A New Class of Ibuprofen Derivatives with Reduced Gastrotoxicity

Marco L. Lolli,[†] Clara Cena,[†] Claudio Medana,[†] Loretta Lazzarato,[†] Giuseppina Morini,[‡] Gabriella Coruzzi,*[,‡] Stefano Manarini,[§] Roberta Fruttero,[†] and Alberto Gasco*[,†]

Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, via Pietro Giuria 9, 10125 Torino, Italy, and Istituto di Farmacologia, Università degli Studi di Parma, via Volturno 39, 43100 Parma, Italy, and Istituto di Ricerche Farmacologiche Mario Negri, Laboratorio di Interazione tra Cellule Ematiche e Vascolari, Dipartimento di Medicina e Farmacologia Vascolare, Consorzio Mario Negri Sud, 66030 Santa Maria Imbaro (CH), Italy

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A new series of nonsteroidal antiinflammatory drugs (NSAIDs) obtained by linking ibuprofen to selected furoxan moieties and to related furazans were synthesized and tested for their antiinflammatory, antiaggregatory, and ulcerogenic properties. All the derivatives are endowed with antiinflammatory activity comparable to that of ibuprofen, but, unlike this drug, they display reduced acute gastrotoxicity. The masking of the ibuprofen-free carboxylic group seems to be principally at the basis of this reduced topical irritant action. The two furoxan derivatives **8** and **9** also trigger potent antiaggregatory effects, principally as a consequence of their NO-donor ability.

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are an important class of compounds that display a number of effects as a consequence of their ability to block cyclooxygenase (COX) involved in the first step of the arachidonic acid cascade.[1] COX exists in two isoforms named COX-1 and COX-2, respectively. The first is constitutively expressed in the stomach, the kidneys, and platelets and is considered important in mucosal protection and platelet function. COX-2 is inducible and plays a major role in prostaglandin biosynthesis in inflammatory cells.[2] Classical NSAIDs, such as ibuprofen **3**, inhibit both the isoforms. The major limitation to long term use of NSAIDs in therapy is the increased risk of gastrointestinal ulceration and complications from ulceration, such as bleeding and perforation.[1] There is strong evidence that inhibition of COX-1 rather than inhibition of COX-2 underlies this gastrointestinal toxicity.[3]

Nitric oxide (NO) is an endogenous mediator that has important functions in mammalian tissues.[4] It is synthesized by the enzyme nitric oxide synthase (NOS), which also exists in constitutive and inducible forms, both present in the stomach. Here NO is able to protect gastric mucosa by a number of mechanisms including promotion of mucus secretion and increasing mucosal blood flow.[3]

On this basis, molecules endowed with mixed cyclooxygenase inhibiting and NO-releasing properties were recently designed.[5,6] These compounds (general structure **1** in Chart 1) were obtained by conjugating traditional NSAIDs to NO-donor nitroxy groups. Very recently nitrosothiol esters of diclofenac were also described as gastrointestinal-sparing prodrugs.[7] The behavior of this class of drugs is very interesting, but quite complex and not completely understood.

There is interesting evidence that furoxans (1,2,5-oxadiazole 2-oxides, general structure **2** in Chart 1) are able to generate nitric oxide NO (family name), in the presence of thiol cofactors.[8,9] By introducing appropriate substituents to the ring, it is possible to modulate the rate and the amount of NO production.[10] The overall reaction mechanism appears to be very complex and direct $NO^{\bullet}$ release or intermediate formation of nitroxyl anion (NO^-) or both could be involved. Both the redox forms are able to activate soluble guanylate cyclase (sGC).[11] The profile of furoxans as vasodilators is similar to that of nitrates, but they, differently from the latter, could lack significant tolerance development.[12,13] All this renders furoxan derivatives flexible tools in designing NO-donor "hybrid" drugs, potentially useful for the treatment of cardiovascular diseases. In previous works, we discussed the use of furoxan substructures to prepare "hybrids" with either α_1-antagonist,[14] or β_1-antagonist,[15] as well as Ca^{2+} blocker[16] and NO-dependent vasodilating properties. We also obtained interesting compounds in which the combination of furoxan moieties with a H_2-antagonist pharmacophoric group resulted in in vivo gastroprotective inhibitors of acid secretion.[17]

As a further development of our research in this field, we have now designed NSAIDs (derivatives **8**, **9**) in which ibuprofen **3** is joined by an ester linkage to two furoxan moieties selected for their ability to release NO. In this paper, we report on the synthesis, antiinflammatory, and antiaggregatory properties as well as gastric effects of these compounds. Related furazan derivatives **8a** and **9a** were taken as controls since they are unable to release NO. Ibuprofen and its γ-nitroxypropyl and propyl esters **11** and **12**, respectively, are also considered as references (Chart 2).

Chemistry. Syntheses of the final compounds considered for biological investigation are outlined in Scheme 1. The starting materials were the propanol

* To whom correspondence should be addressed: Dipartimento di Scienza e Tecnologia del Farmaco, Via P. Giuria, 9, I-10125 Torino, Italy. Telephone: 0039 011 6707670. Fax: 0039 011 6707972. E-mail: gasco@pharm.unito.it.

[†] Università degli Studi di Torino.

[‡] Università degli Studi di Parma.

