); 1702 (C=O); 1223 (Ar–O); 1125 (C–O). ^1H NMR ($(CD_3)_2CO$, 200 MHz) δ (ppm): 3.50 (s, 2H, CH_2–Ar); 4.29 (s, 4H, CH_2–O–); 6.31 (m, 1H, H-4′ pyrrole); 6.94 (m, 4H, Ar); 7.08 (m, 1H, H-2′ pyrrole). ^{13}C NMR ($(CD_3)_2CO$, 50.3 MHz) δ (ppm): 32.7 (CH_2, CH_2–Ar); 65.7 (CH_2, CH_2–O–); 65.9 (CH_2, CH_2–O); 110.3 (CH, C-4′ pyrrole); 112.5 (CH, C-5); 114.1 (CH, C-8); 116.5 (C, C-3′ pyrrole); 119.1* (CH, C-2′ pyrrole); 119.4* (CH, C-5′ pyrrole); 120.4 (CH, C-7); 136.5 (C, C-6); 143.0 (C, C-4a); 145.6 (C, C-8a); 173.9 (C, COOH). Anal. Calcd for $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40. Found: C, 64.73; H, 5.18; N, 5.59.

3.17. 2-(*N*-(2-Fluorophenyl)pyrrol-2-yl) acetic acid (16)

Taking as starting material the thioamide (200 mg, 0.65 mmol) and following the general procedure the carboxylic acid **16** was obtained as a white solid with 95% yield, mp: 92–94 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 2920 (OH); 1708 (C=O); 1311 (Ar–N); 1170 (C–O); 763 (C–F). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 3.54 (s, 2H, CH_2–Ar); 6.28 (m, 2H, H-3 and H-4 pyrrole); 6.75 (m, 1H, H-5 pyrrole); 7.23 (m, 4H, H-3′, H-4′, H-5′, H-6′). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 32.0 (CH_2, CH_2–Ar); 108.9 (CH, C-3 pyrrole); 109.9 (CH, C-4 pyrrole); 116.5 (CH, J = 20, C-3′); 123.2 (CH, C-6′); 124.6 (CH, C-5′); 124.8 (C, C-2 pyrrole); 127.1 (C, J = 12,8 Hz, C-1′); 129.4 (CH, C-5 pyrrole); 129.7 (CH, J = 7.2 Hz, C-4′); 157.3 (C, J = 250.8 Hz, C-2); 176.6 (C, COOH). Anal. Calcd for $C_{12}H_{10}FNO_2$: C, 65.75; H, 4.60; N, 6.39. Found: C, 65.89; H, 4.71; N, 6.63.

3.18. 2-(*N*-(2-Fluorophenyl)pyrrol-3-yl) acetic acid (17)

The preparation of the acetic acid **17** was carried out from the corresponding thioamide (200 mg, 1.11 mmol) following the general procedure for the hydrolysis of thiocarboxamides. The carboxylic acid was obtained as a white solid, 96% yield, mp: 103–105 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 2965 (OH); 1712 (C=O); 1291 (Ar–N); 1143 (C–O); 776 (C–F). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 3.60 (s, 2H, CH_2–Ar); 6.31 (t, J = 2.2 Hz, 1H, H-4 pyrrole); 6.97 (t, J = 2.2 Hz, 2H, H-2 and H-5H pyrrole); 7.17 (m, 3H, Ar); 7.28 (m, 1H, Ar). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 32.8 (CH_2, CH_2–Ar); 110.9 (CH, C-4 pyrrole); 116.4 (C, C-3 pyrrole); 116,9 (CH, J = 20.6 Hz, C-3′); 120.1 (CH, C-6′); 121.5 (CH, C-5′); 124.6 (CH, C-2 and C-5 pyrrole); 127.0 (CH, J = 7,8 Hz, C-4′); 127.2 (C, C-1′); 154.1 (C, J = 250 Hz, C-2′); 176.9 (C, COOH). Anal. Calcd for $C_{12}H_{10}FNO_2$: C, 65.75; H, 4.60; N, 6.39. Found: C, 65.56; H, 4.77; N, 6.67.

