Antiinflammatory, Gastrosparing, and Antiplatelet Properties of New NO-Donor Esters of Aspirin

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A new series of NSAIDs in which aspirin is joined by an ester linkage to furoxan moieties, with different ability to release NO, were synthesized and tested for NO-releasing, antiinflammatory, antiaggregatory, and ulcerogenic properties. Related furazan derivatives, aspirin, its propyl ester, and its γ-nitrooxypropyl ester were taken as references. All the products described present an antiinflammatory trend, maximized in derivatives **12**, **16**, and **17**, they are devoid of acute gastrotoxicity, principally due to their ester nature, and show an antiplatelet activity primarily determined by their ability to release NO. They do not behave as aspirin prodrugs in human serum.

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used to treat the effects of inflammation through inhibition of cyclooxygenase enzymes (COX).[1] The major limitation to long term therapeutic use of these products is their gastrotoxicity; in 1997, 16500 patients with rheumatoid arthritis or osteoarthritis died from the gastrointestinal toxic effects of NSAIDs in the US.[2] Two approaches have emerged over recent years to improve the safety profile of these drugs. First, selective inhibitors of the inducible isoform of cyclooxygenase, COX-2, which plays a major role in prostaglandin biosynthesis in inflammatory cells, have been described.[3] Celecoxib **1** and Rofecoxib **2** (see compounds in Chart 1) are typical examples that were recently marketed. Second, (NO)-releasing NSAIDs are being investigated.[4] (In this paper we use NO as a family name, embracing all the NO-redox species. When necessary we specify which redox form we refer to.) The fundamental rational of this approach is that NO is able to protect the gastric mucosa by a number of mechanisms, including promotion of mucous secretion, increased mucosal blood flow, and decreased adherence of neutrophils to the gastric vascular endothelium.[5] NO−NSAIDs are still in development and have yet to reach the market. The prototype of NSAIDs is aspirin **3**. This drug is commonly used to alleviate the symptoms of inflammation and for the long-term prophylaxis against myocardial infarction and stroke, on account of its antithrombotic properties.[1] Aspirin displays gastrotoxicity as a consequence of topical injury, due to its acidic nature, and of systemic effects largely related to its ability to inhibit both COX isoforms (COX-1, COX-2).[6] Among NO-releasing NSAIDs, NO-aspirins have received particular attention[7,8] and NCX-4016 **4** has been shown to have a number of

Chart 1
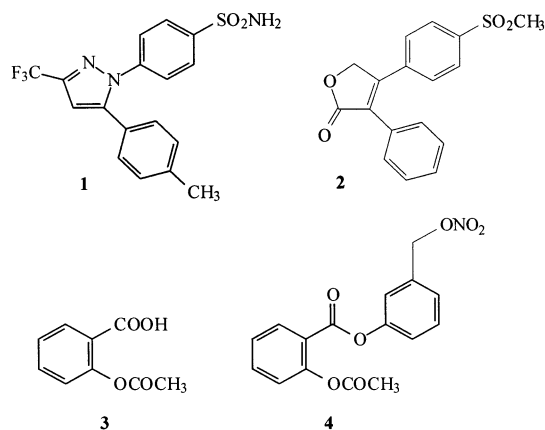
potential benefits over the parent compound.[9] The behavior of this class of drugs is complex and not fully understood.

As a development of our previous work on NO-releasing drugs,[10] we have now designed new NSAIDs (Scheme 1, derivatives **9**, **12**, **14**, **16**, **17**) in which aspirin is joined by an ester linkage to furoxan moieties, selected on the basis of their different NO-releasing properties. In this paper, we report the synthesis and antiinflammatory and antiaggregatory properties of these products, as well as their gastric ulcerogenic effects. Related furazan derivatives (Scheme 1, derivatives **9a**, **12a**, **14a**, **16a**, **17a**) were used as NO-deficient references. Aspirin **3**, its propyl ester **5**, as well as its γ-nitrooxypropyl ester **6** (Scheme 1), were also considered as references.

Chemistry

All the products considered for biological investigation were prepared by standard methods as outlined in Scheme 1. Furoxanyloxypropyl esters of aspirin **9**, **12** and furazanyloxypropyl analogues **9a**, **12a** were synthesized by treatment of the appropriate alcohol (**7**, **7a**,
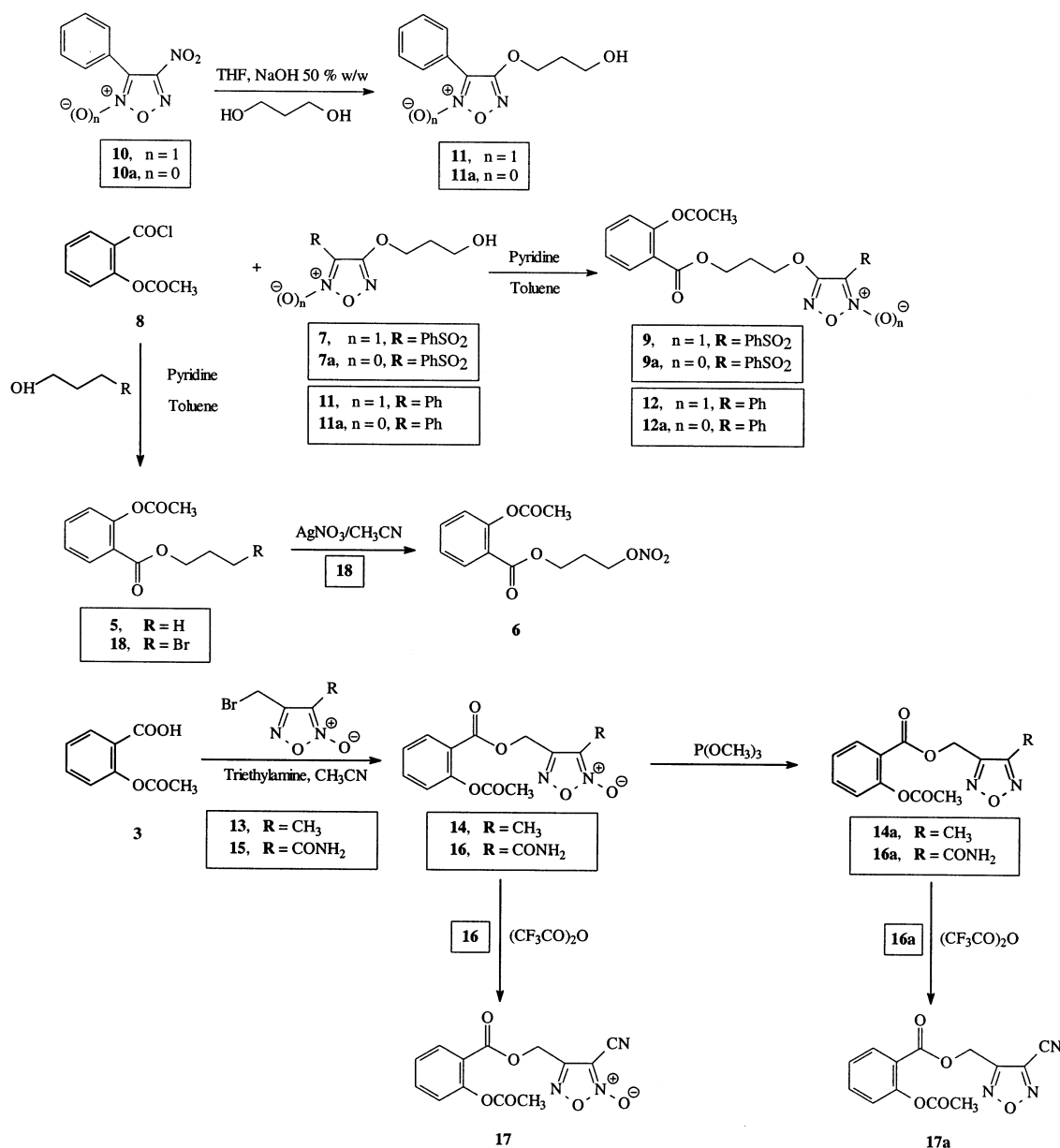
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Scheme 1

11, **11a**) with acetylsalicylic acid chloride (**8**) in toluene solution, in the presence of pyridine. Furoxanylmethyl esters **14**, **16** were prepared by reaction of **3** with the corresponding (bromomethyl)furoxans **13**, **15** in acetonitrile as solvent and in the presence of triethylamine as base. The related furazans **14a**, **16a** were obtained from **14**, **16**, respectively, by reduction with trimethyl phosphite. Cyano derivatives **17**, **17a** were obtained by trifluoroacetic anhydride dehydration of parent amides (**16**, **16a**) in THF solution, in the presence of pyridine. Finally, nitroxy derivative **6** was synthesized by action of $AgNO_3$ on 3-bromopropyl ester of aspirin (**18**) using acetonitrile as solvent.