[§] Istituto di Ricerche Farmacologiche Mario Negri, Consorzio Mario Negri Sud.

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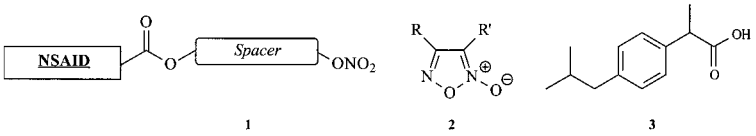
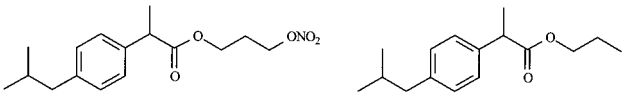
Chart 1



1 2 3

Chart 2



11 12

derivatives **5** and **5a**, obtained by action of 1,3-propandiol on 3,4-bis(benzenesulfonyl)furoxan **4** and on related furazan **4a**, in the presence of sodium hydroxide. Nucleophilic displacement, by action of thiophenol in MeOH/THF solution (potassium salt), of the remaining benzenesulfonyl group of these compounds afforded intermediates **6** and **6a**, respectively. Final structures **8**, **9** and **8a**, **9a** were synthesized by coupling ibuprofen chloride **7** with the appropriate intermediates, in methylene chloride solution in the presence of pyridine. Structure **11** was obtained following the standard reaction sequence reported at the bottom of Scheme 1.

All the structures were confirmed by ^{1}H and ^{13}C NMR spectroscopy. For the intermediate **5**, we proposed a 3-benzenesulfonyl structure[18] on the basis of the reactivity of **4** with ethanol in the presence of sodium hydroxide.[19] ^{13}C NMR resonances of the endocyclic C-3 (110.35) and C-4 (158.79) carbons supported this assignment.[20] Structures **6** and **8** are consequently fixed.

To evaluate the thiol-induced NO-generation, the appropriate NO-donor compound was 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 cysteine. The use of a cosolvent was necessary because of the low water solubility of the compounds. Nitrite, which is an important air oxidation product of nitric oxide, was determined by Griess reaction.[10] Nitrite detection may indicate the previous presence of NO. While it is true that in our experimental conditions other competitive reactions can occur in addition to oxidation,[8] for our purposes nitrite is a convenient index of NO production trend. The results, expressed as percent NO_2^- (mol/mol), are summarized in Table 1. These same products were unable to afford nitrite under the same conditions in the acidic medium (pH = 1).

Table 1. NO Release Data and Ibuprofen Detected in Human Plasma and in Acidic Medium (pH = 1) after Hydrolysis of Esters **8**, **8a**, **9**, **9a**, **11**, and **12**

		% ibuprofen[a]	
compound	% NO_2^- (mol/mol)[a,b]	human plasma 6 h	pH = 1 2 h
8	34.7 ± 1.8	46.8 ± 1.8	12.9 ± 0.9
8a		26.0 ± 1.0	13.0 ± 1.1
9	9.5 ± 0.2	35.6 ± 1.2	6.5 ± 0.5
9a		8.9 ± 1.0	6.1 ± 0.5
11	3.2 ± 0.1	27.1 ± 3.4	22.3 ± 2.8
12		22.9 ± 1.5	11.7 ± 1.0

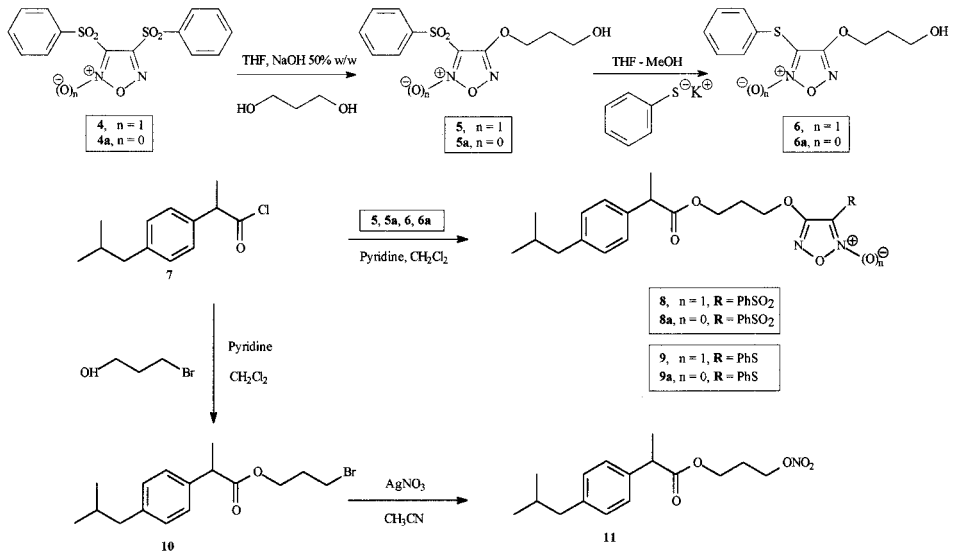
[a] All values are mean ± SEM.[10] [b] Determined by Griess reaction.

The amount of ibuprofen produced by each ibuprofen ester after 6 h of incubation in human plasma at 37 °C and over 2 h in acidic medium (pH = 1) was detected by HPLC. The results are reported in Table 1.