3.19. 6-Oxo-2,3,6,7,8,9-hexahydrobenzo[*g*][1,4]benzodioxine (22)

To a stirring solution of the butyric acid **5** (2.5 g, 11.25 mmol) in dry CH_2Cl_2 (30 mL) cooled to 0 °C was added $(CF_3CO)_2O$ (2 mL). The mixture was stirred at room temperature for 6 h. The crude of reaction was diluted with water and extracted with CH_2Cl_2. The organic layers were washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. A purification on silica gel column chromatography (hexane/ethyl acetate 90:10 as eluent) gave the ketone **22** (87%, yield), mp: 105–106 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 1762 (C=O); 1294 (Ar–O); 1064 (C–O–). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.08 (qt, J = 6.8 Hz, 2H, C-8H_2); 2.55 (t, J = 6.8 Hz, 2H, C-7H_2); 2.84 (t, J = 6.8 Hz, 2H, C-9H_2); 4.27 (m, 4H, CH_2–O–); 6.70 (s, 1H, H-10); 7.55 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 23.5 (CH_2, C-9); 29.0 (CH_2, C-8); 38.7 (CH_2, C-7); 63.9 (CH_2, CH_2–O); 64.7 (CH_2, CH_2–O); 115.7 (CH, C-5); 116.3 (CH, C-10); 126.6 (C, C-5a); 138.7 (C, C-9a); 142.3 (C, C-4a); 148.1 (C, C-10a); 196.9 (C, CO). MS (EI) *m*/*z* (relative intensity): 204 (M^+, 68).

3.20. 6-Cyano-2,3,8,9-tetrahydrobenzo[*g*][1,4]benzodioxine (23)

Under an argon atmosphere, a solution of the ketone **22** (400 mg, 1.95 mmol) in toluene (10 mL) was cooled (−15 °C) and a 1 M solution of $(Et)_2AlCN$ in toluene was dropwise added. The resulting mixture was stirred at −15 °C for 2 h. Then, a mixture of MeOH/HCl concd (10 mL/5 mL) was added and the mixture was stirred at room temperature for 1 h. The crude product was extracted with CH_2Cl_2 (3× 20 mL). The organic layer was dried over Na_2SO_4, filtered off and evaporated to dryness. The residue obtained is a mixture of the desired compound **23** and starting material.

A mixture of the crude product (400 mg) and $KHSO_4$ (200 mg) was heated at 100 °C for 1 h. The residue was washed with water and extracted with CH_2Cl_2 (3× 20 mL). The organic layer was dried over Na_2SO_4, filtered off and the solvent evaporated to dryness. The brown oil obtained was purified by column chromatography (hexane/ethyl acetate 85:15) to give **23** as yellow oil (50%, yield), mp: 94–96 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 2219 (CN); 1503 (C=C); 1287 (Ar–O); 1066 (C–O). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.44 (m, 2H, CH_2); 2.72 (m, 2H, CH_2); 4.23 (m, 4H, CH_2–O–); 6.64 (s, 1H, H-10); 6.75 (t, J = 5 Hz, 1H, H-7); 6.97 (s, 1H, H-5). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 23.9 (CH_2, C-8); 25.6 (CH_2, C-9); 64.3 y 64.6 (CH_2, CH_2–O–); 113.6 (C, C-6); 113.9 (CH, C-5); 116.8 (CH, C-10); 117.2 (C, CN); 122.2 (C, C-5a); 127.5 (C, C-9a); 141.8 (CH, C-7); 142.2 (C, C-4a); 143.9 (C, C-10a). MS (EI) *m*/*z* (relative intensity): 213 (M^+, 42).

3.21. 6-Cyano-2,3-dihydrobenzo[*g*][1,4]benzodioxine (24)

A solution of the ketone **22** (400 mg, 1.95 mmol) in toluene (10 mL) was cooled (−15 °C) and a 1 M solution of $(Et)_2AlCN$ in toluene was added. The resulting mixture was stirred at −15 °C for 2 h. After hydrolysis with MeOH/HCl concd (10 mL/5 mL), the extracted nitrile was stirred at room temperature for 1 h. The obtained residue was extracted with CH_2Cl_2 (3× 20 mL). The organic layers were dried (Na_2SO_4), filtered and evaporated to dryness. The crude product is a mixture of the desired compound and starting compound. Then, $KHSO_4$ (200 mg) was added to the crude product, and the resulting mixture was heated at 170 °C for 5 h. The mixture was washed with water and extracted with CH_2Cl_2 (3× 20 mL). The organic layers were dried over Na_2SO_4, filtered off and evaporated to dryness. The crude product was subjected to silica gel column chromatography (hexane/ethyl acetate (80:20) as an eluent). Thus **24** was obtained as a white solid (48% yield), mp: 157–159 °C (hexane/ethyl acetate). ^1H NMR ($(CD_3)_2CO$, 200 MHz) δ (ppm): 4.39 (m, 4H, CH_2–O–); 7.36 (m, 2H, C-8H, H-10); 7.65 (s, 1H, H-5); 7.73 (d, J = 8.4 Hz, 1H, H-9); 7.86 (d, J = 8.4 Hz, 1H, H-7). MS (EI) m/z (relative intensity): 211 (M^+, 12).