NO-Release and Hydrolysis. NO (family name) release was evaluated by detection of nitrite (Griess reaction).[11] The use of a cosolvent was necessary because of the low water solubility of the compounds. Therefore, NO-donor compounds were dissolved in pH 7.4 buffered water/methanol mixture and incubated at 37 °C for 1 h, in the presence of 1:5 molar excess of L-cysteine. These same products were unable to generate nitrite

Table 1. NO Release Data

compound	% NO_2^- (mol/mol)[a,b] L-Cys 5×10^{-4} M
6	0.18 ± 0.05
9	37.1 ± 0.3
12	0.35 ± 0.08
14	0
16	3.8 ± 0.1
17	32.3 ± 0.8

[a] All values are mean ± SEM. [b] Determined by Griess reaction, after incubation for 1 h at 37 °C in pH 7.4 buffered water/methanol mixture, in the presence of 1:5 molar excess of L-cysteine.

under the same conditions but at pH = 1. The results, expressed as % mol/mol of NO_2^-, are presented in Table 1. Nitrite detection is only a crude measure of NO release. NO_2^- production is, in fact, strongly dependent on the medium, the concentration, and the nature of the thiol employed, and it is accompanied by other reactions between furoxan system and thiol cofactor. Nitrite detection does not give information about the single NO-redox forms involved in the release and on
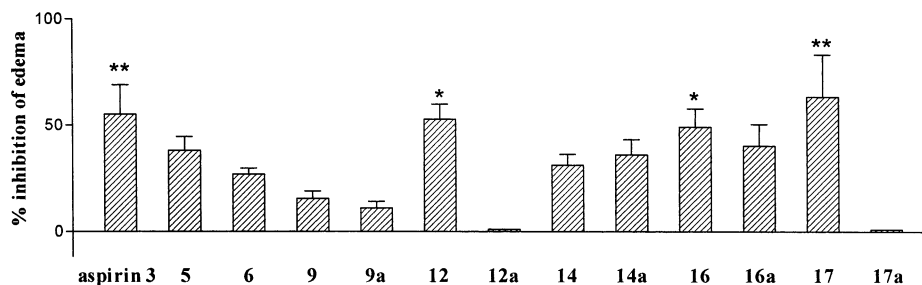
Figure 1. Antiinflammatory effects of tested compounds on carrageenan-induced paw edema in rat. The compounds were administered by intragastric route at a dose equimolar with aspirin **3**, 120 mg/kg, at the same time as carrageenan, and their effects were evaluated 3 h later. Results are expressed as % of inhibition of paw volume increase. Edema volume in vehicle-treated group was taken as 100. $*P < 0.05$; $**P < 0.01$ vs vehicle (ANOVA and Newman-Keuls test). Values are mean $\pm$ SEM ($n = 6-8$ per group).

Table 2. Amount of Unaltered Compound Detected in Human Serum after 1 min and 1 h Hydrolysis

compound	% unaltered compound[a,b] 1 min	% unaltered compound[a,b] 1 h
aspirin (**3**)[c]	95.0 ± 3.9	43.1 ± 1.9
5	0	0
6	0	0
9	43.7 ± 3.0	9.3 ± 2.2
9a	47.6 ± 5.6	8.1 ± 1.4
12	38.8 ± 5.8	3.8 ± 2.1
12a	39.3 ± 5.9	3.9 ± 2.0
14	2.5 ± 1.2	0
14a	0	0
16	0	0
16a	0	0
17	0	0
17a	0	0

[a] All values are mean ± SEM. [b] Detected by HPLC after incubation in serum at 37 °C. [c] Half-time values evaluated for aspirin in these hydrolysis conditions were in keeping with data reported in ref 25.

the eventual formation of NO_2^- directly from furoxan system. Moreover, under the adopted conditions, the different NO-redox forms eventually involved in the process can undergo a variety of reactions in addition to nitrite production.[12] Since the NO produced by endothelial cells is generally assumed to be nitric oxide (NO•), we also determined the ability of the products to afford this redox form, when dissolved in plasma. These measurements were performed in fresh human plasma for compounds **9** and **17** by an electrochemical method, using a Clark type electrode.[13] NO signals obtained with derivatives **9** and **17** were integrated and the area under the curve reported in Figure 3.

HPLC detection was used to determine the amount of unaltered compound remaining after incubation in human serum for 1 min and 1 h (Table 2). Over this period, no production of aspirin was observed. All the products were largely unaltered after 1 h incubation in mixtures of acetonitrile and HCl.

Since NO-releasing aspirins do not produce nitrite at low pH and they are scarcely hydrolyzed in acidic conditions, it is unlikely that NO release occurs in the gastric lumen. It is more likely to happen following systemic absorption.

Pharmacological Results and Discussion

Antiinflammatory Activity. All the compounds were tested for their antiinflammatory activity in the carrageenan rat paw edema assay. The injection of carrageenan into the hind paw produced a marked increase in paw volume within 1 h. Aspirin **3**, administrated by intragastric route at a dose of 120 mg/kg at the same time as carrageenan, significantly reduced (55.17% ± 13.88) paw edema at 3 h. Analysis of Figure 1 shows a trend toward antiinflammatory action for all of the compounds tested, at a dose equimolar with 120 mg/kg of aspirin. In particular, furoxan derivatives **12**, **16**, **17** displayed significant antiinflammatory properties, comparable to that of aspirin. This activity cannot reside in their behavior as prodrugs of **3**. Indeed, all the products were completely or largely transformed within 1 min, and negligible amounts of derivatives **9**, **9a**, **12**, and **12a** were observed after 1 h when incubated in human serum (Table 2), but formation of aspirin among metabolites was never observed. Only salicylic acid and salicylate were qualitatively detected in different ratios for each compound. This behavior is in keeping with the knowledge that the elimination of the negative charge of the aspirin molecule ($pK_a = 3.5$) by esterification renders the acetyl group extremely susceptible to enzymatic cleavage.[14]

