

(Nitrooxyacyloxy)methyl Esters of Aspirin as Novel Nitric Oxide Releasing Aspirins

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A series of (nitrooxyacyloxy)methyl esters of aspirin were synthesized and evaluated as new NO-donor aspirins. Different amounts of aspirin were released in serum from these products according to the nature of nitrooxyacyloxy moiety present. In the aromatic series, there is a rather good linear correlation between the amount of aspirin released and the potencies of the products in inhibiting platelet aggregation induced by collagen. Both the native compounds and the related nitrooxy-substituted acid metabolites were able to relax rat aorta strips precontracted with phenylephrine, in keeping with a NO-induced activation of the sGC as a mechanism that underlies the vasodilator effect. The products here described are new improved examples of NO-donor aspirins containing nitrooxy groups. They could represent an alternative to the use of aspirin in a variety of clinical applications.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs^a) are the most commonly used agents for the treatment of pain and inflammation. Over 30 million people are treated with these products every day.¹ The prototype of NSAIDs is aspirin, which, in addition to anti-inflammatory and analgesic properties, displays antithrombotic effects and, consequently, protects against ischemic vascular disorders, including myocardial and cerebral infarction. It also exerts some beneficial effects against colorectal cancer.^{2–5} The activities of aspirin are largely due to its ability to inhibit irreversibly COX enzymes, preferentially the COX-1 isoform with respect to COX-2.^{6,7} The most important limit in using this drug is its strong gastrotoxicity due to systemic and local irritant effects.^{6,7} The former are thought to be dependent on the inhibition of COX-1 isoform, which is largely expressed in the gastric epithelial cells, and the latter are closely associated with the presence in the molecule of a free carboxylic group. A strategy followed to reduce the local gastric drawback was the masking of this function through pro-drug formation. Indeed, a number of these compounds display reduced gastrotoxicity.⁸ However, the problem with this approach is the high enzymatic lability of the *o*-acetyloxy group in these products. This is due to the loss of the negative charge that is present in aspirin at physiologic conditions following dissociation of its carboxylic function ($pK_a = 3.5$).⁹ Consequently a large part of these substances are not true pro-drugs because they are rapidly metabolized to salicylic acid in human serum without any formation of relevant amounts of aspirin. Another strategy to decrease the gastrotoxicity of aspirin is the combination of aspirin with nitric oxide (NO)-donor moieties. Indeed, it is known that NO is able to display gastrosparring actions through

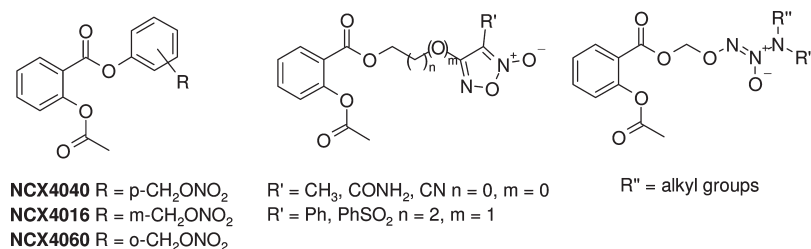
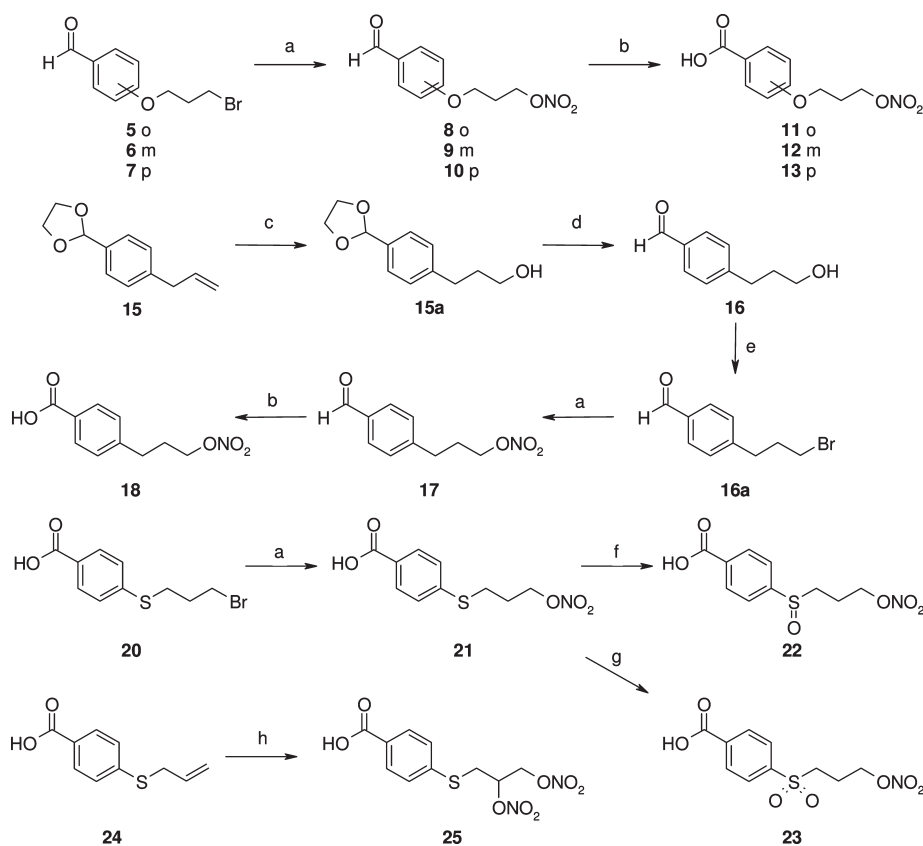
a number of mechanisms.¹⁰ In addition, it is able to maintain micro- and macrovascular homeostasis^{11–13} as well as to trigger anti-inflammatory and analgesic effects.¹⁴ Therefore, this “gaseous solution” to the old problem of aspirin gastrotoxicity received great attention. A number of NO-donor moieties have been joined through an ester linkage to the carboxylic group of the drug. They include substructures containing nitrooxy functions,^{15,16} furoxan,¹⁷ and *N*-diazoniumdiolate moieties (Chart 1).^{18–20} The former two classes of products are rapidly metabolized in serum, plasma, and a number of cell fractions with little or no formation of aspirin,^{16,21–23} while for the latter no specific study of aspirin release has been reported thus far. In this paper, we describe a new class of (nitrooxyacyloxy)methyl esters of this drug, showing that a number of them are stable in acid and in physiological pH solution but are able to release relevant amount of aspirin when incubated in human serum. This double ester moiety was chosen in view of its rapid hydrolysis in serum.²⁴ Antiaggregatory and NO-dependent vasodilator properties of all these new products are discussed as well.

Results and Discussion

Chemistry. The development of this new class of NO-donor aspirins required the availability of a number of nitrooxy substituted carboxylic acids. Products **1–4**, **14**, **19**, and **26** were obtained following procedures already described in literature, while acids **11–13**, **18**, **21–23**, and **25** were synthesized according to the pathways reported in Scheme 1. The compounds **11–13** were obtained starting from the 3-bromopropoxy substituted benzaldehydes **5–7**, which after treating with AgNO₃ in acetonitrile solution afforded the corresponding 3-nitrooxypropoxy analogues **8–10**. These latter intermediates treated with KMnO₄ in acetone solution gave the expected corresponding acids. *p*-Nitrooxypropyl substituted benzoic acid **18** was prepared starting from allylphenyl substituted dioxolane **15**,

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^aAbbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; PRP, platelet rich plasma; NO, nitric oxide; sGC, soluble guanylate cyclase; ODO, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one.