Pharmacological Results and Discussion

Antiinflammatory Activity. All the compounds were tested for their antiinflammatory activity in the carrageenan-induced rat foot paw edema assay. The injection of carrageenan into the paw pad produced a marked increase in paw volume within 1 h of its administration and reached its maximal effect after 4−6 h. Ibuprofen **3**, administered by intragastric route at 120 mg/kg just prior to the carrageenan injection, significantly reduced (48.5%) paw edema at 4 h (Table 2).

Scheme 1



7 10 11

Table 2. Antiinflammatory Activity of Tested Compounds on Carragenan-Induced Rat Paw Edema and Gastrotoxicity Studies

	antiinflammatory activity[a]			gastrotoxicity[b]
	% inhibition of paw volume increase[c]			lesion index (mm)[c]
compound	dose regimen	4 h	6 h	dose regimen,[e] 6 h
ibuprofen **3**	[d]	19.6 ± 7.4	11.7 ± 6.6	25.7 ± 3.5
	[e]	48.5 ± 8.8[f]	30.3 ± 8.9	
8	[d]	37.4 ± 8.9[f]	41.5 ± 6.3[f]	0[g]
8a	[d]	52.8 ± 6.1[f]	63.3 ± 6.6[h]	5.7 ± 1.7[g]
9	[e]	40.7 ± 14.5[f]	36.2 ± 11.5[f]	0[g]
9a	[e]	48.3 ± 10.1[f]	54.4 ± 9.5[h]	1.3 ± 0.9[g]
11	[e]	41.8 ± 10.0[f]	40.0 ± 11.2[f]	0.7 ± 0.7[g]
12	[d]	37.6 ± 6.9[f]	49.6 ± 7.7[f]	3.8 ± 0.9[g]

[a] Determined in conscious rat in the carrageenan rat paw model. [b] Determined in conscious rat after i.g. administration of test compounds. [c] All values are mean ± SEM. [d] Dose equimolar to 40 mg/kg of ibuprofen. [e] Dose equimolar to 120 mg/kg of ibuprofen. Compounds were administered to 6 rats/groups. [f] $P < 0.05$ vs vehicle (ANOVA and Dunnett's test). [g] $P < 0.01$ vs ibuprofen-treated group (ANOVA and Newman-Keuls test). [h] $P < 0.01$ vs vehicle (ANOVA and Dunnett's test).

However, its inhibitory effect did not reach significance after 6 h. Ibuprofen propyl ester **12**, at 4 h, induced a significant reduction (37.6%) of the edema volume, this effect being actually increased after 6 h (49.6%). All the other products of the series displayed significant antiinflammatory activity, with a comparable efficacy (Table 2). Compounds differed in terms of the dose needed to achieve a significant antiinflammatory effect. In fact, benzenesulfonyl derivatives **8** and **8a** elicited their action at the lower dose tested, and their effect was evident at 4 and 6 h. Phenylthiofuroxan **9**, phenylthiofurazan **9a**, and the nitroxy ester **11** were effective at the higher dose tested, and again the effect was present at 4 and 6 h. The antiinflammatory activity of all the compounds of the series, could partly reside in their behavior as prodrugs of **3**. Indeed, as shown in Table 1, after a 6 h incubation in human plasma, the compounds afforded a significant amount of ibuprofen (Table 1), although to different degrees. In any case, other factors could be involved; for example, the products themselves could directly inhibit COX.

Acute Gastric Mucosal Damage. All the compounds were assessed for their ulcerogenic properties in conscious rats. The extent of gastric lesions were measured 4 and 6 h after the intragastric administration of the compounds, and the results were expressed as "lesion index". Ibuprofen **3**, administered at 120 mg/kg, time-dependently induced macroscopically visible lesions, characterized by mucosal necrosis and haemorrhage, lesion index being 15.4 and 25.7 at 4 and 6 h, respectively (Table 2). By contrast, propyl ester **12** was significantly less ulcerogenic than the parent compound at 6 h (lesion index = 3.8, Table 2).

There are two main components of gastric damaging properties of NSAIDs, an irritating effect dependent upon direct contact with the mucosa, related to the presence of acidic groups in the molecules, and a systemic effect attributable to the inhibition of prostaglandin synthesis.[21−23] Both components contribute to the ulcerogenic activity of ibuprofen **3**.[21] Each of these mechanisms has a different time course for the injury induction, and the acute toxicity should be principally influenced by the former component. The reduced toxicity of **12** could be partly due to the masking of the free carboxylic group in **3**, by ester prodrug formation, with the consequent reduction of the topical irritating action. Also, all the other esters of the series were definitely less irritating than ibuprofen, when studied in the same conditions (Table 2). In fact, the ibuprofen production by all the compounds during their lumen transit is likely to be low as a consequence of their quite good stability in acidic medium (Table 1).