Acute Gastric Mucosal Damage. All the products were assessed for their ulcerogenic properties in conscious rats. The development of gastric lesions was assessed 3 h after the intragastric administration of the compounds and lesions were quantified by determination of the "lesion index", on the basis of their greatest length in millimeters. Aspirin **3**, administrated at 120 mg/kg, produced macroscopically detectable gastric damage, characterized by mucosal necrosis and haemorrhage (lesion index = 22.0 ± 1.0; Figure 2). Acute gastric toxicity of aspirin is reported to be largely dependent on the presence of the carboxylic group.[15] As a consequence, ester derivatives of aspirin could be presumed to produce markedly less gastric damage than the parent compound. In agreement with our expectations, the present results provide evidence that all the ester derivatives, when administered at doses equimolar with aspirin, were almost devoid of ulcerogenic properties (Figure 2). On account of the fact that compounds devoid of NO exhibited impressive reduced acute toxicity, the ability to release NO appears to be of little relevance to this effect. NSAIDs are largely reported to damage gastric and intestinal mucosa by both local and systemic action, each of these mechanisms having a different time course for the induction of the injury.[16] While tested compounds appear to be almost devoid of acute ulcerogenic toxicity, their effects following chronic administration remain to be determined.
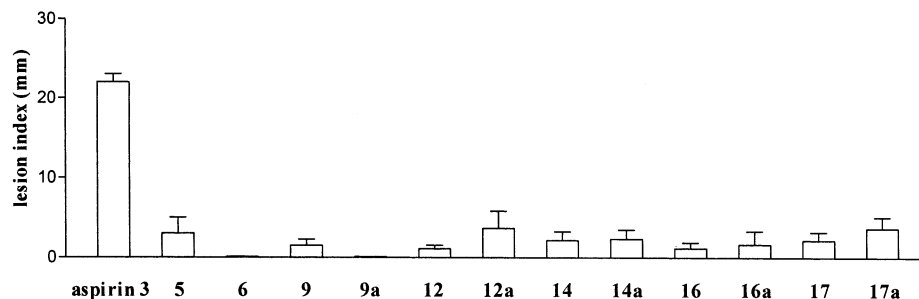
Figure 2. Gastric ulcerogenic effects of tested compounds in rat. The compounds were administered by intragastric route at a dose equimolar with aspirin **3**, 120 mg/kg, and the stomachs were examined 3 h later. Gastric lesions were quantified by determination of the lesion index, on the basis of their greatest length in millimeters. They all produced significantly less gastric damage than aspirin **3** ($P < 0.01$; ANOVA and Newman−Keuls test). Values are mean $\pm$ SEM ($n = 6-8$ per group).

Table 3. Antiaggregatory Activity of the Tested Compounds on ADP[a] -Induced PRP Aggregation

			+ HbO$_2$ 40 μM		+ODQ 100 μM	
compound	pIC$_{50}$ (μM) $\pm$ SEM	% inhibition $\pm$ SEM (50 μM)[b]	pIC$_{50}$ (μM) $\pm$ SEM	% inhibition $\pm$ SEM (50 μM)[b]	pIC$_{50}$ (μM) $\pm$ SEM	% inhibition $\pm$ SEM (50 μM)[b]
5	c	18.1 $\pm$ 9.0				
6	c	18.3 $\pm$ 9.2				
9	5.95 $\pm$ 0.10	d	5.17 $\pm$ 0.03	d	4.86 $\pm$ 0.10	d
9a	c	3.3 $\pm$ 4.2				
12	c	18.9 $\pm$ 2.9				
12a	c	16.9 $\pm$ 5.6				
14	c	17.8 $\pm$ 9.5				
14a	c	23.2 $\pm$ 12.5				
16	4.52 $\pm$ 0.13	d	c	28.6 $\pm$ 7.3	c	22.6 $\pm$ 10.9
16a	c	7.6 $\pm$ 3.7				
17	7.21 $\pm$ 0.09	d	6.46 $\pm$ 0.07	d	5.37 $\pm$ 0.10	d
17a	c	14.8 $\pm$ 7.0				d

[a] Aspirin **3** was not taken as a reference compound since it displays negligible effects on ADP-induced aggregation. [b] Maximal concentration tested. [c] Inhibition did not reach 50% of control effect: pIC$_{50}$s could not be calculated. [d] For these compounds a complete concentration−response curve could be performed, therefore pIC$_{50}$s are reported in the previous column.

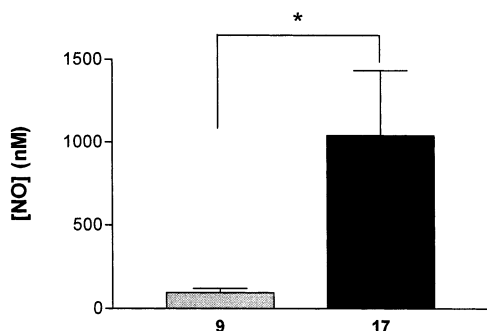**Table 4.** Antiaggregatory Activity of the Tested Compounds on Collagen-Induced PRP Aggregation

			+ HbO$_2$ 40 μM		+ODQ 100 μM	
compound	pIC$_{50}$ (μM) $\pm$ SEM	% inhibition $\pm$ SEM (50 μM)[a]	pIC$_{50}$ (μM) $\pm$ SEM	% inhibition $\pm$ SEM (50 μM)[a]	pIC$_{50}$ (μM) $\pm$ SEM	% inhibition $\pm$ SEM (50 μM)[a]
aspirin (**3**)	4.68 $\pm$ 0.11	c				
5	b	14.4 $\pm$ 7.0				
6	b	12.3 $\pm$ 5.5				
9	5.54 $\pm$ 0.20	c	4.84 $\pm$ 0.14	c	5.08 $\pm$ 0.14	c
9a	b	14.8 $\pm$ 10.1				
12	b	1.9 $\pm$ 2.5				
12a	b	0.7 $\pm$ 6.9				
14	b	18.6 $\pm$ 17.5				
14a	b	1.5 $\pm$ 1.7				
16	4.20 $\pm$ 0.02	c	b	14.4 $\pm$ 6.0	b	18.5 $\pm$ 7.5
16a	b	16.7 $\pm$ 10.1				
17	7.22 $\pm$ 0.10	c	5.97 $\pm$ 0.05	c	5.21 $\pm$ 0.17	c
17a	b	27.9 $\pm$ 17.8				

[a] Maximal concentration tested. [b] Inhibition did not reach 50% of control effect: pIC$_{50}$s could not be calculated. [c] For these compounds a complete concentration−response curve could be performed, therefore pIC$_{50}$s are reported in the previous column.
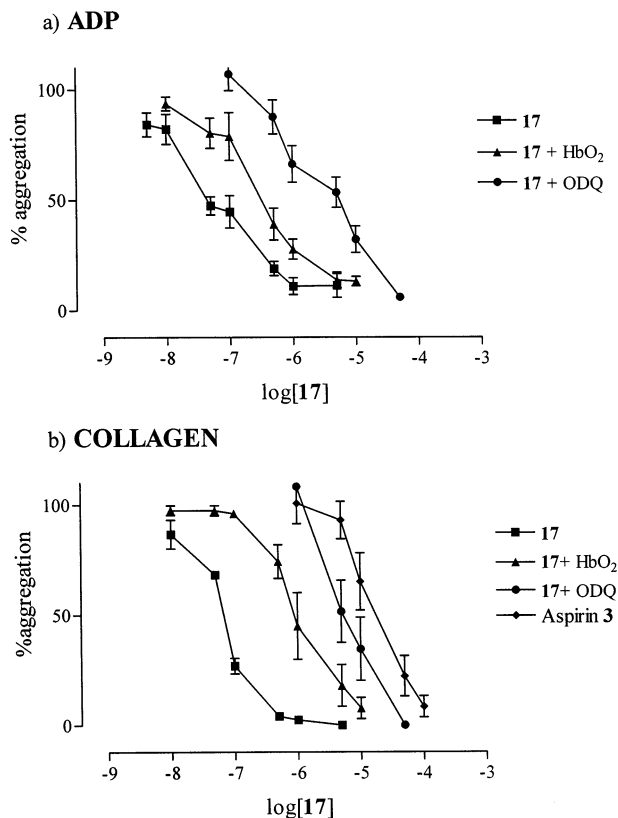
Platelet Aggregation. Antiaggregatory effects of the compounds were studied either on ADP or collagen-induced platelet aggregation of human platelet rich plasma (PRP). ADP induces aggregation by a pathway independent of the arachidonic acid cascade, while collagen is arachidonic acid-dependent. In the case of NO-donor furoxan derivatives, the experiments were repeated in the presence of either the NO-scavenger oxyhemoglobin (HbO$_2$), or the selective inhibitor of soluble guanylate cyclase (sGC) ODQ. The results are shown in Tables 3 and 4. Where possible, results are expressed as pIC$_{50}$ $\pm$ SEM, as determined by nonlinear regression analysis. In experiments where pIC$_{50}$ estimation was precluded because inhibition of maximal response did not reach 50%, we report % inhibition at maximal concentration tested (50 μM). Inspection of Tables 3 and 4 indicates that the antiaggregatory potency of furoxan derivatives is strongly dependent on their ability to produce NO. In this respect, the studied NO-donors can be classified in three groups: good NO-donors (derivatives **9**, **17**), intermediate NO-donors (derivative **16**) and poor NO-donors (derivatives **6**, **12**, **14**). In effect, the most potent antiaggregatory compounds of the series are the benzenesulfonyl and the cyano substituted furoxans **9** and **17** respectively. These compounds generated correspondingly high levels of nitrite, while the cyano derivative **17** released the most NO in plasma (Figure 3) and proved to be the most