Chart 1. Examples of NO-donors Aspirin**Scheme 1^a**

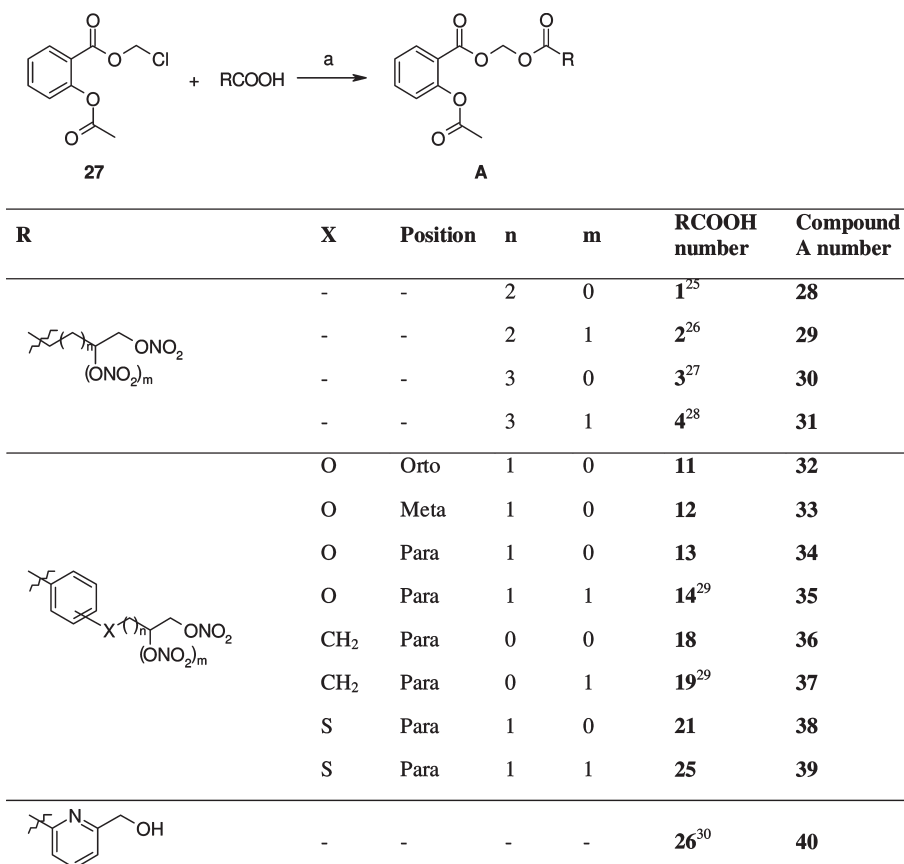
^a Reagents and conditions: (a) AgNO₃, CH₃CN, 70 °C; (b) KMnO₄, acetone; (c) (Sia)₂BH, dry THF then NaOH, H₂O₂; (d) H₂O/MeOH, 4 M HCl, 60 °C; (e) Ph₃P, NBS, CH₃CN; (f) mCPBA, CH₂Cl₂, -78 °C; (g) 85% H₂O₂, CF₃COOH; (h) I₂, AgNO₃, CH₃CN rt then AgNO₃, CH₃CN reflux.

which was transformed into alcohol **15a** by action of disiamylborane ((Sia)₂BH) and then of H₂O₂ in the presence of NaOH. Compound **15a**, after partial purification, was immediately hydrolyzed with 4 M HCl to the aldehyde **16**. This intermediate was subjected to the action of *N*-bromosuccinimide (NBS) in acetonitrile solution in the presence of triphenylphosphine (Ph₃P) to give **16a** that was immediately treated with AgNO₃, giving rise to the aldehyde **17**. The related acid **18** was obtained following the same procedure used to prepare **11–13**. The compound **21** was synthesized from the corresponding bromo derivative **20** by action of AgNO₃ in acetonitrile solution. Acids **22** and **23** were prepared from **21** for *m*-chloroperbenzoic acid and H₂O₂ oxidation, respectively. Acid **25** was prepared by action of I₂ and AgNO₃ in acetonitrile solution on 4-allylthiobenzoic acid (**24**).

Most of the NO-aspirins described in the present work, namely products **28–39**, and the intermediate **40** were prepared by action of the cesium salt of appropriate acid prepared in situ on chloromethyl 2-(acetyloxy)benzoate

(**27**) in DMF solution (Scheme 2). The remaining products were obtained according to the pathways outlined in Scheme 3. Oxidation of **38** with *m*-chloroperbenzoic acid and oxone provided sulfoxide and sulfone models **41** and **42**, respectively. The action of acetaldehyde on acetylsalicylic acid chloride **43** produced the adduct **44** that was coupled with acid **21** in DMF solution in the presence of Cs₂CO₃ to produce the final compound **45**. Nitration of intermediate **40** with 65% HNO₃ in the presence of acetic anhydride gave rise to the pyridine derivative **46**. Finally, product **32** afforded **47** through HCl hydrolysis. This latter was used as a reference to follow the hydrolysis of **32** in human serum.

Hydrolysis Studies. The possible hydrolytic routes of (nitrooxyacyloxy) aspirin esters are reported in Scheme 4. For compounds to release a meaningful amount of aspirin, the rate constant of the deacetylation *k*₂ must be slower than the hydrolytic constant *k*₁. The stabilities of the target products were assessed by high-performance liquid chromatography (HPLC) in buffered solutions at pH 1.0

Scheme 2^a

^a Reagents and conditions: (a) Cs₂CO₃, DMF.

and 7.4 as well as in human serum. The results are reported in Table 1. After 3 h of incubation in acid solution, both the aliphatic and the aromatic NO-donor aspirins resulted unchanged for about 98% with the only exception of **46** containing the electron poor pyridine ring. This product was transformed slightly more extensively (unchanged 84%). A similar behavior was observed at physiological pH, but in this case, in addition to **46** (unchanged for 60%) also **41** and **42**, containing the electron-withdrawing SO or SO₂ groups on the benzene ring, displayed less stability compared to the other terms of the series (unchanged for 90% and for 70%, respectively). Quite different results were obtained in serum in which the hydrolysis of a variety of esters is catalyzed by carboxylesterases. These enzymes are ubiquitous and display a broad substrate specificity. Frequently the same ester can be hydrolyzed by more than one of these enzymes.³¹ It was assumed that (acyloxy)alkyl esters are hydrolyzed to the corresponding hydroxyalkyl esters, which immediately and spontaneously decompose to the related carboxylic acids and aldehydes.²⁴ According to Scheme 4, aspirin, salicylates, nitrooxy-substituted carboxylic acids, and salicylic acid were detected during the hydrolysis. The time course of the metabolites detected over 10 min and 2 h in the case of products **32** and **36** is reported in Figure 1 and Figure 2 as examples. For all the products, the final metabolites (6 h) were salicylic acid and nitrooxy-substituted carboxylic acids. The hydrolysis strictly followed first-order kinetics. The observed pseudo-first-order rate constants (k_{obs}) were calculated from the slopes of linear plots of the logarithm of the remaining

ester against time: the corresponding half-lives were obtained from eq 1.