Table 3. Antiaggregatory Activity of the Tested Compounds on Arachidonic Acid Induced PRP Aggregation

	antiaggregatory activity		
	% inhibition (100 μM)[a,b]	pIC_{50}[b]	
compound			+ HbO_2 40 μM
ibuprofen **3**	[c]	4.94 ± 0.005	
8	[c]	6.21 ± 0.01	5.57 ± 0.03
8a	[c]	4.07 ± 0.001	
9	[c]	5.30 ± 0.07	[d]
9a	13.9 ± 3.8	[d]	
11	[c]	4.24 ± 0.13	[d]
12	5.0 ± 2.2	[d]	
aspirin	[c]	4.89 ± 0.26	

[a] Maximal concentration tested. [b] All values are mean ± SEM ($n = 5$). [c] For these compounds, a complete concentration−response curve could be performed; therefore, pIC_{50}s are reported in the next column. [d] Aggregation did not reach 50% of control effect: pIC_{50}s could not be calculated.

Another possible reason for the low ulcerogenicity of the compounds could be a consequence of their inhibitory effects on COX-2 rather than COX-1. A recent report shows that this is the case of a series of ester and amide derivatives of indomethacin.[24] Preliminary results obtained using a human whole blood test[25] show that our compounds display a certain degree of COX-2 selective inhibition. However, the interpretation of this behavior is complex because, owing to different incubation times required in the COX inhibition experiments (1 h for COX-1, 24 h for COX-2), enzyme inhibition is the result of a combined action of the prodrugs as well as ibuprofen derived from them, and the relative importance of these two components is different in the two tests. To overcome this problem, we are extending the study by employing ovine isolated COX-1 and COX-2 enzymes.

Finally, it is known that nitric oxide is able to trigger gastroprotection by a number of mechanisms and NO-donors are capable of reducing gastrotoxicity in some experimental models.[26] Thus, this third component should be taken into account to explain the behavior of the NO-donor esters **8**, **9**, and **11**. However, the inability of NO-releasing ibuprofen derivatives to produce nitrite in the acidic medium suggests that NO release is unlikely to occur in the gastric lumen.

Platelet Aggregation. Antiaggregatory effects of the compounds were studied on arachidonic acid (AA) induced platelet aggregation of human platelet rich plasma (PRP); in the case of NO-donor derivatives **8**, **9**, **11**, the experiments were repeated in the presence of oxyhaemoglobin (HbO_2), a known scavenger of NO. The results are shown in Table 3, where the antiaggregatory activity of aspirin is also reported for comparison. Data are expressed as % of inhibition of aggregation at maximal concentration tested or as pIC_{50} ± SEM, when

it could be calculated by nonlinear regression analysis. All the derivatives were able to inhibit the aggregation induced by AA in PRP in a concentration-dependent manner. Furazan and propyl derivatives **8a**, **9a**, and **12** were less potent than ibuprofen. By contrast, the NO-donor **8** was more potent than ibuprofen, while compounds **9** and **11** were approximately equipotent with the parent drug. The potency of these three NO-donors parallels their ability to produce nitrite and, in comparison with reference compounds, it follows the sequence **8** > **9** > aspirin = ibuprofen ≫ **11**. The antiaggregatory activity of the NO-donors **8**, **9**, and **11** was reversed by oxyhaemoglobin, indicating an involvement of nitric oxide.

Conclusions

The introduction of furoxan and furazan moieties into the molecule of ibuprofen does not alter the antiinflammatory activity. The products described in the present investigation exhibit reduced gastric ulcerogenicity when compared with ibuprofen. A number of heterogeneous mechanisms could underlie the loss of ulcerogenic properties. Since both the NO donor and the non-NO-donor derivatives are devoid of these effects, it is assumable that the prodrug formation is primarily at the basis of reduced topical irritant action, by masking the free carboxylic group of ibuprofen.

Ibuprofen derivatives also display antiaggregatory activity to a different degree. It is evident that the furoxan analogues, which are able to release NO, are endowed with an antiaggregatory activity superior to ibuprofen and aspirin.

In conclusion, some of the products described herein might be potential clinical candidates as safer antiinflammatory and antiaggregatory drugs.

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; *n* = nine lines; *m* = multiplet) and mass spectrometry (Finnigan-Mat TSQ-700). Silica gel (Merck Kieselgel 60) 70-230 mesh ASTM was employed for column chromatography. HPLC analyses were performed by using a diode array UV detector (Shimadzu LC10A); the figures in brackets are standard errors (± SEM). Anhydrous magnesium sulfate was used as drying agent of the organic phases. Analysis (C, H, N) of the new compounds was performed by REDOX (Monza), and the results are within ± 0.4% of the theoretical. Structures **4**,[19] **4a**,[15] **5**,[18] **7**,[27] and **12**[28] were synthesized according to literature methods.

3-(4-(Benzenesulfonyl)furazan-3-yloxy)propanol (5a). The title compound was obtained in the same manner as analogue **5** (lit.[18]). Yield 58%, mp 67 °C from isopropyl ether. ^{1}H NMR ($CDCl_3$) δ 1.61 (*t*, 1H, -CH_2OH); 2.10 (*qi*, 2H, −CH_2-CH_2OH); 3.82 (*qt*, 2H, -CH_2CH_2OH); 4.55 (*t*, 2H, FurazanOCH_2-CH_2-); 7.6−8.1 (*m*, 5H, $C_6H_5SO_2$-). ^{13}C NMR ($CDCl_3$) δ 31.22 (-CH_2CH_2OH); 58.81 (−CH_2CH_2OH); 71.12 (FurazanOCH_2-CH_2-); 128.60, 129.53, 135.37, 137.88 ($C_6H_5SO_2$-); 148.67 (Furazan *C*(4)); 161.18 (Furazan *C*(3)). MS (CI) *m*/*z* 285 $(M+1)^+$. Anal. (drying conditions: 40 °C; 18 h, pressure < 1 mmHg) for $C_{11}H_{12}N_2O_5S$ C, H, N.