Figure 3. NO generation from derivatives **9** and **17** in fresh human plasma at 37 °C, as assessed by electrochemical determination.[13] *P < 0.05 (unpaired *t*-test, *n* = 5).

Figure 4. Antiaggregatory activity of derivative **17** compared to aspirin **3** in (a) ADP (8 μM) and (b) collagen (2.5 mg/mL)-induced platelet aggregation in human platelet rich plasma (PRP). The role of NO and soluble guanylate cyclase in the inhibitory effect of **17** was investigated using the NO scavenger, oxyhaemoglobin (10 μM), and the soluble guanylate cyclase inhibitor, ODQ (100 μM), respectively.

powerful antiaggregatory agent. Its potency with respect to collagen-induced aggregation was about 100-fold greater than that of aspirin (Figure 4). In addition, derivative **17** showed potent COX-1-independent inhibition of ADP-induced aggregation. In both tests, the inhibition was concentration-dependent and the concentration-effect curve is right shifted in the presence of either HbO$_2$ or ODQ. When ADP was used as an agonist, the product still displayed poor antiaggregatory properties in the presence of ODQ, suggesting that it might inhibit aggregation by a cGMP independent mechanism, e.g., nitrosation of cysteine residues of ADP receptors[17] and/or activation of sGC by a heme-independent mechanism, e.g., through the sulfydryl sites of enzyme.[18] The product also showed poor activity in the

presence of ODQ when collagen was used as an agonist. This activity was very similar to that detected in ADP-induced aggregation. This residual activity could again be due to heme independent activation of sGC and/or to inhibition of aggregation consequent to deactivation of collagen receptors, e.g. by nitration of tyrosine residues of the protein.[18] The parent furazan was practically inactive at the maximal concentration tested (50 μM), irrespective of the agonist used. The behavior of **9** paralleled that of **17** but, in keeping with its minor ability to release NO in plasma, was less active. Furoxans **12** and **14**, which are very poor NO-donors, display very poor antiaggregatory properties, similar to the analogue furazans **12a**, **14a**. This is also the behavior of the nitrooxy derivative **6** and of the related nitrooxy-deficient compound **5**. The furoxan amide **16**, which has intermediate capacity to produce NO, has an intermediate potency as an antiaggregatory agent. As expected, the related furazan was inactive.

Conclusions

Several key points emerge from the results of this study: first, the products described in the present work present an antiinflammatory trend, maximized in derivatives **12**, **16**, and **17**; second, the lack of acute gastrotoxicity is principally due to the ester nature of the compounds; third, their antiplatelet action is primarily determined by their ability to release NO; finally, they do not behave as aspirin prodrugs in human serum.

Experimental Section

Chemistry. Melting points were measured with a capillary apparatus (Büchi 530) and are uncorrected. All the compounds were routinely checked by IR (Shimadzu FT-IR 8101 M), ^{1}H and ^{13}C NMR (Bruker AC-200; the following abbreviations were used to indicate the peak multiplicity: s = singlet; d = doublet; t = triplet; qt = quartet; qi = quintet; si = sixtet; n = nine lines; m = multiplet), and mass spectrometry (Finnigan-Mat TSQ-700). Flash column chromatography was performed on silica gel (Merck Kiesekgel 60, 230−400 mesh ASTM) using the indicated eluents. Anhydrous magnesium sulfate was used as the drying agent of the organic extracts. Elemental analysis of the new compounds was performed by REDOX (Monza), and the results are within ±0.4% of the theoretical values. HPLC analyses were performed by using a diode array UV detector (Shimadzu LC10A); the figures in brackets are standard errors (±SEM). Structures 7,[10] 7a,[10] 8,[19] 10,[20] 10a,[20] 13,[21] 15[21] were synthesized according to literature methods. Inert atmosphere indicates that the reaction was performed in flame- or oven-dried glassware under a positive pressure of dry nitrogen or argon.

3-(3-Phenylfuroxan-4-yloxy)propanol (11). NaOH (50% w/w; 1.16 g; 14.48 mmol) was added over a period of 40 min to a stirred solution of 4-nitro-3-phenylfuroxan **10** (3.00 g; 14.48 mmol) and 1,3-propandiol (11.4 mL; 14.50 mmol) in THF (50 mL). The resulting mixture was stirred at room temperature for 12 h and then an excess of 50% w/w NaOH (400 mg; 5.00 mmol) was added to complete the reaction. The mixture was concentrated under reduced pressure and then poured into ice−water (40 mL). The resulting mixture was extracted with CH$_2$Cl$_2$ (20 + 10 + 10 mL), the organic layers were dried and concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent: petroleum ether (40−60)/EtOAc from 8/2 v/v to 6/4 v/v). The pure title compound was obtained as a white crystalline solid. Yield 27%. Mp 57 °C from diisopropyl ether. ^{1}H NMR (CDCl$_3$) δ 1.74 (s, 1H, CH$_2$O*H*); 2.18 (qi, 2H, C*H*$_2$CH$_2$OH); 3.89 (m, 2H, CH$_2$C*H*$_2$-OH); 4.67 (*t*, 2H, furoxan-OC*H*$_2$CH$_2$); 7.48−8.14 (m, 5H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 31.4 (*C*H$_2$CH$_2$OH); 58.8

(CH$_2$CH$_2$OH); 67.7 (furoxan-OCH$_2$CH$_2$); 107.5 (furoxan C(3)); 122.3 (C$_6$H$_5$ C(1)); 126.0/128.7 (C$_6$H$_5$ C(2)/C(3)); 130.4 (C$_6$H$_5$ C(4)); 162.2 (furoxan C(4)). MS (EI) *m/z* 236 (M)$^+$. Anal. (drying conditions: 40 °C, 13 h, pressure < 1 mmHg) for C$_{11}$H$_{12}$N$_2$O$_4$ C, H, N.

3-(4-Phenylfurazan-3-yloxy)propanol (11a). The title compound was obtained from 3-nitro-4-phenylfurazan **10a** in the same manner as analogue **11**. Pale yellow oil. Yield 77%. ^{1}H NMR (CDCl$_3$) δ 1.67 (broad, 1H, CH$_2$O*H*); 2.17 (qi, 2H, C*H*$_2$-CH$_2$OH); 3.88 (*t*, 2H, CH$_2$C*H*$_2$OH); 4.63 (*t*, 2H, furazan-OC*H*$_2$-CH$_2$−); 7.45−7.99 (m, 5H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 31.6 (*C*H$_2$CH$_2$OH); 58.8 (CH$_2$*C*H$_2$OH); 69.7 (furazan-O*C*H$_2$-CH$_2$); 125.0 (C$_6$H$_5$ C(1)); 127.2/128.8 (C$_6$H$_5$ C(2)/C(3)); 130.5 (C$_6$H$_5$ C(4)); 145.0 (furazan C(3)); 163.5 (furazan C(4)). MS (EI) *m/z* 220 (M)$^+$. Anal. (drying conditions: 42 °C, 13 h, pressure < 1 mmHg) for C$_{11}$H$_{12}$N$_2$O$_3$ C, H, N.