3.22. 6-Cyano-2,3,6,7,8,9-hexahydrobenzo[*g*][1,4]benzodioxine (25)

$NaBH_4$ (227 mg, 6 mmol) was added slowly to a solution of the nitrile derivative **23** (300 mg, 1.40 mmol) in methanol (30 mL), under an argon atmosphere. The resulting mixture was stirred at room temperature for 24 h. The solvent was removed, and the residue was partitioned between CH_2Cl_2 and H_2O. The two layers were separated and the aqueous layer was extracted with dichloromethane. The organic layer was dried and the solvent removed. The crude product was purified by column chromatography (silica gel, hexane/ethyl acetate 50:50 as eluent) to give **25** (89% yield) as a white solid, mp: 96–97 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 2223 (CN); 1504 (C=C); 1295 (Ar–O); 1067 (C–O). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 1.60 (m, 2H, CH_2–); 2.10 (m, 2H, CH_2–); 2.69 (m, 2H, CH_2–); 3.67 (t, J = 6 Hz, CH–); 4.05 (s, 4H, CH_2–O); 6.60 (s, 1H, Ar); 6.85 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 20.9 (CH_2, C-8); 27.4 (CH_2, C-7); 27.8 (CH_2, C-9); 30.2 (CH, C-6); 64.3 and 64.6 (CH_2, CH_2–O–); 116.9 (C, C-5); 117.5 (CH, C-10); 121.8 (C, CN); 122.4 (C,C-5a); 129.3 (C, C-9a); 142.1 (C, C-4a); 143.3 (C, C-10a).

3.23. Preparation of methylketones: general procedure

To a cooled solution (0 °C) of the corresponding heterocyclic aromatic system (5 mmol) in dried CH_2Cl_2 (30 mL), acetic anhydride (1.4 mL, 7.5 mmol) and $BF_3.(CH_3CH_2)_2O$ (2 mL) were successively added and the reddish solution obtained was stirred at room temperature for 6 h. The resulting mixture was poured into ice (20 mL) and washed with water. The combined extracts were dried over Na_2SO_4, filtered off and evaporated under reduced pressure to give the corresponding ketone. The crude product was purified by silica gel column chromatography using hexane/ethyl acetate as eluent.

3.24. Preparation of the 6-(2-acetylpyrrol-1-yl)-2,3-dihydro-1,4-benzodioxine (32) and 6-(3-acetylpyrrol-1-yl)-2,3-dihydro-1,4-benzodioxine (33)

Starting from 6-(pyrrol-1-yl)-2,3-dihydro-1,4-benzodioxine **31** (1 g, 5 mmol) and following the general procedure described above for the preparation of methylketones were obtained **32** (604 mg, 2.48 mmol, yield 50%) and **33** (363 mg, 1.49 mmol, yield 30%). 6-(2-Acetylpyrrol-1-yl)-2,3-dihydro-1,4-benzodioxine (**32**), mp: 111–113 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 1662 (C=O); 1507 (C=C); 1226 (Ar–O); 1064 (C–O). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.40 (s, 3H, CH_3); 4.26 (s, 4H, CH_2–O–); 6.24 (dd, J_1 = 4, J_2 = 2.4 Hz, 1H, H-4′ pyrrole); 6.78 (m, 3H, Ar); 6.88 (m, 1H, H-3′ pyrrole); 6.90 (dd, J_1 = 2.2, J_2 = 1.4 Hz, 1H, H-5′ pyrrole). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 64.3 (CH_2, CH_2–O); 108.9 (CH, C-4′ pyrrole); 115.2 (CH, C-7); 116.9 (CH, C-5); 119.2 (CH. C-8); 120.2 (CH, C-3′ pyrrole); 131.2 (CH, C-5′ pyrrole); 131.6 (C, C-2′ pyrrole); 134.3 (C, C-6); 143,1 (C, C-4a, C-8a); 186,9 (C, C=O). Anal. Calcd for $C_{14}H_{13}NO_3$: C, 69.12; H, 5.39; N, 5.76. Found: C. 69.42; H. 5.76; N, 5.97. 6-(3-Acetylpyrrol-1-yl)-2,3-dihydro-1,4-benzodioxine (**33**). IR (NaCl) ν (cm^{-1}): 1659 (C=O); 1503 (C=C); 1231 (Ar–O); 1045 (C–O). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.43 (s, 3H, CH_3); 4.27 (s, 4H, CH_2–O); 6.70 (m, 1H, H-4′ pyrrole); 6.90 (m, 4H, H-5′ pyrrole, H-5, H-7, H-8); 7.54 (t, J = 2.2 Hz, 1H, H-2′ pyrrole). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 64.3 (CH_2, CH_2–O); 64.5 (CH_2, CH_2–O); 110.3* (CH, C-4′ pyrrole); 110.6* (CH, C-5′ pyrrole); 114.2 (CH, C-8); 117.9 (CH, C-5); 121.5 (CH, C-7); 124.3 (CH, C-2′ pyrrole); 127.2 (C, C-3′ pyrrole); 133.6 (C, C-6); 142.6 (C, C-4a); 143.9 (C, C-8a); 193.4 (C, C=O). * Interchangeable. Anal. Calcd for $C_{14}H_{13}NO_3$: C, 69.12; H, 5.39; N, 5.76. Found: C. 69.49; H. 5.12; N, 5.38.