3-(3-(Phenylthio)furoxan-4-yloxy)propanol (6). A solution of potassium benzenethiolate (20 mL), prepared by mixing under nitrogen and stirring benzenethiol (0.70 mL, 6.6 mmol) dissolved in dry MeOH (10 mL) and potassium *tert*-butoxide (0.75 g, 6.6 mmol) dissolved in dry MeOH (10 mL), was added under nitrogen over 2 h to a stirred solution of **5** (2.0 g, 6.6 mmol) in dry THF (30 mL) kept at −15 °C. The reaction mixture was stirred for an additional hour at −15 °C. The solution was allowed to reach room temperature and then was concentrated under vacuum without heating. The residue was treated with EtOAc, and the resulting mixture was washed twice with water. The organic layer dried and evaporated under vacuum at room temperature gave a residue that was purified by flash chromatography (petroleum ether (40−60)/EtOAc 7.5/2.5 v/v). The pure title compound was obtained as a pale yellow oil, yield 45%. ^{1}H NMR ($CDCl_3$) δ 1.59 (*s*, 1H, -CH_2OH); 2.04 (*qi*, 2H,-CH_2CH_2OH); 3.73 (*t*, 2H, -CH_2CH_2OH); 4.52 (*t*, 2H, FuroxanOCH_2CH_2-); 7.3−7.5 (*m*, 5H, C_6H_5S-). ^{13}C NMR ($CDCl_3$) δ 31.25 (-CH_2CH_2OH); 58.68 (-CH_2CH_2OH); 67.53 (FuroxanOCH_2CH_2-); 104.79 (Furoxan *C*(3)); 128.63, 129.02, 129.50, 131.81 (C_6H_5S-); 163.4 (Furoxan *C*(4)). MS (EI) *m*/*z* 268 $(M)^+$. Anal. (drying conditions: room temperature; 15 days; pressure < 1 mmHg) for $C_{11}H_{12}N_2O_4S$ C, H, N.

3-(4-(Phenylthio)furazan-3-yloxy)propanol (6a). The title compound was obtained as pale yellow oil in the same manner as analogue **6**. In this case, the addition of potassium benzenethiolate solution was performed at room temperature and the resulting mixture was heated at 40 °C for 48 h to complete the reaction. Yield 68%. ^{1}H NMR ($CDCl_3$) δ 1.50 (*s*, 1H, -CH_2OH); 1.98 (*qi*, 2H, -CH_2CH_2OH); 3.66 (*t*, 2H, -CH_2CH_2-OH); 4.45 (*t*, 2H, FurazanOCH_2CH_2-); 7.4−7.6 (*m*, 5H, C_6H_5S-). ^{13}C NMR ($CDCl_3$) δ 31.36 (-CH_2CH_2OH); 58.67 (-CH_2CH_2-OH); 69.66 (-FurazanOCH_2CH_2-); 127.86, 129.13, 129.38, 132.67 (C_6H_5S-); 144.06 (Furazan *C*(4)); 163.39 (Furazan *C*(3)). MS (EI) *m*/*z* 252 $(M)^+$. Anal. (drying conditions: room temperature; 15 days; pressure < 1 mmHg) for $C_{11}H_{12}N_2O_3S \cdot 0.2$ H_2O C, H, N.

General Procedure for the Preparation of Esters 8, 8a, 9, 9a, 10. Dry pyridine (0.55 mL, 6.8 mmol) and a solution of the appropriate propanol derivative (3.4 mmol) in dry CH_2Cl_2 (7.0 mL) were added dropwise to a solution of 2-(4-isobutylphenyl)propionyl chloride **7** (0.78 g, 3.4 mmol) in dry CH_2Cl_2 (5.0 mL). The mixture was stirred for 2 h, then diluted with CH_2Cl_2 (20 mL). The resulting solution was washed in sequence with 0.5 M HCl (4 × 10 mL), 1 M NaOH (2 × 10 mL), and saturated aqueous NaCl and then dried. Solvent removal under reduced pressure at room temperature afforded a crude oily residue that was purified by flash chromatography. The expected pure compound was obtained as an oily pale yellow material. Chromatographic eluents and yields of the products were as follows.