General Procedure for the Preparation of the Esters 9, 9a, 12, and 12a. A solution of **8** (2.20 g; 11 mmol) in dry toluene (25 mL) and a solution of dry pyridine (900 μL; 11 mmol) in dry toluene (25 mL) were added, over a period of 40 min, to a stirred solution, kept under inert atmosphere at room temperature, of the appropriate propanol derivative (compounds **7**, **7a**, **11**, **11a**; 9.99 mmol). The mixture was then stirred at room temperature. When necessary an excess of the two reagents was added to complete the reaction. The mixture was washed in sequence with water (20 mL), 1 M HCl (2 × 20 mL), and saturated solution of Na$_2$CO$_3$ (2 × 20 mL), dried, and concentrated under reduced pressure. The crude material was purified by flash chromatography to give the pure compound as white solid.

3-(3-(Benzenesulfonyl)furoxan-4-yloxy)propyl 2-Acetoxybenzoate (9). The mixture was stirred for 12 h, an excess (30%) of the two reagents was added, and then the mixture was stirred for 24 h. Flash chromatography eluent: petroleum ether (40−60)/EtOAc 8/2 v/v. Yield 77%. White crystalline solid. Mp 94 °C from diisopropyl ether. ^{1}H NMR (CDCl$_3$) δ 2.35/2.35 (s, 3H, OCOC*H*$_3$)/(qi, 2H, COOCH$_2$C*H*$_2$CH$_2$−); 4.49 (*t*, 2H, COOC*H*$_2$CH$_2$CH$_2$); 4.58 (*t*, 2H, COOCH$_2$CH$_2$C*H*$_2$); 7.11−8.09 (m, 9H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 20.9 (COOCH$_2$-*C*H$_2$CH$_2$); 27.9 (OCO*C*H$_3$); 60.8 (COO*C*H$_2$CH$_2$CH$_2$); 67.8 (COOCH$_2$CH$_2$*C*H$_2$); 110.3 (furoxan C(3)); 122.6 (C$_6$H$_4$ C(1)); 123.8 (C$_6$H$_4$ C(3)); 126.0 (C$_6$H$_4$ C(5)); 126.4 (SO$_2$C$_6$H$_5$ C(3)); 129.6 (SO$_2$C$_6$H$_5$ C(2)); 131.5 (C$_6$H$_4$ C(6)); 134.0 (C$_6$H$_4$ C(4)); 135.6 (SO$_2$C$_6$H$_5$ C(4)); 137.9 (SO$_2$C$_6$H$_5$ C(1)); 150.7 (C$_6$H$_4$ C(2)); 158.7 (furoxan C(4)); 164.0 (*C*OOCH$_2$CH$_2$CH$_2$); 169.6 (O*C*OCH$_3$). MS (CI) *m/z* 463 (M + 1)$^+$. Anal. (drying conditions: rt, 12 h, pressure < 1 mmHg) for C$_{20}$H$_{18}$N$_2$O$_9$S C, H, N.

3-(4-(Benzenesulfonyl)furazan-3-yloxy)propyl 2-Acetoxybenzoate (9a). The mixture was stirred for 12 h. Flash chromatography eluent: petroleum ether (40−60)/EtOAc from 8/2 to 75/25 v/v. Yield 84%. White crystalline solid. Mp 56 °C from diisopropyl ether. ^{1}H NMR (CDCl$_3$) δ 2.30 (qi, 2H, COOCH$_2$C*H*$_2$CH$_2$); 2.36, (s, 3H, OCOC*H*$_3$); 4.41/4.53 (t, 2H, COOC*H*$_2$CH$_2$CH$_2$)/(t, 2H COOCH$_2$CH$_2$C*H*$_2$); 7.11−8.12 (m, 9H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 20.9 (COOCH$_2$*C*H$_2$CH$_2$); 28.0 (OCO*C*H$_3$); 60.7 (COO*C*H$_2$CH$_2$CH$_2$); 70.2 (COOCH$_2$-CH$_2$*C*H$_2$); 122.7 (C$_6$H$_4$ C(1)); 123.8 (C$_6$H$_4$ C(3)); 126.0 (C$_6$H$_4$ C(5)); 128.8 (SO$_2$C$_6$H$_5$ C(3)); 129.6 (SO$_2$C$_6$H$_5$ C(2)); 131.5 (C$_6$H$_4$ C(6)); 134.5 (C$_6$H$_4$ C(4)); 135.4 (SO$_2$C$_6$H$_5$ C(4)); 137.79 (SO$_2$C$_6$H$_5$ C(1)); 148.7 (furazan C(4)); 150.7 (C$_6$H$_4$ C(2)); 161.1 (furazan C(3)); 164.0 (*C*OOCH$_2$CH$_2$CH$_2$); 169.5 (O*C*OCH$_3$). MS (CI) *m/z* 447 (M + 1)$^+$. Anal. (drying conditions: rt, 12 h, pressure < 1 mmHg) for C$_{20}$H$_{18}$N$_2$O$_8$S C, H, N.

3-(3-Phenylfuroxan-4-yloxy)propyl 2-Acetoxybenzoate (12). The mixture was stirred for 3 h, an excess (20%) of the two reagents was added, and then the mixture was stirred for 2 h. Flash chromatography eluent: petroleum ether (40−60)/EtOAc from 8/2 to 7/3 v/v. Yield 77%. White crystalline solid. Mp 84 °C from diisopropyl ether. ^{1}H NMR (CDCl$_3$) δ 2.34 (s, 3H, OCOC*H*$_3$); 2.39 (qi, 2H, COOCH$_2$C*H*$_2$CH$_2$); 4.52/4.67 (t, 2H, COOC*H*$_2$CH$_2$CH$_2$)/(t, 2H, COOCH$_2$CH$_2$C*H*$_2$); 7.08−8.10 (m, 9H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 20.8 (COOCH$_2$*C*H$_2$-CH$_2$); 28.1 (OCO*C*H$_3$); 61.1 (COO*C*H$_2$CH$_2$CH$_2$); 67.4 (COOCH$_2$-CH$_2$*C*H$_2$); 107.4 (furoxan C(3)); 122.2/122.7 (C$_6$H$_5$ C(1))/(C$_6$H$_4$

C(1)); 123.7 (C$_6$H$_4$ C(3)); 125.9 (C$_6$H$_4$ C(5)); 126.0 (C$_6$H$_5$ C(3)); 126.4 (C$_6$H$_5$ C(2)); 130.3 (C$_6$H$_4$ C(6)); 131.4 (C$_6$H$_4$ C(4)); 134.0 (C$_6$H$_5$ C(4)); 150.6 (C$_6$H$_4$ C(2)); 162.0 (furoxan C(4)); 164.0 (*C*OOCH$_2$CH$_2$CH$_2$); 169.5 (O*C*OCH$_3$). MS (EI) *m/z* 398 (M)$^+$. Anal. (drying conditions: rt, 24 h, pressure < 1 mmHg) for C$_{20}$H$_{18}$N$_2$O$_7$ C, H, N.