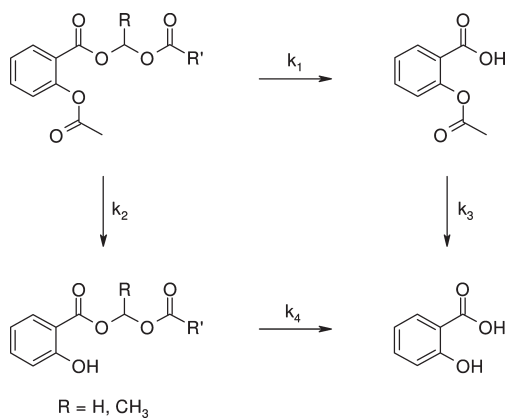
$$t_{1/2} = 0.693/k_{\text{obs}} \quad (1)$$

In Table 1, the maximal amounts of aspirin detected for each product, expressed as % of the initial ester concentration, are also collected. Analyses of the data show that all the compounds are very quickly hydrolyzed in serum. A number of products display $t_{1/2} < 1$ min, and this figure does not exceed 5.4 min in the others. The peak of aspirin released in the aliphatic nitrooxyacyl series (**28**–**31**) ranges from 11% to 20%. The products of the aromatic series are definitively better aspirin releasing compounds. In the set of mononitrooxy-alkyloxy substituted models, the release follows the series **33** > **34** > **32**. The very low release of aspirin from the *o*-derivative is probably due to the negative effect of steric hindrance on hydrolysis exerted by the substituent group in proximity of the ester function. The dinitrooxy substituted compound **35** appears to be more susceptible to the enzymatic cleavage of acetyloxy group than the mononitrooxy structurally related **34**. All the other compounds we considered bear a *p*-substituent at the phenyl ring of benzoic acid. This is due to their easier synthetic availability, but the good results obtained with **33** indicates that the *m*-substitution could also be worthy of investigation. The products **36** and **38**, bearing mononitrooxypropyl- and mononitrooxypropylthio- chains, respectively, are among the best aspirin releasing products of the series. Moving to the dinitrooxy analogues **37** and **39**, this capacity decreases. The change in the oxidation level of the sulfur chain of **38** affords the

Scheme 3^a

^a Reagents and conditions: (a) mCPBA, CH₂Cl₂, -78 °C; (b) oxone, H₂O/MeOH; (c) CH₃CHO, ZnCl₂, CH₂Cl₂; (d) **21**, Cs₂CO₃, DMF; (e) (CH₃CO)₂O, 65% HNO₃, CH₂Cl₂; (f) 6 M HCl, 1,4-dioxane, 70 °C.

Scheme 4. Possible Hydrolytic Routes of Aspirin (Acyloxy)alkyl Esters to Salicylic Acid



sulfoxide **41** and the sulfone **42**, which are very good aspirin releasing compounds. As expected, the introduction of a methyl group on the methylene moiety of the acyloxymethyl substructure of **38** to give **45** weakens the production of aspirin, reasonably for steric reason. Finally, the good behavior of the pyridine derivative **46** indicates that other interesting NO-donor aspirins could be designed from the use of other π -deficient heterocycle carboxylic acids.

Platelet Antiaggregatory Activity. Antiaggregatory effects of the new NO-aspirins were studied on collagen induced platelet aggregation of human platelet rich plasma (PRP),

taking aspirin as a reference. The inhibitory activity of the compounds was tested by addition of product to PRP 10 min before addition of the stimulus. All the products displayed a concentration dependent inhibitory effect. Their antiaggregatory potency, expressed as IC₅₀, are reported in Table 1. By contrast, no antiaggregatory activity was observed when the related nitrooxy substituted carboxylic acids, which are rapidly formed from the parent drugs under the action of plasma esterases, were tested at 300 μ M concentration. This finding is consistent with the known inability of platelets to efficiently effect NO-release from organic nitrates.³² As expected, the products of the aromatic series are definitively more active than the products of aliphatic series. The low activity of these latter compounds prevented us from determining their IC₅₀ values. The areas under the aspirin release curves (AUC) of all the products, measured after 10 min of incubation, are reported in Table 1. In the aromatic series there is a rather good linear correlation ($r^2 = 0.8732$) between these values and the corresponding IC₅₀ values (Figure 3), despite the different medium in which they were measured, serum and plasma, respectively (eq 2, the standard error of regression coefficients is given within parentheses).

$$\text{IC}_{50} = -0.27(\pm 0.03)\text{AUC} + 174(\pm 12) \quad (2)$$

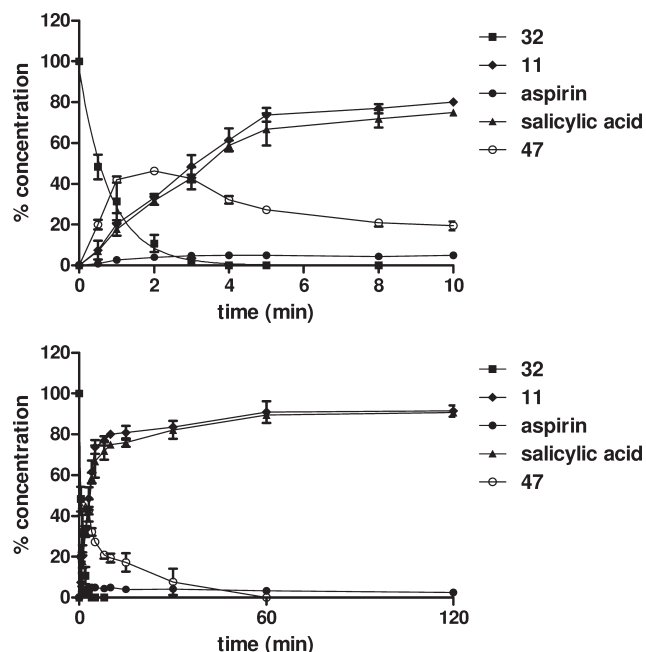
$$n = 11, \quad r^2 = 0.8732, \quad F = 61.98$$

Vasodilator Activities. The vasodilator activity of the new NO-donor aspirins was evaluated on endothelium denuded

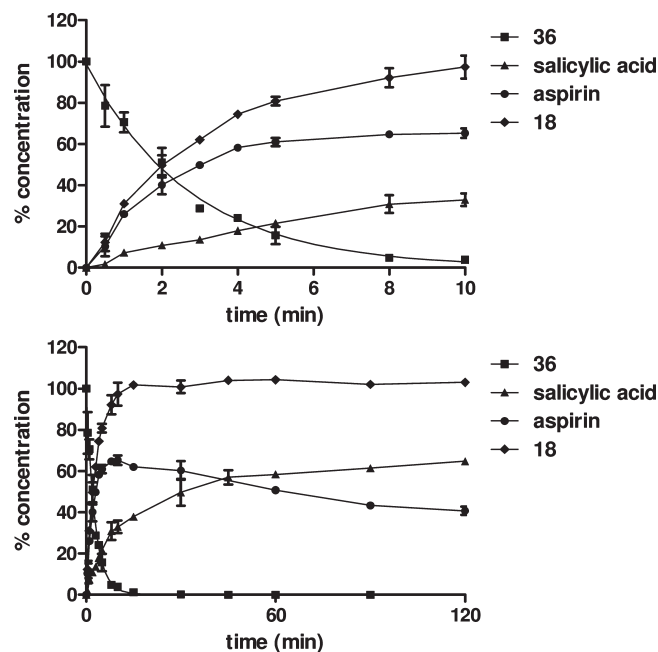
Table 1. Stability of the Compounds **28–39**, **41**, **42**, **45**, and **46** in Buffered Solutions (Percentage of Unchanged Compound after 3 h); Stability in Human Serum (Half-Life, Percent of Maximal Amounts of Aspirin Released and AUC Values at 10 min) and Antiaggregatory Activities

compd	stability in buffered solutions % unchanged at 3 h ^a		stability in human serum			platelet aggregation	
	pH 1.0	pH 7.4	<i>t</i> _{1/2} (min) ^b	% max of aspirin released ^c	AUC at 10 min	IC ₅₀ (μM) (CL 95%)	% inhibition ± SEM at 300 μM ^d
aspirin	90	90	63			54 (49–60)	
28	99	99	< 1	10.1	93.6	^d	17 ± 3
29	99	99	< 1	10.6	84.6	^d	8.6 ± 5.8
30	99	100	< 1	17.6	153.7	^d	29 ± 3
31	99	99	< 1	9.8	86.6	^d	36 ± 14
32	99	98	< 1	5.0	43.6	163 (132–198)	
33	99	98	1.4	48.3	387.6	100 (81–122)	
34	99	99	3.4	39.8	236.7	138 (105–181)	
35	99	99	5.4	22.4	105.2	140 (119–163)	
36	98	100	1.9	65.2	521.6	41 (35–49)	
37	99	99	3.4	34.2	220.1	97 (82–115)	
38	99	99	2.4	61.7	403.3	45 (36–56)	
39	98	98	4.6	43.0	192.2	129 (116–145)	
41	98	90	< 1	70.7	301.0	72 (66–78)	
42	98	70	< 1	59.2	515.8	38 (34–44)	
45	98	99	4.4	29.4	144.6	^d	27 ± 4
46	84	60	< 1	58.0	542.8	20 (15–25)	

^a SEM ≤ 1%. ^b SEM ≤ 0.2. ^c SEM ≤ 2.5%. ^d Due to the low activity of the compound, IC₅₀ could not be calculated: in this case the percent of inhibition is reported at 300 μM.