3-(3-(Benzenesulfonyl)furoxan-4-yloxy)propyl 2-(4-isobutylphenyl)propionate (8): eluent (petroleum ether (40−60)/EtOAc 8/2 v/v); yield 55%. ^{1}H NMR ($CDCl_3$) δ 0.87 (*d*, 6H, $(CH_3)_2$CH-); 1.50 (*d*, 3H, Ar(CH_3)CH-); 1.82 (*n*, 1H, $(CH_3)_2CHCH_2$-); 2.15 (*qi*, 2H, -$COOCH_2CH_2$-); 2.42 (*d*, 2H, $(CH_3)_2CHCH_2$-); 3.71 (*qt*, 1H, Ar(CH_3)C*H*-); 4.26 (*m*, 2H, −$COOCH_2CH_2$-); 4.33 (*t*, 2H, −CH_2CH_2OFuroxan); 7.0−7.2 (*m*, 4H, $(CH_3)_2CHCH_2C_6H_4$-); 7.6−8.1 (*m*, 5H, $C_6H_5SO_2$-). ^{13}C NMR ($CDCl_3$) δ 18.16 (Ar(*C*H_3)CH-); 22.19 ($(CH_3)_2CHCH_2$-); 27.63 (-$COOCH_2CH_2$-); 29.99 ($(CH_3)_2CHCH_2$-); 44.81, 44.90 (Ar(CH_3)-*C*H-/$(CH_3)_2CHCH_2$-); 60.07 ($COOCH_2CH_2$); 67.52 (-CH_2CH_2-OFuroxan); 110.30 (Furoxan *C*(3)); 126.88, 129.21, 137.39, 140.55 (Phenyl); 128.37, 129.56, 135.47, 137.91 ($C_6H_5SO_2$-); 158.56 (Furoxan *C*(4)); 174.39 (-*C*$OOCH_2CH_2$-). MS (EI) *m*/*z* 488 $(M)^+$. Anal. (drying conditions: 40 °C; 15 days; pressure < 1 mmHg) for $C_{24}H_{28}N_2O_7S$ C, H, N.

3-(4-(Benzenesulfonyl)furazan-3-yloxy)propyl 2-(4-isobutylphenyl)propionate (8a): eluent (petroleum ether (40−60)/EtOAc 8.5/1.5 v/v); yield 66%. ^{1}H NMR ($CDCl_3$) δ 0.88 (*d*, 6H, $(CH_3)_2$CH-); 1.49 (*d*, 3H, Ar(CH_3)CH-); 1.86 (*n*, 1H, $(CH_3)_2CHCH_2$-); 2.11 (*qi*, 2H, -$COOCH_2CH_2$-); 2.43 (*d*, 2H, $(CH_3)_2CHCH_2$-); 3.69 (*qt*, 1H, Ar(CH_3)C*H*-); 4.17 (*t*, 2H, -$COOCH_2CH_2$-); 4.29 (*t*, 2H, -CH_2CH_2OFurazan); 7.0−7.2 (*m*, 4H, $(CH_3)_2CHCH_2C_6H_4$-); 7.6−8.1 (*m*, 5H, $C_6H_5SO_2$-). ^{13}C NMR ($CDCl_3$) δ 18.17 (Ar(*C*H_3)CH-); 22.24 ($(CH_3)_2CHCH_2$-); 27.73 (-$COOCH_2CH_2$-); 30.04 ($(CH_3)_2CHCH_2$-); 44.86 (Ar(CH_3)CH-)/ ($(CH_3)_2CHCH_2$-); 60.07 (-$COOCH_2CH_2$-); 69.96 (-CH_2CH_2-

OFurazan); 126.87, 129.21, 137.40, 140.58 (Phenyl); 128.76, 129.57, 135.34, 137.72 ($C_6H_5SO_2$-); 149.74 (Furazan *C*(4)); 160.92 (Furazan *C*(3)); 174.37 (*C*OOCH$_2$CH$_2$). MS (EI) *m*/*z* 472 $(M)^+$. Anal. (drying conditions: 40 °C; 15 days; pressure <1 mmHg) for $C_{24}H_{28}N_2O_6S$ C, H, N.

3-(3-(Phenylthio)furoxan-4-yloxy)propyl 2-(4-Isobutylphenyl)propionate (9): eluent (petroleum ether (40−60)/EtOAc 9.5/0.5 v/v); yield 64%. ^{1}H NMR ($CDCl_3$) δ 0.89 (*d*, 6H, (C*H*$_3$)$_2$CH-); 1.49 (*d*, 3H, Ar(C*H*$_3$)CH-); 1.84 (*n*, 1H, (CH$_3$)$_2$C*H*CH$_2$-); 2.05 (*qi*, 2H, -COOCH$_2$C*H*$_2$-); 2.44 (*d*, 2H, (CH$_3$)$_2$CHC*H*$_2$-); 3.69 (*qt*, 1H, Ar(CH$_3$)C*H*-); 4.11 (*t*, 2H, -COOC*H*$_2$CH$_2$-); 4.29 (*t*, 2H, -CH$_2$C*H*$_2$OFuroxan); 7.0−7.2 (*m*, 4H, (CH$_3$)$_2$CHCH$_2$C$_6$*H*$_4$-); 7.3−7.4 (*m*, 5H, C$_6$*H*$_5$S-). ^{13}C NMR ($CDCl_3$) δ 16.17 (Ar(*C*H$_3$)CH-); 22.21 ((*C*H$_3$)$_2$CHCH$_2$-); 27.65 (-COOCH$_2$*C*H$_2$-); 30.04 ((CH$_3$)$_2$*C*HCH$_2$-); 44.84, 44.92 ((CH$_3$)$_2$-CH*C*H$_2$-/Ar(CH$_3$)*C*H-)); 60.14 (−COO*C*H$_2$CH$_2$-); 66.61 (-CH$_2$*C*H$_2$-OFuroxan); 104.75 (Furoxan *C*(3)); 126.91, 129.52, 137.39, 140.59 (Phenyl); 128.48, 129.04, 129.23, 131.92 (*C*$_6$H$_5$S-); 163.75 (Furoxan *C*(4)); 174.37 (-*C*OOCH$_2$CH$_2$-). MS (EI) *m*/*z* 456 $(M)^+$. Anal. (drying conditions: room temperature; 15 days; pressure < 1 mmHg) for $C_{24}H_{28}N_2O_5S$ C, H, N; Calculated C 63.14; H 6.18; N 6.14; Found: C 63.70; H 6.34; N 6.06.