3-(4-Phenylfurazan-3-yloxy)propyl 2-Acetoxybenzoate (12a). The mixture was stirred for 3 h. Flash chromatography eluent: petroleum ether (40−60)/EtOAc 85/15 v/v. Yield 83%. White crystalline solid. Mp 54 °C from diisopropyl ether. ^{1}H NMR (CDCl$_3$) δ 2.33 (s, 3H, OCOC*H*$_3$); 2.41 (qi, 2H, COOCH$_2$-C*H*$_2$CH$_2$); 4.51/4.63 (t, 2H, COOC*H*$_2$CH$_2$CH$_2$)/(t, 2H, COOCH$_2$-CH$_2$C*H*$_2$); 7.08−8.02 (m, 9H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 20.8 (COOCH$_2$*C*H$_2$CH$_2$); 28.2 (OCO*C*H$_3$); 61.2 (COO*C*H$_2$CH$_2$CH$_2$); 69.3 (COOCH$_2$CH$_2$*C*H$_2$); 122.8 (C$_6$H$_4$ C(1)); 123.7 (C$_6$H$_4$ C(3)); 124.9 (C$_6$H$_5$ C(1)); 125.9 (C$_6$H$_4$ C(5)); 127.2 (C$_6$H$_5$ C(3)); 128.8 (C$_6$H$_5$ C(2)); 130.5 (C$_6$H$_4$ C(6)); 131.4 (C$_6$H$_4$ C(4)); 133.9 (C$_6$H$_5$ C(4)); 145.0 (furazan C(4)); 150.6 (C$_6$H$_4$ C(2)); 163.3 (furazan C(3)); 164.1 (*C*OOCH$_2$CH$_2$CH$_2$); 169.4 (O*C*OCH$_3$). MS (CI) *m/z* 383 (M + 1)$^+$. Anal. (drying conditions: rt, 13 h, pressure <1 mmHg) for C$_{20}$H$_{18}$N$_2$O$_6$ C, H, N.

(3-Methylfuroxan-4-yl)methyl 2-Acetoxybenzoate (14). A solution of 4-bromomethyl-3-methylfuroxan **13** (1.00 g; 5.18 mmol) in dry CH$_3$CN (25 mL) was added dropwise to a stirred solution, under inert atmosphere at room temperature, of **3** (2.54 g; 13.50 mmol) and triethylamine (1.88 mL; 13.50 mmol) in dry CH$_3$CN (50 mL). The mixture was stirred for 24 h and then concentrated under reduced pressure. The crude solid material was dissolved with Et$_2$O (40 mL), and the resulting solution was washed with water (20 mL) and a saturated solution of NaHCO$_3$ (20 mL), dried, and concentrated under reduced pressure. The crude material was purified by flash chromatography (eluent: petroleum ether (40−60)/AcOEt 8:2 v/v) to give the title compound as a pale yellow oil. Yield 81%. ^{1}H NMR (CDCl$_3$) δ 2.23/2.29 (s, 3H, OCOC*H*$_3$)/(s, 3H, furoxan-C*H*$_3$); 5.36 (s, 2H, COOC*H*$_2$); 7.12−8.04 (m, 4H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 7.6 (furoxan-*C*H$_3$); 20.8 (OCO*C*H$_3$); 56.5 (COO*C*H$_2$); 112.1 (furoxan C(3)); 121.7 (C$_6$H$_4$ C(1)); 123.9 (C$_6$H$_4$ C(3)); 126.1 (C$_6$H$_4$ C(5)); 131.5 (C$_6$H$_4$ C(6)); 134.7 (C$_6$H$_4$ C(4)); 150.8 (C$_6$H$_4$ C(2)); 153.3 (furoxan C(4)); 163.3 (*C*OOCH$_2$); 169.4 (O*C*OCH$_3$). MS (EI) *m/z* 292 (M)$^+$. Anal. (drying conditions: 42 °C, 13 h, pressure < 1 mmHg) for C$_{13}$H$_{12}$N$_2$O$_6$ C, H, N.

(3-Carbamoylfuroxan-4-yl)methyl 2-Acetoxybenzoate (16). The title compound was obtained in the same manner as **14** using 4-bromomethyl-3-carbamoylfuroxan **15**. Reaction time: 48 h. CH$_2$Cl$_2$ was used in the workup of the reaction mixture. Flash chromatography eluent: petroleum ether (40−60)/EtOAc 7:3 v/v. White crystalline solid. Yield 66%. Mp 139 °C from water. ^{1}H NMR (CDCl$_3$) δ 2.35 (s, 3H, OCOC*H*$_3$); 5.65 (s, 2H, COOC*H*$_2$); 6.30 (broad, 1H, CON*H*$_2$); 7.12−8.09 (m, 4H, aromatic protons); 7.50 (broad, 1H, CON*H*$_2$). ^{13}C NMR (CDCl$_3$) δ 20.9 (OCO*C*H$_3$); 57.1 (COO*C*H$_2$); 110.3 (furoxan C(3)); 122.0 (C$_6$H$_4$ C(1)); 123.9 (C$_6$H$_4$ C(3)); 126.0 (C$_6$H$_4$ C(5)); 131.8 (C$_6$H$_4$ C(6)); 134.4 (C$_6$H$_4$ C(4)); 150.8 (C$_6$H$_4$ C(2)); 154.0/155.6 (furoxan-*C*ONH$_2$)/(furoxan C(4)); 169.5 (O*C*OCH$_3$); 163.2 (*C*OOCH$_2$). MS (CI) *m/z* 322 (M + 1).$^+$ Anal. (drying conditions: rt, 24 h, pressure < 1 mmHg) for C$_{13}$H$_{11}$N$_3$O$_7$ C, H, N.

(4-Methylfurazan-3-yl)methyl 2-Acetoxybenzoate (14a). A solution of **14** (3.84 g; 13.14 mmol) in trimethyl phosphite (50 mL) was refluxed for 24 h and then cooled at room temperature. Trimethyl phosphite was distilled off at atmospheric pressure by using a Claisen distillation head. The residue was dissolved in CH$_2$Cl$_2$ (50 mL), and the resulting solution was washed with H$_2$O (2 × 50 mL), dried, and concentrated under reduced pressure. The crude material was purified by flash chromatography (eluent: petroleum ether (40−60)/EtOAc 8:2 v/v). The title compound was obtained as a pale yellow oil. Yield 75%. ^{1}H NMR (CDCl$_3$) δ 2.26/2.45 (s, 3H, OCOC*H*$_3$)/(s, 3H, furazan-C*H*$_3$); 5.47 (s, 2H, COOC*H*$_2$); 7.11−8.10 (m, 4H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 8.1 (furazan-*C*H$_3$); 20.7 (OCO*C*H$_3$); 54.7 (COO*C*H$_2$); 121.8 (C$_6$H$_4$ C(1)); 123.9 (C$_6$H$_4$ C(3)); 126.1 (C$_6$H$_4$ C(5)); 131.7 (C$_6$H$_4$ C(6)); 134.6 (C$_6$H$_4$ C(4)); 150.1 (C$_6$H$_4$ C(2)); 150.1/150.8 (furazan C(3))/

(furazan $C(4)$); 163.5 (COOCH$_2$); 169.4 (OCOCH$_3$). MS (CI) *m/z* 277 (M + 1)$^+$. Anal. (drying conditions: 40 °C for 8 h then rt for 48 h, pressure < 1 mmHg) for C$_{13}$H$_{12}$N$_2$O$_5$ C, H, N.