**Figure 1.** Time course of the metabolites of product **32** at 10 min and at 2 h incubation time in human serum: values are mean ± SEM (SEM ≤ 5; number of determinations = 3).

rat aorta strips precontracted with phenylephrine. The vasodilator action of the nitrooxy-substituted acids, intermediates in the synthesis of the target compounds and which are, together with salicylic acid, the final metabolites of the hydrolysis of these products, were assessed as well. As a nitrooxy-substituted acid for compound **46**, we used 6-(nitrooxymethyl)pyridine-2-carboxylic acid (**48**). All the products were able to relax the contracted tissue in a concentration-dependent manner. Their potencies, expressed as EC₅₀, are collected in Table 2. The nitrooxy-substituted acids are always less potent than the parent compounds. The case of **36** and of the related acid **18** is reported in Figure 4 as an example. This is likely due to their

**Figure 2.** Time course of the metabolites of product **36** at 10 min and at 2 h incubation time in human serum: values are mean ± SEM (SEM ≤ 5; number of determinations = 3).

greater hydrophilicity, which limits the penetration into the vascular smooth muscle cell. When the experiments were repeated in the presence of 1 μM ODQ (1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one), a decrease in the potencies was observed, in keeping with a NO-induced activation of the sGC as a mechanism which underlies the vasodilator effect.

Conclusions

We were able to develop a class of (nitrooxyacyloxy)methyl esters of aspirins which behave as true NO-donor aspirins. The aspirin release, evaluated in serum, follows a pseudo-first-order kinetic. The amount of aspirin released is strongly

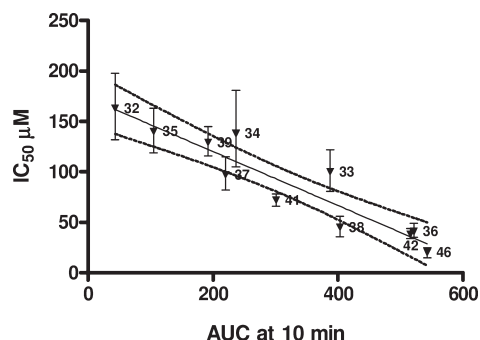


Figure 3. Linear correlation between AUC values of aspirin released in human serum from the indicated compounds and the corresponding antiaggregatory IC_{50} values.

dependent on the nature of acyloxy moiety. The best results were obtained using aromatic nitrooxyacyloyl moieties. In the case of the benzoyl derivatives, the most active compounds are those bearing NO-donor chains at *p*- or at *m*-position. All the products display in vitro vasodilator activities included the nitrooxy-substituted acids formed following metabolism. The products here described are new improved examples of NO-donor aspirins containing nitrooxy groups. They could represent an improved alternative to the use of aspirin in a variety of clinical applications. Studies are in progress aimed at examining their oral and dermal delivery characteristics.

Experimental Section

Synthesis. 1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 at 300 and 75 MHz, respectively, using SiMe₄ as the internal standard, and the following abbreviations were used to indicate the peak multiplicity: s = singlet, d = doublet, t = triplet, qt = quartet, qi = quintet, m = multiplet, br s = broad signal. Low resolution mass spectra were recorded with a Finnigan-Mat TSQ-700. Melting points were determined with a capillary apparatus (Buchi 540). Flash column chromatography was performed on silica gel (Merck Kiesegel 60, 230–400 mesh ASTM); PE stands for 40–60 petroleum ether. The progress of the reactions was followed by thin layer chromatography (TLC) on 5 cm × 20 cm plates with a layer thickness of 0.25 mm. Anhydrous magnesium sulfate was used as the drying agent for the organic phases. Organic solvents were removed under vacuum at 30 °C. Preparative HPLC was performed on a Lichrospher C₁₈ column (250 mm × 25 mm, 10 μm) (Merck Darmstadt, Germany) with a Varian ProStar mod-210 with Varian UV detector mod-325. Elemental analyses (C, H, N) were performed by REDOX (Monza), and the results are within ±0.4% of the theoretical values. Compounds **1**,²⁵ **2**,²⁶ **3**,²⁷ **4**,²⁸ **5**,³³ **6**,³³ **7**,³⁴ **14**,²⁹ **19**,²⁹ **20**,³⁵ **24**,³⁶ **26**,³⁰ **27**,³⁷ **43**,³⁸ and **48**³⁰ were synthesized according to literature. Compound **15** was synthesized with standard method³⁹ starting from 4-allylbenzaldehyde.²⁹

General Procedure for the Preparation of 8, 9, 10. A solution of the appropriate bromo derivative (2.20 g, 9.05 mmol) and AgNO₃ (3.10 g, 18.10 mmol) in CH₃CN (25 mL) was stirred at 70 °C for 1 h. Then brine was added to precipitate the excess of AgNO₃, the mixture was filtered through celite and concentrated under reduced pressure. The residue was treated with CH₂Cl₂ (50 mL) and H₂O (50 mL). After separation, the aqueous layer was extracted twice with CH₂Cl₂ (50 mL). The combined organic layers were dried, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE/EtOAc 90/10 v/v) to give the compound as a pale-yellow oil.

3-(2-Formylphenoxy)propyl Nitrate (8). Yield 70%. 1H NMR (CDCl₃) δ 2.31 (qi, 2H, $-CH_2CH_2ONO_2$), 4.20 (t, 2H,

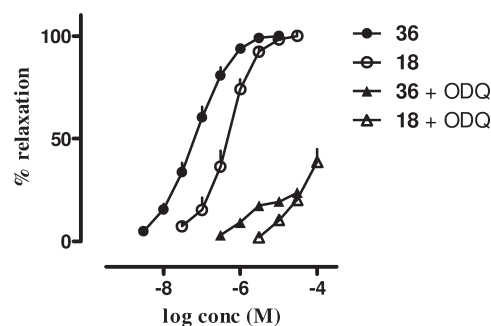


Figure 4. Concentration response curves for vasodilator activity of the compound **36** and of its corresponding nitrooxy-substituted acid **18** in the absence and in the presence of ODQ.

$-OCH_2-$, $^3J_{HH} = 6.0$ Hz), 4.71 (t, 2H, $-CH_2ONO_2$, $^3J_{HH} = 6.0$ Hz), 6.98 (d, 1H, C₆H₄), 7.06 (t, 1H, C₆H₄), 7.53–7.59 (m, 1H, C₆H₄), 7.82–7.85 (m, 1H, C₆H₄), 10.48 (s, 1H, $-CHO$). ^{13}C NMR (CDCl₃) δ 27.0, 64.2, 69.6, 112.3, 121.2, 124.9, 128.8, 136.0, 160.6, 189.3. MS (CI) m/z 226 ($M + 1$)⁺.