3-(4-(Phenylthio)furazan-3-yloxy)propyl 2-(4-isobutylphenyl)propionate (9a): eluent (petroleum ether (40−60)/EtOAc 9.5/0.5 v/v); yield 74%. ^{1}H NMR ($CDCl_3$) δ 0.89 (*d*, 6H, (C*H*$_3$)$_2$CH-); 1.49 (*d*, 3H, Ar(C*H*$_3$)CH-); 1.85 (*n*, 1H, (CH$_3$)$_2$C*H*CH$_2$-); 1.98 (*qi*, 2H, -COOCH$_2$C*H*$_2$-); 2.45 (*d*, 2H, (CH$_3$)$_2$CHC*H*$_2$-); 3.69 (*qt*, 1H, Ar(CH$_3$)C*H*-); 4.03 (*t*, 2H, −COOC*H*$_2$CH$_2$-); 4.24 (*t*, 2H, -CH$_2$C*H*$_2$OFurazan); 7.1−7.2 (*m*, 4H, (CH$_3$)$_2$CHCH$_2$C$_6$*H*$_4$-); 7.3−7.5 (*m*, 5H, C$_6$*H*$_5$S-). ^{13}C NMR ($CDCl_3$) δ 18.13 (Ar(*C*H$_3$)CH-); 22.21 ((*C*H$_3$)$_2$CHCH$_2$-); 27.76 (-COOCH$_2$*C*H$_2$-); 30.00 ((CH$_3$)$_2$*C*HCH$_2$-); 44.85, 44.92 (Ar(CH$_3$)-*C*H-/(CH$_3$)$_2$CH*C*H$_2$-)); 60.21 (-COO*C*H$_2$CH$_2$-); 66.78 (−CH$_2$*C*H$_2$-OFurazan); 126.92, 129.37, 137.40, 140.56 (Phenyl); 127.72, 129.20, 129.20, 132.91 (*C*$_6$H$_5$S-); 144.02 (Furazan *C*(4)); 163.30 (Furazan *C*(3)); 174.33 (-*C*OOCH$_2$CH$_2$-). MS (EI) *m*/*z* 440 $(M)^+$. Anal. (drying conditions: 40 °C; 48 h; pressure < 1 mmHg) $C_{24}H_{28}N_2O_4S$ C, H, N.

3-Bromopropyl 2-(4-Isobutylphenyl)propionate (10): eluent (petroleum ether (40−60)/EtOAc 9.5/0.5 v/v); yield 67%. ^{1}H NMR ($CDCl_3$) δ 0.90 (*d*, 6H, (C*H*$_3$)$_2$CH-); 1.50 (*d*, 3H, Ar(C*H*$_3$)CH-); 1.85 (*n*, 1H, (CH$_3$)$_2$C*H*CH$_2$-); 2.11 (*qi*, 2H, −COOCH$_2$C*H*$_2$-); 2.45 (*d*, 2H, (CH$_3$)$_2$CHC*H*$_2$-); 3.28 (*t*, 2H, −CH$_2$CH$_2$C*H*$_2$Br); 3.70 (*qt*, 1H, Ar(CH$_3$)C*H*-); 4.21 (*m*, 2H, −COOC*H*$_2$CH$_2$-); 7.1−7.3 (*m*, 4H, (CH$_3$)$_2$CHCH$_2$C$_6$*H*$_4$-). ^{13}C NMR ($CDCl_3$) δ 18.15 (Ar(*C*H$_3$)CH-); 22.22 ((*C*H$_3$)$_2$CH-); 30.41 (-COOCH$_2$*C*H$_2$-); 31.47 ((CH$_3$)$_2$*C*HCH$_2$-); 32.59 (-CH$_2$CH$_2$*C*H$_2$-Br); 44.87, 44.96 ((CH$_3$)$_2$CH*C*H$_2$-/Ar(CH$_3$)*C*H-); 62.08 (-*C*H$_2$CH$_2$-CH$_2$Br); 126.94, 129.22, 137.52, 140.49 (Phenyl); 174.40 (-*C*OOCH$_2$-). MS (EI) *m*/*z* 326/328 $(M)^+$. Anal. (drying conditions: 40 °C; 3 days; pressure < 1 mmHg) for $C_{16}H_{23}BrO_2$ C, H.