(4-Carbamoylfurazan-3-yl)methyl 2-Acetoxybenzoate (16a). The title compound was obtained from **16** in the same manner as **14a**. Reaction time: 13 h. White crystalline solid. Yield 64%. Mp 106−107° C from diisopropyl ether. ^{1}H NMR (CDCl$_3$) δ 2.33 (s, 3H, OCOCH_3); 5.73 (s, 2H, COOCH_2); 6.22 (broad, 1H, CONH_2); 6.78 (broad, 1H, CONH_2); 7.11−8.09 (m, 4H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 20.9 (OCOCH$_3$); 55.7 (COOCH$_2$); 122.1 (C$_6$H$_4$ $C(1)$); 123.8 (C$_6$H$_4$ $C(3)$); 126.0 (C$_6$H$_4$ $C(5)$); 131.9 (C$_6$H$_4$ $C(6)$); 134.3 (C$_6$H$_4$ $C(4)$); 147.2 (furazan $C(4)$); 150.8 (C$_6$H$_4$ $C(2)$); 151.4 (furazan $C(3)$); 158.1 (furazan-CONH$_2$); 163.3 (COOCH$_2$); 169.6 (OCOCH$_3$). MS (CI) *m/z* 306 (M + 1)$^+$. Anal. (drying conditions: rt, 24 h, pressure < 1 mmHg) for C$_{13}$H$_{11}$N$_3$O$_6$ C, H, N.

(3-Cyanofuroxan-4-yl)methyl 2-Acetoxybenzoate (17). Trifluoroacetic anhydride (1.73 mL; 7.04 mmol) was added, over a period of 90 min, to a ice-cold stirred solution, kept under inert atmosphere, of **16** (1.2 g; 3.74 mmol) and dry pyridine (600 μL; 7.48 mmol) in dry THF (40 mL). The mixture was slowly warmed at room temperature and then concentrated under reduced pressure. Water (20 mL) and Et$_2$O (40 mL) were added to the residue, and, after separation, the organic layer was washed with 0.5 M HCl (2 × 20 mL), dried, and concentrated under reduced pressure. The crude material was purified by flash chromatography (eluent: petroleum ether (40−60)/EtOAc 85:15 v/v) obtaining the title compound as a sticky oil that becomes a white solid during drying. Yield 78%. Mp 57 °C (trituration with diisopropyl ether). ^{1}H NMR (CDCl$_3$) δ 2.37 (s, 3H, OCOCH_3); 5.44 (s, 2H, COOCH_2); 7.14−8.10 (m, 4H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 20.8 (OCOCH$_3$); 55.95 (COOCH$_2$); 96.1 (furoxan $C(3)$); 104.7 (furoxan-CN); 120.9 (C$_6$H$_4$ $C(1)$); 124.0 (C$_6$H$_4$ $C(3)$); 126.1 (C$_6$H$_4$ $C(5)$); 131.6 (C$_6$H$_4$ $C(6)$); 135.0 (C$_6$H$_4$ $C(4)$); 151.1 (C$_6$H$_4$ $C(2)$); 152.2 (furoxan $C(4)$); 162.8 (COOCH$_2$); 169.4 (OCOCH$_3$). MS (CI) *m/z* 304 (M + 1)$^+$. Anal. (drying conditions: 42 °C, 15 h, pressure < 1 mmHg) for C$_{13}$H$_9$N$_3$O$_6$ C, H, N.

(4-Cyanofurazan-3-yl)methyl 2-Acetoxybenzoate (17a). The title compound was obtained from **16a** in the same manner as **17**. White crystalline solid. Yield 80%. Mp 52−53 °C. ^{1}H NMR (CDCl$_3$) δ 2.34 (s, 3H, OCOCH_3); 5.60 (s, 2H, COOCH_2); 7.13−8.13 (m, 4H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 20.6 (OCOCH$_3$); 54.2 (COOCH$_2$); 106.6 (furazan-CN); 121.0 (C$_6$H$_4$ $C(1)$); 123.9 (C$_6$H$_4$ $C(3)$); 126.1 (C$_6$H$_4$ $C(5)$); 131.7 (C$_6$H$_4$ $C(6)$); 132.2 (furazan $C(4)$); 135.0 (C$_6$H$_4$ $C(4)$); 151.1 (C$_6$H$_4$ $C(2)$); 152.0 (furazan $C(3)$); 162.9 (COOCH$_2$); 169.5 (OCOCH$_3$). MS (EI) *m/z* 287 (M)$^+$. Anal. (drying conditions: 40 °C, 48 h, pressure < 1 mmHg) for C$_{13}$H$_9$N$_3$O$_5$ 0.25 H$_2$O C, H, N.

3-Bromopropyl 2-Acetoxybenzoate (18). A solution of **8** (1.00 g; 5.03 mmol) in dry toluene (25 mL) and a solution of dry pyridine (410 μL; 5.03 mmol) in dry toluene (25 mL) were added, over a period of 2 h, to a stirred solution, kept under inert atmosphere, of 3-bromopropanol (1.40 g; 910 μL; 10.06 mmol) in dry toluene (20 mL). The mixture was stirred at room temperature for 48 h and then washed with water (2 × 40 mL), 1 M HCl (2 × 40 mL), and saturated solution of NaHCO$_3$ (2 × 40 mL), dried, and concentrated under reduced pressure. The crude material was purified by flash chromatography (eluent: petroleum ether (40−60)/EtOAc 95/5 v/v) to give the title compound as a colorless oil (lit.[22]). Yield 66%. ^{1}H NMR (CDCl$_3$) δ 2.33 (qi, 2H, COOCH$_2$CH_2); 2.40 (s, 3H, OCOCH_3); 3.53 (t, 2H, CH$_2$CH$_2$CH_2-Br); 4.44 (t, 2H, COOCH_2CH$_2$); 7.10−8.04 (m, 4H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 20.9 (COOCH$_2$CH_2); 29.2 (OCOCH$_3$); 31.6 (CH$_2$CH$_2$$CH_2$Br); 62.64 (COO$CH_2CH_2$); 122.9 (C$_6H_4$ $C(1)$); 123.7 (C$_6$H$_4$ $C(3)$); 125.9 (C$_6$H$_4$ $C(5)$); 131.5 (C$_6$H$_4$ $C(4)$); 133.9 (C$_6$H$_4$ $C(6)$); 150.6 (C$_6$H$_4$ $C(2)$); 164.1 (COOCH$_2$); 169.6 (OCOCH$_3$). MS (CI) *m/z* 301−303 (M + 1)$^+$. Anal. (drying conditions: 40 °C, 48 h, pressure < 1 mmHg) for C$_{12}$H$_{13}$BrO$_4$ C, H.

3-Nitrooxypropyl 2-Acetoxybenzoate (6). AgNO$_3$ (11.28 g; 66.4 mmol) was added to a solution of **18** (2.50 g; 8.30 mmol)

in CH$_3$CN (50 mL). The resulting clear solution was heated at 40 °C for 60 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in CH$_2$Cl$_2$ (50 mL), and the resulting solution washed with H$_2$O (2 × 50 mL), dried, and concentrated under reduced pressure. The crude material was purified by flash chromatography (eluent: petroleum ether (40−60)/EtOAc 8/2 v/v) to give the title compound as a pale yellow oil (lit.[23]). Yield 80%. ^{1}H NMR (CDCl$_3$) δ 2.36 (s, 3H, OCOCH_3); 2.19 (qi, 2H, COOCH$_2$CH_2); 4.40 (t, 2H, COOCH_2CH$_2$); 4.61 (t, 2H, CH$_2$-CH$_2$CH_2ONO$_2$); 7.10−8.03 (m, 4H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 20.8 (COOCH$_2$CH_2); 26.3 (OCOCH$_3$); 60.8 (COOCH$_2$); 69.6 (CH$_2$CH$_2$$CH_2ONO_2$); 122.7 (C$_6H_4$ $C(1)$); 123.7 (C$_6$H$_4$ $C(3)$); 125.9 (C$_6$H$_4$ $C(5)$); 131.4 (C$_6$H$_4$ $C(4)$); 134.0 (C$_6$H$_4$ $C(6)$); 150.6 (C$_6$H$_4$ $C(2)$); 164.0 (COOCH$_2$); 169.5 (OCOCH$_3$). MS (CI) *m/z* 284 (M + 1)$^+$. Anal. (drying conditions: 40 °C, 48 h, pressure < 1 mmHg) for C$_{12}$H$_{13}$NO$_7$ C, H, N.