3-(3-Formylphenoxy)propyl Nitrate (9). Yield 90%. 1H NMR (CDCl₃) δ 2.25 (qi, 2H, $-CH_2CH_2ONO_2$), 4.14 (t, 2H, $-OCH_2-$, $^3J_{HH} = 6.0$ Hz), 4.69 (t, 2H, $-CH_2ONO_2$, $^3J_{HH} = 6.3$ Hz), 7.13–7.21 (m, 1H, C₆H₄), 7.38–7.50 (m, 3H, C₆H₄), 9.97 (s, 1H, $-CHO$). ^{13}C NMR (CDCl₃) δ 26.9, 63.9, 69.8, 112.6, 121.8, 124.0, 130.2, 137.8, 159.0, 192.0. MS (CI) m/z 226 ($M + 1$)⁺.

3-(4-Formylphenoxy)propyl Nitrate (10). Yield 93%. 1H NMR (CDCl₃) δ 2.26 (qi, 2H, $-CH_2CH_2ONO_2$), 4.16 (t, 2H, $-OCH_2-$, $^3J_{HH} = 6.0$ Hz), 4.69 (t, 2H, $-CH_2ONO_2$, $^3J_{HH} = 6.3$ Hz), 7.00 (d, 2H, C₆H₄), 7.84 (d, 2H, C₆H₄), 9.89 (s, 1H, $-CHO$). ^{13}C NMR (CDCl₃) δ 26.9, 64.0, 69.7, 115.2, 130.3, 132.0, 163.4, 190.8. MS (CI) m/z 226 ($M + 1$)⁺.

3-(4-Formylphenyl)propyl Nitrate (17). A solution of NaBH₄ (11.3 g, 0.30 mol) in dry THF (250 mL) was slowly added to amylene (115 mL, 1.1 mol) stirred at 0 °C. Then BF₃·Et₂O (27.5 mL, 0.22 mol) was added in 30 min to the mixture maintained at 0 °C. After 5.5 h, a solution of **15** (4.20 g, 22.1 mmol) in dry THF (100 mL) was slowly added and the stirring was continued for 24 h. Then to the mixture, cooled at 0 °C, H₂O (140 mL), NaOH 3 M (140 mL), and H₂O₂ 30% (210 mL) were added and the resulting mixture was heated at 40 °C for 1.5 h. After separation, the organic layer was washed with H₂O (100 mL), dried, filtered, and concentrated under reduced pressure. The crude product so obtained was purified by flash chromatography (PE/EtOAc 8/2 to 6/4 v/v) to give 3-[4-(1,3-dioxolan-2-yl)phenyl]propan-1-ol (**15a**) as a colorless oil. HCl (4 M, 20 mL) was added to a stirred solution of **15a** (4.50 g, 21.61 mmol) in MeOH/H₂O 1/1 (90 mL). After 2 h, the reaction was completed; the mixture was concentrated under reduced pressure and extracted twice with CH₂Cl₂ (50 mL). The combined organic layers were washed with brine (20 mL), dried, filtered, and concentrated under reduced pressure to give **16** as a pale-yellow oil. Yield 97%.

NBS (4.49 g, 25.21 mmol) was added portionwise to a solution of **16** (3.45 g, 21.01 mmol) and Ph₃P (6.06 g, 23.11 mmol) in dry CH₃CN (50 mL), stirred at 0 °C. After 30 min, the reaction was completed, the mixture was diluted with CH₂Cl₂ (50 mL), washed twice with H₂O (50 mL), dried, filtered, and concentrated under reduced pressure. AgNO₃ (7.14 g; 42.02 mmol) was added to a solution of the crude in CH₃CN (50 mL), and the mixture was heated at 70 °C for 1 h. Then brine was added to precipitate the excess of AgNO₃, the mixture was filtered through celite, and the residue was diluted with CH₂Cl₂ (50 mL) and washed with H₂O (50 mL). The organic layer was dried, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE/EtOAc 80/20 v/v) to give the title compound as a pale-yellow oil.

Table 2. Vasodilator Activities of the Products **1–4**, **11–14**, **18**, **19**, **21–23**, **25**, **28–39**, **41**, **42**, **45**, **46**, and **48**

R	X	Position	n	m	Y	Compound	Vasodilator activity	
							EC ₅₀ (μM) ± SEM	EC ₅₀ (μM) ± SEM + 1 μM ODO
	-	-	2	0	Y ₁	1	8.3 ± 1.4	a)
	-	-	2	0	Y ₂	28	1.2 ± 0.2	b)
	-	-	2	1	Y ₁	2	5.8 ± 0.8	a)
	-	-	2	1	Y ₂	29	0.39 ± 0.06	b)
	-	-	3	0	Y ₁	3	20 ± 3	a)
	-	-	3	0	Y ₂	30	1.6 ± 0.2	b)
	-	-	3	1	Y ₁	4	6.8 ± 0.4	a)
	-	-	3	1	Y ₂	31	0.52 ± 0.09	b)
	O	Orto	1	0	Y ₁	11	20 ± 2	a)
	O	Orto	1	0	Y ₂	32	4.0 ± 1.2	b)
	O	Meta	1	0	Y ₁	12	3.8 ± 0.5	a)
	O	Meta	1	0	Y ₂	33	0.14 ± 0.03	b)
	O	Para	1	0	Y ₁	13	0.62 ± 0.07	66 ± 12
	O	Para	1	0	Y ₂	34	0.017 ± 0.003	b)
	O	Para	1	1	Y ₁	14	0.28 ± 0.04	67 ± 6
	O	Para	1	1	Y ₂	35	0.041 ± 0.007	b)
	CH ₂	Para	0	0	Y ₁	18	0.51 ± 0.08	a)
	CH ₂	Para	0	0	Y ₂	36	0.075 ± 0.014	b)
	CH ₂	Para	0	1	Y ₁	19	0.33 ± 0.06	a)
	CH ₂	Para	0	1	Y ₂	37	0.052 ± 0.007	b)
	S	Para	1	0	Y ₁	21	0.27 ± 0.05	53 ± 15
	S	Para	1	0	Y ₂	38	0.15 ± 0.02	b)
	S	Para	1	0	Y ₃	45	0.14 ± 0.03	b)
	S	Para	1	1	Y ₁	25	0.27 ± 0.04	56 ± 16
	S	Para	1	1	Y ₂	39	0.10 ± 0.03	b)
	SO	Para	1	0	Y ₁	22	6.0 ± 0.7	a)
	SO	Para	1	0	Y ₂	41	0.47 ± 0.07	b)
	SO ₂	Para	1	0	Y ₁	23	20 ± 2	a)
	SO ₂	Para	1	0	Y ₂	42	0.36 ± 0.09	b)
	-	-	-	-	Y ₁	48	12 ± 3	a)
-	-	-	-	-	Y ₂	46	0.82 ± 0.10	b)

^a The maximal concentration tested (100 μM) cannot reach the 50% of the effect. ^b The maximal concentration tested (30 μM due to insolubility limits) cannot reach the 50% of the effect.

Yield 76%. ¹H NMR (CDCl₃) δ 2.04–2.14 (m, 2H, –CH₂CH₂ONO₂), 2.83 (t, 2H, –CH₂–, ³J_{HH} = 7.5 Hz), 4.47 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.6 Hz), 7.36 (d, 2H, C₆H₄), 7.83 (d, 2H, C₆H₄), 9.99 (s, 1H, –CHO). ¹³C NMR (CDCl₃) δ 28.3, 32.3, 72.3, 129.4, 130.5, 135.3, 147.8, 192.2. MS (CI) *m/z* 210 (M + 1)⁺.

General Procedure for the Preparation of 11, 12, 13, 18. KMnO₄ (2.00 g, 12.58 mmol) was added to a solution of the appropriate aldehyde (8.39 mmol) in acetone (25 mL) and stirred at 0 °C. The reaction was allowed to reach rt, and it was completed after 1 h. Oxalic acid was added until the color of the mixture became green, and then the mixture was filtered and the filtrate was diluted with CH₂Cl₂ (50 mL). The organic layer was washed with H₂O (50 mL) and then dried, filtered, and concentrated under reduced pressure.