3-Nitroxypropyl 2-(4-Isobutylphenyl)propionate (11). A solution of compound **10** (0.88 g, 2.7 mmol) in a small amount of dry CH_3CN was added at room temperature to a stirred solution of $AgNO_3$ (1.83 g, 10.7 mmol) in dry CH_3CN (10 mL). Stirring was continued over 24 h and then for 4 h at 40 °C. The reaction mixture was filtered, and the collected solid was washed with CH_3CN. The combined organic phases were dried and treated with CH_2Cl_2 (20 mL). The solution was washed with water, dried, and evaporated under vacuum. The residue was purified by flash chromatography [petroleum ether (40−60)/isopropyl ether 9.5/0.5 v/v] to give the expected product as yellow oil (lit.[29]). Yield 49%. ^{1}H NMR ($CDCl_3$) δ 0.92 (*d*, 6H, (C*H*$_3$)$_2$CH-); 1.51 (*d*, 3H, Ar(C*H*$_3$)CH-); 1.84 (*n*, 1H, (CH$_3$)$_2$C*H*CH$_2$-); 1.98 (*qi*, 2H, -COOCH$_2$C*H*$_2$-); 2.47 (*d*, 2H, (CH$_3$)$_2$CHC*H*$_2$-); 3.72 (*qt*, 1H, Ar(CH$_3$)C*H*-); 4.19 (*m*, 2H, -COOC*H*$_2$CH$_2$-); 4.36 (*t*, 2H, −CH$_2$C*H*$_2$ONO$_2$); 7.1−7.3 (*m*, 4H, (CH$_3$)$_2$CHCH$_2$C$_6$*H*$_4$-). ^{13}C NMR ($CDCl_3$) δ 17.98 (Ar(*C*H$_3$)-CH-); 22.13 ((*C*H$_3$)$_2$CH-); 26.07 (−COOCH$_2$*C*H$_2$-); 29.97 ((CH$_3$)$_2$-*C*HCH$_2$-); 44.79, 44.84 ((CH$_3$)$_2$CH*C*H$_2$-/Ar(CH$_3$)CH-); 60.15 (-*C*H$_2$CH$_2$CH$_2$ONO$_2$); 69.44 (-CH$_2$*C*H$_2$ONO$_2$); 126.85, 129.19, 137.36, 140.49 (Phenyl); 174.22 (-*C*OOCH$_2$-). MS (EI) *m*/*z* 309 $(M)^+$. Anal. (drying conditions: 40 °C; 3 days; pressure < 1 mmHg) for $C_{16}H_{23}NO_5$ C, H, N.

Hydrolysis in Human Plasma. A solution of each compound (0.0438 μmol) in acetonitrile (5 μL) was added to human plasma (495 μL) at 37 °C. After 6 h of incubation, the reaction mixture was diluted with acetonitrile containing 0.1% trifluoroacetic acid (1:1.5 v/v). Serum was separated by centrifugation (9600*g*, 15 min) and assayed by HPLC for ibuprofen production, using a Merck Purospher RP-18 column (250 × 4 mm; 5 μm particles) at 40 °C, eluting (1 mL/min) with water/acetonitrile/TFA 25/75/0.1 (v/v/v).

Hydrolysis in Acidic Medium. A solution of each compound (0.25 mM) in acetonitrile (560 μM) was added to a HCl solution (pH 1, 1440 μL) at 37 °C. After 2 h of incubation, ibuprofen and corresponding ester were detected by HPLC, according to the previously described method.

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 either of 50 mM phosphate buffer (pH 7.4) or of a HCl solution (pH 1) with MeOH, containing 5×10^{-4} M L-cysteine. The final concentration of drug 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 expressed as NO_2^- % (mol/mol) ± SEM. No production of nitrite was observed in the absence of L-cysteine.

Pharmacology. (a) 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.

(1) 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 injecting 0.1 mL of 1% carrageenan suspended in 1% carboxymethylcellulose, into the plantar surface of the right hind foot of each rat. Foot volume was measured 4 h after carrageenan injection; foot 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 ($n = 6$) were given ibuprofen 40 and 120 mg/kg or equimolar doses of the other compounds. The higher dose was tested only when the lower one was inactive. All compounds were administered immediately before a carrageenan injection. Control rats received the vehicle only. The results obtained are presented as mean ± SEM. Statistical analysis was performed with ANOVA followed by Dunnett's test.

(2) 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 ($n = 6$) were given ibuprofen 120 mg/kg, or equimolar doses of the other compounds. The rats were sacrified 6 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 ± SEM. Statistical analysis was performed with ANOVA followed by Newman-Keuls test.

(b) Platelet Aggregation Assay. Venous blood was obtained from consenting healthy human subjects 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. PRP was prepared by centrifugation of citrated blood at 200*g* for 20 min. Aliquots (300 μL) of PRP were added into an aggregometer cuvette (Elvi) and aggregation was recorded as increased light transmission under continuous stirring (1000 rpm) at 37 °C for 5 min after addition of the stimulus. AA was used as platelet activator at a concentration producing 85−95% of the maximal aggregation (0.5−1 mM). The inhibitory activity of the compounds was tested by adding their solution or the solvent (DMSO) to PRP 10 min before addition of the stimulus at 37 °C. Haemoglobin was added to the platelets 12 min before addition of the platelet activator at 40 μM final concentration. The maximal amount (0.5%) of DMSO added to PRP or to the platelet suspension did not affect platelet function. The anti-aggregating activity of tested compounds was evaluated as % of inhibition of platelet aggregation in respect with the control samples. When inhibition at maximal concentration exceeded 50%, pIC_{50} values were calculated by nonlinear regression analysis.

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