3.25. Preparation of the *N*-(2-fluorophenyl)-2-acetylpyrrole (36) and *N*-(2-fluorophenyl)-3-acetylpyrrole (37)

Starting from the *N*-(2-fluorophenyl)pyrrole **35** (806 mg, 5 mmol) and following the general procedure described above, the 2-substituted pyrrole **36** 559 mg (55%) and the 3-substituted pyrrole **37** 305 mg (30%) were obtained. *N*-(2-Fluorophenyl-2-acetylpyrrole) (**36**), mp: 118–120 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 1653 (C=O); 1505 (C=C); 1214 (Ar–O); 1024 (C–O). ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.43 (s, 3H, CH_3); 6.35 (m, 1H, H-4 pyrrole); 6.93 (m, 1H, H-5 pyrrole); 7.08 (m, 1H, H-3 pyrrole); 7.22 (m, 4H, H-3′, H-4′, H-5′, H-6′). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 26.9 (CH_3, CH_3–); 109.7 (CH, C-3); 115.8 (CH, J = 22 Hz, C-3′); 119,8 (CH, C-4); 124.1 (CH, J = 8 Hz, C-4′); 127.8 (CH, C-5); 129.0 (C, C-1′); 129.2 (CH, J = 8 Hz, C-6′); 130.6 (CH, C-5′); 130.1 (C, C-2); 157,0 (C, J = 250 Hz, C-2′); 187,2 (C, C=O). Anal. Calcd for $C_{12}H_{10}FNO$: C, 70.93; H, 4.97; N, 6.89. Found: C. 70.65; H. 5.23; N, 6.67. *N*-(2-Fluorophenyl)-3-acetylpyrrole (**37**). IR (KBr) ν (cm^{-1}): 1661 (C=O); 1512 (C=C); 1207

(Ar–O); 1035 (C–O). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 2.47 (s, 3H, CH_3); 6.73 (m, 1H, H-5 pyrrole); 7.02 (m, 1H, H-4 pyrrole); 7.25 (m, 4H, H-3′, H-4′, H-5′, H-6′); 7.61 (m, 1H, H-2 pyrrole). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ (ppm): 27.3 (CH_3, CH_3–); 110.1 (CH, C-4); 117.4 (CH, J = 20 Hz, C-3′); 123.2 (CH, C-2); 125.2 (CH, C-5); 126.3 (CH, C-5′); 127.2 (C, C-3); 127.3 (C, J = 8 Hz, C-4′); 128.7 (CH, J = 8 Hz, C-6′); 130.0 (CH, C-1′); 155,2 (C, J = 250 Hz, C-2′); 163.5 (C, C=O). Anal. Calcd for $C_{12}H_{10}FNO$: C, 70.93; H, 4.97; N, 6.89. Found: C. 71.23; H. 4.76; N, 6.59.

3.26. Preparation of arylacetic acids from the corresponding acetyl derivatives. Willgerodt–Kindler reaction

A mixture of the acetyl compound (1 mmol), morpholine (2.5 mmol) and sulfur (S_8) (0.25 mmol) was stirred at 140 °C for 12 h. The reaction mixture was poured into ice and extracted with CH_2Cl_2 (3× 30 mL). The organic phase was dried, filtered off and the solvent was removed in vacuum to give brown oil which was purified by silica gel column chromatography using hexane/ethyl acetate as an eluent to afford the corresponding thioamide.