2-[3-(Nitrooxy)propoxy]benzoic Acid (11). The crude product so obtained was purified by flash chromatography (PE/EtOAc/HCOOH 90/10/0.1 v/v/v) to give the title compound as a colorless oil; yield 47%. ¹H NMR (CDCl₃) δ 2.34 (qi, 2H, –CH₂CH₂ONO₂), 4.30 (t, 2H, –OCH₂–, ³J_{HH} = 6.0 Hz), 4.73 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.0 Hz), 7.03 (d, 1H, C₆H₄), 7.11 (t, 1H, C₆H₄), 7.53–7.59 (m, 1H, C₆H₄), 8.09–8.12 (m, 1H, C₆H₄), 11.0 (br s, 1H, –COOH). ¹³C NMR (CDCl₃) δ 26.9,

65.5, 69.5, 112.8, 118.3, 121.9, 133.6, 135.0, 157.8, 167.7. MS (CI) *m/z* 242 (M + 1)⁺.

3-[3-(Nitrooxy)propoxy]benzoic Acid (12). Melting point: 110–111 °C (from *i*Pr₂O); white solid; yield 75%. ¹H NMR (DMSO-*d*₆) δ 2.16 (qi, 2H, –CH₂CH₂ONO₂), 4.12 (t, 2H, –OCH₂–), 4.70 (t, 2H, –CH₂ONO₂), 7.19–7.21 (m, 1H, C₆H₄), 7.40–7.71 (m, 3H, C₆H₄), 13.0 (br s, 1H, –COOH). ¹³C NMR (DMSO-*d*₆) δ 26.6, 64.6, 71.3, 114.9, 119.7, 122.2, 130.1, 132.6, 158.7, 167.5. MS (CI) *m/z* 242 (M + 1)⁺.

4-[3-(Nitrooxy)propoxy]benzoic Acid (13). Melting point: 137–138 °C (from *i*Pr₂O/*i*PrOH); white solid; yield 81%. ¹H NMR (DMSO-*d*₆) δ 2.20 (qi, 2H, –CH₂CH₂ONO₂), 4.18 (t, 2H, –OCH₂–, ³J_{HH} = 6.0 Hz), 4.73 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.3 Hz), 7.05 (d, 2H, C₆H₄), 7.93 (d, 2H, C₆H₄), 12.7 (s, 1H, –COOH). ¹³C NMR (DMSO-*d*₆) δ 26.5, 64.7, 71.3, 114.6, 123.5, 131.8, 162.3, 167.4. MS (CI) *m/z* 242 (M + 1)⁺.

4-[3-(Nitrooxy)propyl]benzoic Acid (18). Melting point: 125.5–126.5 °C (from *i*Pr₂O); white solid; yield 89%. ¹H NMR (CDCl₃) δ 2.09 (qi, 2H, –CH₂CH₂ONO₂), 2.82 (t, 2H, C₆H₄CH₂–, ³J_{HH} = 7.5 Hz), 4.46 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.3 Hz), 7.30 (d, 2H, C₆H₄), 8.06 (d, 2H, C₆H₄), 11.7 (br s, 1H, –COOH). ¹³C NMR (CDCl₃) δ 28.0, 31.9, 72.0, 127.6, 128.6, 130.7, 146.7, 171.9. MS (CI) *m/z* 226 (M + 1)⁺.

4-[3-(Nitrooxy)propylthio]benzoic Acid (21). A solution of **20** (2.70 g, 10.0 mmol) and AgNO₃ (3.40 g, 20.0 mmol) in CH₃CN (50 mL) was stirred at 70 °C for 5 h. Then brine was added to precipitate the excess of AgNO₃, and the mixture was filtered through celite and concentrated under reduced pressure. The residue was treated with CH₂Cl₂ (50 mL) and H₂O (50 mL). After separation, the aqueous layer was extracted twice with CH₂Cl₂ (50 mL). The combined organic layers were dried, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE/EtOAc/HCOOH 80/20/0.1 v/v/v) to give the title compound as a white solid. Yield 80%; mp 103.5–105.5 °C (from iPr₂O/iPrOH). ¹H NMR (CD₃OD) δ 2.07 (q, 2H, –CH₂CH₂ONO₂), 3.13 (t, 2H, –SCH₂–, ³J_{HH} = 7.2 Hz), 4.60 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.3 Hz), 7.38 (d, 2H, C₆H₄), 7.93 (d, 2H, C₆H₄). ¹³C NMR (CD₃OD) δ 27.4, 28.9, 72.8, 127.9, 128.8, 131.3, 144.4, 169.4. MS (CI) *m/z* 258 (M + 1)⁺.

4-[3-(Nitrooxy)propyl-1-sulfinyl]benzoic Acid (22). A solution of 70% mCPBA (0.25 g, 1.0 mmol) in dry CH₂Cl₂ (10 mL) was slowly added to a solution of **21** (0.26 g, 1.0 mmol) in dry CH₂Cl₂ (15 mL), stirred at –78 °C. At the end of the addition, the reaction was completed. The mixture was poured in 10% Na₂SO₃ (50 mL), the layers separated, and the aqueous layer extracted twice with Et₂O (50 mL). The organic layers were dried, filtered, and concentrated under reduced pressure. The crude product so obtained was purified by flash chromatography (PE/EtOAc/HCOOH 80/20/0.1 v/v/v) to give the title compound as a white solid. Yield 55%; mp 154.5–155.0 °C. ¹H NMR (DMSO-*d*₆) δ 1.70–1.90 (m, 1H, –CH_aH_bCH₂ONO₂), 2.00–2.14 (m, 1H, –CH_aH_bCH₂ONO₂), 2.86–2.95 (m, 1H, SOCH_aH_b–), 3.15–3.35 (m, 1H, SOCH_aH_b–), 4.58 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.3 Hz), 7.80 (d, 2H, C₆H₄), 8.12 (d, 2H, C₆H₄), 13.32 (br s, 1H, COOH). ¹³C NMR (DMSO-*d*₆) δ 19.7, 51.7, 73.0, 125.1, 131.1, 133.7, 149.4, 167.4. MS (CI) *m/z* 274 (M + 1)⁺.

4-[3-(Nitrooxy)propyl-1-sulfonyl]benzoic Acid (23). A solution of 85% H₂O₂ (0.31 g, 7.76 mmol) in CF₃COOH (2 mL) was slowly added to a suspension of **21** (0.50 g, 1.94 mmol) in CF₃COOH (5 mL) stirred at 0 °C. The reaction was allowed to reach rt, and it was completed after 1.5 h. The mixture was poured into ice/water and the white solid so obtained was filtered. The crude product was purified by flash chromatography (CH₂Cl₂/EtOAc/HCOOH 95/5/0.1 v/v/v) to give the title compound as a white solid. Yield 74%; mp 172–173.5 °C. ¹H NMR (DMSO-*d*₆) δ 1.97 (q, 2H, –CH₂CH₂ONO₂), 3.51 (t, 2H, SO₂CH₂CH₂–, ³J_{HH} = 7.5 Hz), 4.56 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.3 Hz), 8.04 (d, 2H, C₆H₄), 8.19 (d, 2H, C₆H₄), 13.6 (br s, 1H, COOH). ¹³C NMR (DMSO-*d*₆) δ 20.1, 50.9, 71.2, 128.1, 130.1, 135.5, 142.0, 166.0. MS (CI) *m/z* 290 (M + 1)⁺.