Propyl 2-Acetoxybenzoate (5). The title compound was obtained from 1-propanol in the same manner as **18**. Reaction time 96 h. Flash chromatography eluent: petroleum ether (40−60)/diisopropyl ether 9/1 v/v. Colorless oil (lit.[24]). Yield 57%. ^{1}H NMR (CDCl$_3$) δ 1.02 (t, 3H, CH$_2$CH$_2$CH_3); 1.77 (si, 2H; COOCH$_2$CH_2); 2.36 (s, 3H, OCOCH_3); 4.24 (t, 2H, COOCH_2-CH$_2$); 7.08−8.06 (m, 4H, aromatic protons). ^{13}C NMR (CDCl$_3$) δ 10.3 (CH$_2$CH$_2$$CH_3$); 20.8/21.8 (OCO$CH_3$)/(COOCH$_2$$CH_2$); 66.5 (COO$CH_2CH_2$); 123.3 (C$_6H_4$ $C(1)$); 123.6 (C$_6$H$_4$ $C(3)$); 125.6 (C$_6$H$_4$ $C(5)$); 131.6 (C$_6$H$_4$ $C(4)$); 133.6 (C$_6$H$_4$ $C(6)$); 150.5 (C$_6$H$_4$ $C(2)$); 164.4 (COOCH$_2$); 169.5 (OCOCH$_3$). MS (CI) *m/z* 223 (M + 1).$^+$ Anal. for C$_{12}$H$_{14}$O$_4$ C, H.

Detection of Nitrite. A solution of the appropriate compound (20 μL) in dimethyl sulfoxide (DMSO) was added to 2 mL of 1/1 v/v mixture of 50 mM phosphate buffer (pH 7.4) with MeOH, containing 5 × 10^{-4} M L-cysteine. The final concentration of the compound was 10^{-4} M. After 1 h at 37 °C, 1 mL of the reaction mixture was treated with 250 μL of Griess reagent [sulfanilamide (4 g), *N*-naphthylethylenediamine dihydrochloride (0.2 g), 85% phosphoric acid (10 mL) in distilled water (final volume: 100 mL)]. After 10 min at room temperature, the absorbance was measured at 540 nm (Shimatzu UV-2501PC spectrophotometer). Sodium nitrite standard solutions (10−80 nmol/mL) were used for the calibration curve. The yields in nitrite were expressed as NO$_2$$^-$ % (mol/mol) ± SEM. No production of nitrite was observed in the absence of L-cysteine.[11]

Detection of NO• in Human Plasma. Citrated venous blood was obtained from healthy volunteers who had not taken any drug for at least two weeks. Platelet poor plasma (PPP) was prepared by centrifugation at 1000g for 10 min. Aliquots (2.0 mL) of PPP were equilibrated at 37 °C for 10 min. Radical NO was detected with a Clark-type electrode (ISO−NO, World Precision Instruments, Stevenage, UK), calibrated using diethylamine diazeniumdiolate (DEA/NO; 100 nM−10 mM) in phosphate-buffered saline (pH 4.0; DEA/NO generates 2 mol equiv of NO spontaneously at pH < 5.0). The area under the curve (AUC) was integrated for each DEA/NO concentration, generating a linear calibration curve. NO generated from our novel compounds in plasma was evaluated by integrating the area under the curve, and the results were expressed as NO concentration (nM).

Hydrolysis in Human Serum. A solution of each compound (1.75 × 10^{-2} M) in acetonitrile (5 μL) was added to human serum (Sigma-Aldrich) (495 μL) preheated at 37 °C. After appropriate time of incubation the reaction mixture was diluted with acetonitrile containing 0.1% trifluoroacetic acid (1:1.5 v/v) in order to deproteinize the serum. Supernatant was separated by centrifugation (9600g, 10 min) and analyzed by HPLC. Chromatographic detection of the unaltered compounds was accomplished using a Merck Purospher RP-18 column (250 × 4 mm; 5 μm particles size) termostatised at 40 °C, eluting with a flow-rate of 1 mL/min. Mobile phase system was consisting of linear gradient of 0.1% acqueous TFA (el. A) and acetonitrile containing 0.1% TFA (el. B): a gradient profile of 25% B to 80% B in 15 min was used. The column effluent was monitored at 224 nm; quantitation of the compounds was done

by measurement of the peak areas in relation to those of standards chromatographed under the same conditions. The absence of aspirin among the hydrolysis products was confirmed according to the methods reported in ref 25.

Pharmacology. Antiinflammatory Activity and Gastrotoxicity. All the compounds were initially dissolved in DMSO and then diluted in 1% carboxymethylcellulose. The final concentration of DMSO was 5%. Each agent was prepared immediately before use and administered intragastrically in a volume of 10 mL/kg.

Carrageenan-Induced Paw Edema. Male Wistar strain rats (Harlan, Italy), weighing 180−200 g, were deprived of food but not of water for 24 h before the experiment. The edema was induced by intraplantar injection of 0.1 mL of 1% carrageenan suspended in 1% carboxymethylcellulose, into the right hind paw of each rat. Hind paw volume was measured 3 h after carrageenan injection; volume increase was recorded with respect to the volume measured before carrageenan. The edema reduction in treated animals was expressed as percentage inhibition of the edema observed in the corresponding control group, considered as 100. Measurements were conducted using a water plethysmometer (Basile, Comerio, Italy). Groups of rats ($n = 6-8$) were given aspirin 120 mg/kg or equimolar doses of the other compounds. All compounds were administered at the same time as carrageenan injection. Control rats received the vehicle only. The results obtained are presented as mean $\pm$ SEM. Statistical analysis was performed with ANOVA followed by Newman-Keuls test.

Gastric Mucosal Damage. Male Wistar strain rats (Harlan, Italy), weighing 180−200 g, were deprived of food but not of water for 24 h before the experiment. Groups of rats ($n = 6-8$) were given aspirin 120 mg/kg, or equimolar doses of the other compounds. The rats were sacrified 3 h after the administration of the compounds. The stomachs were removed, opened along the lesser curvature, and examined under a stereomicroscope for the presence of macroscopically visible lesions. Each individual haemorrhagic lesion was measured along its greatest length (< 1 mm = rating of 1; 1−2 mm = rating of 2; > 2 mm = rating according to their length in mm). The overall total was designated as the "lesion index". The results obtained are presented as mean $\pm$ SEM. Statistical analysis was performed with ANOVA followed by Newman− Keuls test.

Inhibition of Platelet Aggregation in Vitro. Venous blood was obtained from healthy volunteers who had not taken any drug for at least two weeks. Volunteers were informed that blood samples were obtained for research purposes and that their privacy would be protected. Platelet rich plasma (PRP) was prepared by centrifugation of citrated blood at $200g$ for 20 min. Aliquots (300 μL) of PRP were added into aggregometer (Elvi) cuvettes and aggregation was recorded as increased light transmission under continuous stirring (1000 rpm) at 37 °C for 5 min after addition of the stimulus. ADP (10 μM) or collagen (2.5 mg/mL) were used as platelet activators in PRP. The inhibitory activity of the compounds was tested by addition of drug to PRP 10 min before addition of the stimulus (ADP or collagen). Drug vehicle (<0.5% DMSO) added to PRP or to the platelet suspension served as a control and did not affect platelet function. The role of NO and sGC in the inhibitory effect of **17** was investigated using the NO scavenger, oxyhaemoglobin (10 μM), and the sGC inhibitor, ODQ (100 μM), respectively.

The antiaggregatory activity of tested compounds was evaluated as % inhibition of platelet aggregation compared to control samples. When inhibition of aggregation at maximal inhibitor concentration exceeded 50%, pIC_{50} values were calculated by nonlinear regression analysis.

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