3.27. Preparation of the 2-(*N*-(2-fluorophenyl)pyrrole-2-yl)thioacetomorpholide (38) and 2-(*N*-(2-fluorophenyl)pyrrol-2-yl)-2-oxo-thioacetomorpholide (39)

Following the general procedure described above, starting from 2-acetyl pyrrole derivative **36** (800 mg, 3.94 mmol), morpholine (0.86 mL, 9.85 mmol) and sulfur S_8 (252 mg, 0.95 mmol), the thioamide **38** was obtained as a brown oil (345 mg, 1.14 mmol, 33% yield) and the ketothioamide **39** as a brown solid (395 mg, 1.24 mmol, 36% yield). 2-(*N*-(2-Fluorophenyl) pyrrole-2-yl)thioacetomorpholide (**38**). IR (KBr) ν (cm^{-1}): 1435 (C=C); 1256 (C=S); 1251 (Ar–O); 1104 (C–O). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 3.52 (s, 4H, CH_2–, CH_{ax}–N); 3.74 (t, J = 5 Hz, 2H, CH_{eq}–N); 4.07 (s, 2H, CH_{ax}–O); 4.30 (t, J = 5 Hz, 2H, CH_{eq}–O); 6.16 (m, 1H, H-4 pyrrole); 6.26 (t, J = 4 Hz, H-3 pyrrole); 6.72 (t, J = 4 Hz, H-5 pyrrole); 7.27 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 42.7 (CH_2, CH_2–Ar); 50.0 (CH_2, CH_2–N); 66.2 (CH_2, CH_2–O–); 108.2 (CH, C-2 pyrrole); 109.1 (CH, C-4 pyrrole); 116.5 (CH, J = 20 Hz, C-3′); 122.8 (CH, C-1 pyrrole); 124.9 (CH, C-4′); 127.8 (C, C-2 pyrrole); 129.4* (CH, C-5′); 129.9* (C, C-6′); 130.2 (C, C-1′); 157.2 (C, J = 250 Hz, C-2′); 198.5 (C, C=S). Anal. Calcd for $C_{16}H_{17}FN_2OS$: C, 63.13; H, 5.63; N, 6.25. Found: C. 63.56; H. 5.98; N, 5.89. 2-(*N*-(2-Fluorophenyl)pyrrol-2-yl)-2-oxo-thioacetomorpholide (**39**), mp: 154–156 °C (hexane/ethyl acetate). IR (KBr) ν (cm^{-1}): 1633 (CO); 1504 (C=C); 1189 (C=S); 1047 (C–O). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 3.67 (s, 4H, CH_2–N); 3.84 (t, J = 5 Hz, 2H, CH_2–O_{ax}); 4.26 (t, J = 5 Hz, 2H, CH_2–O_{eq}); 6.39 (dd, J_1 = 4 Hz, J_2 = 2.4 Hz, 1H, H-4 pyrrole); 7.02 (t, J = 2 Hz, 1H, H-5 pyrrole); 7.11 (dd, J_1 = 4 Hz, J_2 = 2 Hz, H-3 pyrrole); 7.32 (m, 4H, Ar). * Interchangeable. MS (EI) *m/z* (relative intensity): 318 (M^+, 73).

3.28. Preparation of the 2-(*N*-(2-fluorophenyl)pyrrol-3-yl)thioacetomorpholide (40)

Starting from 2-acetyl pyrrole **37** (200 mg, 1.11 mmol) and following the general procedure the thioamide **40** was obtained as a brown oil (240 mg, 0.79 mmol, 71% yield). IR (NaCl) ν (cm^{-1}): 1467 (C=C); 1253 (Ar–O); 1210 (C=S); 1133 (C–O). ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 3.55 (t, J = 5.2 Hz, 2H, CH_{ax}–N); 3.75 (dd, J_1 = 5.2 Hz, J_2 = 4.6 Hz, 4H, CH_{eq}–N, CH_2–Ar); 4.23 (s, 2H, CH_{ax}–O); 4.35 (t, J = 5.2 Hz, 2H, CH_{eq}–O); 6.28 (t, J = 2 Hz, 1H, H-4 pyrrole); 6.94 (m, 2H, H-2 and H-5 pyrrole); 7.26 (m, 4H, H-3′, H-4′, H-5′, H-6′). MS (EI) *m/z* (relative intensity): 304 (M^+, 8).