4-[[2,3-Bis(nitrooxy)propyl]thio]benzoic Acid (25). Iodine (8.2 g, 32.38 mmol) was added portionwise to a stirred solution of **24** (6.30 g, 32.38 mmol) and AgNO₃ (5.50 g, 32.38 mmol) in CH₃CN (100 mL) kept at –15 °C. At the end of the addition, the stirring was continued for 1 h. Then AgNO₃ (11.0 g, 64.76 mmol) was added, and the mixture was heated at 70 °C for 16 h. After cooling, the mixture was filtered through celite. The filtrate was concentrated under reduced pressure, dissolved in water (50 mL), and extracted with CH₂Cl₂ (3 × 100 mL). The combined organic layers were washed with brine (50 mL), dried, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE/EtOAc/HCOOH 80/20/0.1 v/v/v) to give the title compound as white solid. Yield 60%; mp 132–133 °C (toluene). ¹H NMR (DMSO-*d*₆) δ 3.50–3.63 (m, 2H, –SCH₂–), 4.77–4.86 (m, 1H, –CH₂H_bONO₂), 5.00–5.05 (m, 1H, –CH_aH_bONO₂), 5.52–5.58 (m, 1H, –CH(ONO₂)–), 7.53 (d, 2H, C₆H₄), 7.87 (d, 2H, C₆H₄), 13.00 (br s, 1H, –COOH). ¹³C NMR (DMSO-*d*₆) δ 30.5, 71.3, 78.6, 127.8, 128.7, 130.5, 141.1, 167.2. MS (CI) *m/z* 319 (M + 1)⁺.

General Procedure for the Preparation of 28–40. To a solution of the appropriate carboxylic acid (1.00 mmol) in DMF (5 mL) were added Cs₂CO₃ (0.50 mmol) and after 10 min **27** (1.00 mmol). The mixture was stirred for 24 h and then poured in H₂O (10 mL) and extracted with Et₂O (3 × 10 mL). The combined organic layers were washed with a saturated solution of NaHCO₃ (10 mL), dried, filtered, and concentrated under reduced pressure. The crude product so obtained was purified by flash chromatography. Chromatographic eluents and yields of the products were as follow.

[[6-(Nitrooxy)hexanoyl]oxy]methyl 2-(Acetyloxy)benzoate (28). Eluent (PE/EtOAc 9/1 v/v); colorless oil; yield 70%. ¹H NMR (CDCl₃) δ 1.41–1.49 (m, 2H), 1.64–1.78 (m, 4H) (–CH₂CH₂CH₂CH₂ONO₂), 2.36 (s, 3H, CH₃COO–), 2.41 (t, 2H, –OCOCH₂–, ³J_{HH} = 7.2 Hz), 4.41 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.6 Hz), 5.95 (s, 2H, –OCH₂O–), 7.12 (d, 1H, C₆H₄), 7.33 (t, 1H, C₆H₄), 7.61 (t, 1H, C₆H₄), 8.07 (d, 1H, C₆H₄). ¹³C NMR (CDCl₃) δ 21.2, 24.3, 25.3, 26.7, 33.8, 73.1, 79.4, 122.2, 124.3, 126.4, 132.4, 135.0, 151.4, 163.2, 169.9, 172.2. MS (CI) *m/z* 370 (M + 1)⁺.

[[5,6-Bis(nitrooxy)hexanoyl]oxy]methyl 2-(Acetyloxy)benzoate (29). Eluent (PE/EtOAc 8/2 v/v); colorless oil; yield 36%. ¹H NMR (CDCl₃) δ 1.74–1.83 (m, 4H, –CH₂CH₂CH–), 2.36 (s, 3H, CH₃COO–), 2.45–2.48 (m, 2H, –OCOCH₂–), 4.39–4.46 (m, 1H, –CH_aH_bONO₂), 4.68–4.74 (m, 1H, –CH_aH_bONO₂), 5.25–5.28 (m, 1H, –CHONO₂–), 5.95 (s, 2H, –OCH₂O–), 7.12 (d, 1H, C₆H₄), 7.34 (t, 1H, C₆H₄), 7.61 (t, 1H, C₆H₄), 8.08 (d, 1H, C₆H₄). ¹³C NMR (CDCl₃) δ 20.0, 21.0, 28.4, 33.0, 71.0, 78.7, 79.2, 121.8, 124.0, 126.2, 132.2, 134.9, 151.1, 163.0, 169.6, 171.3. MS (CI) *m/z* 431 (M + 1)⁺.

[[7-(Nitrooxy)heptanoyl]oxy]methyl 2-(Acetyloxy)benzoate (30). Eluent (PE/EtOAc 8/2 v/v); colorless oil; yield 52%. ¹H NMR (CDCl₃) δ 1.34–1.43 (m, 4H), 1.61–1.71 (m, 4H) (–CH₂CH₂CH₂CH₂CH₂ONO₂), 2.36–2.42 (m, 5H, CH₃COO– + –OCOCH₂–), 4.41 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.6 Hz), 5.94 (s, 2H, –OCH₂O–), 7.12 (d, 1H, C₆H₄), 7.33 (t, 1H, C₆H₄), 7.61 (t, 1H, C₆H₄), 8.07 (d, 1H, C₆H₄). ¹³C NMR (CDCl₃) δ 21.0, 24.3, 25.3, 26.5, 28.4, 33.7, 73.2, 79.2, 122.0, 124.1, 126.2, 132.2, 134.7, 151.2, 163.0, 169.7, 171.1. MS (CI) *m/z* 384 (M + 1)⁺.

[[6,7-Bis(nitrooxy)heptanoyl]oxy]methyl 2-(Acetyloxy)benzoate (31). The crude product was purified partially by flash chromatography (PE/EtOAc 8/2 v/v) and then by RP18 preparative HPLC (flow 39 mL/min, λ 226 nm, CH₃CN/H₂O 65/35 v/v, injection 2 mL, solution 100 mg/mL); colorless oil; yield 30%. ¹H NMR (CDCl₃) δ 1.45–1.51 (m, 2H), 1.65–1.76 (m, 4H) (–CH₂CH₂CH₂CH–), 2.35 (s, 3H, CH₃COO–), 2.42 (t, 2H, –OCOCH₂–, ³J_{HH} = 6.9 Hz), 4.38–4.45 (m, 1H, –CH_aH_bONO₂), 4.67–4.72 (m, 1H, –CH_aH_bONO₂), 5.15–5.28 (m, 1H, –CHONO₂–), 5.95 (s, 2H, –OCH₂O–), 7.12 (d, 1H, C₆H₄), 7.34 (t, 1H, C₆H₄), 7.60 (t, 1H, C₆H₄), 8.08 (d, 1H, C₆H₄). ¹³C NMR (CDCl₃) δ 20.9, 24.0, 24.2, 29.0, 33.4, 71.1, 78.9, 79.2, 121.9, 124.1, 126.2, 132.2, 134.8, 151.1, 163.0, 169.7, 171.8. MS (CI) *m/z* 445 (M + 1)⁺.

[[2-[3-(Nitrooxy)propoxy]benzoyl]oxy]methyl 2-(Acetyloxy)benzoate (32). Eluent (PE/EtOAc 90/10 v/v); colorless oil; yield 73%. ¹H NMR (CDCl₃) δ 2.21 (q, 2H, –CH₂CH₂ONO₂), 2.36 (s, 3H, CH₃COO–), 4.12 (t, 2H, –OCH₂CH₂–, ³J_{HH} = 5.7 Hz), 4.72 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.3 Hz), 6.16 (s, 2H, –OCH₂O–), 6.94 (d, 1H, C₆H₄), 7.01 (t, 1H, C₆H₄), 7.12 (d, 1H, C₆H₄), 7.33 (t, 1H, C₆H₄), 7.50 (t, 1H, C₆H₄), 7.60 (t, 1H, C₆H₄), 7.91 (d, 1H, C₆H₄), 8.11 (d, 1H, C₆H₄). ¹³C NMR (CDCl₃) 20.9, 26.9, 64.3, 69.9, 79.5, 113.1, 118.6, 120.7, 122.0, 124.0, 126.2, 132.3, 132.6, 134.6, 134.7, 151.2, 158.7, 163.1, 164.4, 169.7. MS (CI) *m/z* 434 (M + 1)⁺.