3.29. Biological assay: anti-inflammatory activity

3.29.1. In vitro assay. 3α-HSD activity was determined by using 5β-dihydrocortisone as substrate and β-nicotinamide adenine dinucleotide phosphate reduced form (β-NADP(H)) as cofactor.

Reduction of 5β-dihydrocortisone was measured at 25 °C by monitoring the decrease in absorbance of β-NADP(H) at 340 nM. The reaction mixture contained 2.5 mL of distilled water, 0.3 mL of 1 M potassium phosphate buffer (pH 6.0), 60 μL of 9 mM NADP(H), 30 μL of 5 mM 5β-dihydrocortisone and 90 μL of compound solution in the tests or DMSO (used to dissolve compounds) in control experiments. Each assay was initiated by the addition of 20 μL of crude preparation of rat liver cytosol[20] and the optical density was followed for 5 min. Enzyme activity was expressed as a variation in optical density decrease per minute (OD minute) and the percentage of inhibition of 3α-HSD was calculated using the following equation:

$$\%\,\text{Inhibition} = (\text{OD min control} - \text{OD min sample}/\text{OD min control}) \times 100$$

Percent inhibition (mean values of at least four independent experiments) of compounds and ibuprofen at screening concentration of 1000 μM is reported in Table 1. None of the compounds showed absorbance at 340 nm at this concentration except for compounds **10**–**12** that were tested at 500 or 250 μM owing to their excessive absorbance at 1000 μM. Moreover the presence at same time of cofactor NADP(H) and substrate 5β-dihydrocortisone was required before the cytosol promoted a change in absorbance. The concentration of compound required to decrease the rate of 5β-dihydrocortisone reduction by 50% (IC_{50}) was calculated from the resulting dose–response curve.

3.29.2. In vivo assay. The carrageenan-induced rat paw oedema assay was carried out using a modified Winter's method as a preliminary screening test.[21] The rats (in groups of six animals weighing 160–200 g, young adult male Sprague–Dawley) were housed in a controlled environment and provided with standard rodent chow and water for 24 h before a dose of the test compounds

(70 and 100 mg/kg po) was administered. One hour later, the volume of the right hind paw was measured, and 0.05 mL of a 1% suspension of carrageenan in sterile pyrogen-free 0.9% NaCl solution was injected subcutaneously into planter aponeurosis of the hind paw. Three hours after the injection of carrageenan, the paw volume was again measured by a water pletysmometer.

The mean increase of paw volume at each time interval was compared with that of control group (six rats treated with carrageenan, but without test compounds) at the same time intervals. The percentage inhibition values were calculated according to the formula:

$$\%\,\text{Anti} - \text{inflammatory activity} = 1 - R_t/R_c \times 100 (R_t = \text{result of tested group}, R_c = \text{result of control group})$$

All experiments involving animals were carried out using protocols approved by the Animal Committee, University of Barcelona (Spain). Animal care was in compliance with *Generalitat of Catalunya* regulations on protection of animals used for experimental and other scientific purposes.

3.29.3. Cyclooxygenase inhibition studies. These compounds were tested for their ability to inhibit COX-1 and COX-2 using the human whole blood assay.[23] The assay for COX-1 and COX-2 enzyme activity was realised according to the protocol described by Young et al.[1f,23] Human blood from male or female donors who had not taken any NSAIDs for 15 days was collected in sodium heparin (20 U/mL of blood) and distributed in 1 mL aliquots, and 24 aliquots tissue culture plate. The plate was placed on a rotating platform shaker in a 5% CO_2 incubator at 37 °C for 15 min. Test compounds were dissolved in DMSO and 1 μL of each dilution of the test compound was added per well in duplicate wells. Cells were stimulated for 24 h, with *Escherichia coli* lipopolysaccharide (LPS, 10 μg/mL) to induce COX-2. The supernatant of the cells was replaced with fresh medium, cells pre-treated with test compounds, at the concentrations previously selected, for 15 min. For the stimulation of COX-1, the calcium ionophore A23187 was added to a concentration of 25 μM to separate wells 4.5 h after the addition of test compounds. After 30 min, incubations were terminated by cooling on ice and adding EGTA to a final concentration of 2 mM. The blood samples were transferred by transfer pipettes to 15 mL propylene tubes and were centrifuged for 10 min at 4 °C. Finally, the supernatants were collected and transferred to glass tubes and evaporated to dryness and reconstituted with EIA buffer and quantified. Concentrations of thromboxanes then were determined.

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References and notes

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