[[3-[3-(Nitrooxy)propoxy]benzoyl]oxy]methyl 2-(Acetyloxy)benzoate (33). Eluent (PE/EtOAc 90/10 v/v); colorless oil; yield 65%. ¹H NMR (CDCl₃) δ 2.23 (q, 2H, –CH₂CH₂ONO₂), 2.36 (s, 3H, CH₃COO–), 4.12 (t, 2H, –OCH₂CH₂–, ³J_{HH} = 6.0 Hz), 4.67 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.3 Hz), 6.19 (s, 2H, –OCH₂O–), 7.12 (d, 2H, C₆H₄), 7.30–7.40 (m, 2H, C₆H₄),

7.58–7.73 (m, 2H, C₆H₄), 7.71 (d, 1H, C₆H₄), 8.09 (d, 1H, C₆H₄). ¹³C NMR (CDCl₃) δ 21.0, 26.9, 63.8, 69.8, 79.9, 114.9, 120.7, 122.0, 123.0, 124.0, 126.2, 129.7, 130.2, 132.3, 134.7, 151.1, 158.5, 163.0, 165.0, 169.7. MS (CI) *m/z* 434 (M + 1)⁺.

(**4**-[3-(Nitrooxy)propoxy]benzoyl]oxy)methyl 2-(Acetyloxy)benzoate (**34**). Eluent (PE/EtOAc 8/2 v/v); colorless oil; yield 47%. ¹H NMR (CDCl₃) δ 2.23 (qi, 2H, –CH₂CH₂ONO₂), 2.35 (s, 3H, CH₃COO–), 4.12 (t, 2H, –OCH₂CH₂–, ³J_{HH} = 5.7 Hz), 4.66 (t, 2H, –CH₂ONO₂), 6.17 (s, 2H, –OCH₂O–), 6.91 (d, 2H, C₆H₄), 7.11 (d, 1H, C₆H₄), 7.32 (t, 1H, C₆H₄), 7.59 (t, 1H, C₆H₄), 8.03–8.10 (m, 3H, C₆H₄). ¹³C NMR (CDCl₃) δ 21.0, 26.8, 63.8, 69.6, 79.8, 114.2, 121.6, 122.1, 124.0, 126.1, 132.3, 132.3, 134.6, 151.1, 162.8, 163.2, 164.8, 169.7. MS (CI) *m/z* 434 (M + 1)⁺.

(**4**-[2,3-Bis(nitrooxy)propoxy]benzoyl]oxy)methyl 2-(Acetyloxy)benzoate (**35**). Eluent (PE/EtOAc 8/2 v/v); colorless oil; yield 37%. ¹H NMR (CDCl₃) δ 2.36 (s, 3H, CH₃COO–), 4.31 (d, 2H, –OCH₂CH–, ³J_{HH} = 5.4 Hz), 4.75–4.81 (m, 1H, –CH_aH_bONO₂), 4.90–4.96 (m, 1H, –CH_aH_bONO₂), 5.61–5.64 (m, 1H, –CHONO₂–), 6.17 (s, 2H, –OCH₂O–), 6.94 (d, 2H, C₆H₄), 7.11 (d, 1H, C₆H₄), 7.32 (t, 1H, C₆H₄), 7.59 (t, 1H, C₆H₄), 8.05–8.09 (m, 3H, C₆H₄). ¹³C NMR (CDCl₃) δ 20.9, 64.7, 68.6, 76.4, 79.8, 114.2, 122.0, 122.7, 124.0, 126.1, 132.2, 132.4, 134.7, 151.0, 161.6, 163.1, 164.6, 169.7. MS (CI) *m/z* 495 (M + 1)⁺.

(**4**-[3-(Nitrooxy)propyl]benzoyl]oxy)methyl 2-(Acetyloxy)benzoate (**36**). Eluent (PE/EtOAc 9/1 v/v); colorless oil; yield 52%. ¹H NMR (CDCl₃) δ 2.07 (qi, 2H, –CH₂CH₂ONO₂), 2.36 (s, 3H, CH₃COO–), 2.80 (t, 2H, –CH₂CH₂CH₂–, ³J_{HH} = 7.2 Hz), 4.44 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.3 Hz), 6.19 (s, 2H, –OCH₂O–), 7.12 (d, 1H, C₆H₄), 7.26–7.35 (m, 3H, C₆H₄), 7.59 (t, 1H, C₆H₄), 8.02–8.11 (m, 3H, C₆H₄). ¹³C NMR (CDCl₃) δ 21.0, 28.0, 31.8, 72.0, 79.7, 122.0, 124.0, 126.1, 127.1, 128.6, 130.5, 132.3, 134.7, 146.6, 151.1, 163.1, 165.0, 169.7. MS (CI) *m/z* 418 (M + 1)⁺.

(**4**-[2,3-Bis(nitrooxy)propyl]benzoyl]oxy)methyl 2-(Acetyloxy)benzoate (**37**). Eluent (PE/EtOAc 8/2 v/v); colorless oil; yield 60%. ¹H NMR (CDCl₃) δ 2.35 (s, 3H, CH₃COO–), 3.03–3.18 (m, 2H, –CH₂CH–), 4.40–4.46 (m, 1H, –CH_aH_bONO₂), 4.70–4.75 (m, 1H, –CH_aH_bONO₂), 5.42–5.50 (m, 1H, –CHONO₂–), 6.19 (s, 2H, –OCH₂O–), 7.12 (d, 1H, C₆H₄), 7.26–7.35 (m, 3H, C₆H₄), 7.59 (t, 1H, C₆H₄), 8.06–8.10 (m, 3H, C₆H₄). ¹³C NMR (CDCl₃) δ 21.0, 35.6, 60.4, 70.1, 78.7, 124.5, 126.4, 128.2, 129.9, 131.3, 132.2, 135.6, 141.4, 151.9, 159.6, 161.6, 169.5, 171.2. MS (CI) *m/z* 479 (M + 1)⁺.

(**4**-[3-(Nitrooxy)propyl]thio]benzoyl]oxy)methyl 2-(Acetyloxy)benzoate (**38**). Eluent (PE/EtOAc 9/1 v/v); colorless oil that became solid on standing; yield 70%. ¹H NMR (CDCl₃) δ 2.10 (qi, 2H, –CH₂CH₂ONO₂), 2.35 (s, 3H, CH₃COO–), 3.09 (t, 2H, –SCH₂CH₂–, ³J_{HH} = 7.9 Hz), 4.57 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.3 Hz), 6.18 (s, 2H, –OCH₂O–), 7.12 (d, 1H, C₆H₄), 7.30–7.34 (m, 3H, C₆H₄), 7.59 (t, 1H, C₆H₄), 7.99 (d, 2H, C₆H₄), 8.08 (d, 1H, C₆H₄). ¹³C NMR (CDCl₃) δ 21.0, 26.1, 28.1, 71.0, 79.8, 122.0, 124.0, 125.9, 126.1, 126.9, 130.6, 132.3, 134.7, 143.7, 151.1, 163.1, 164.8, 169.7. MS (CI) *m/z* 450 (M + 1)⁺.

(**4**-[2,3-Bis(nitrooxy)propyl]thio]benzoyl]oxy)methyl 2-(Acetyloxy)benzoate (**39**). Eluent (PE/EtOAc 8/2 v/v); colorless oil; yield 36%. ¹H NMR (CDCl₃) δ 2.36 (s, 3H, CH₃COO–), 3.20–3.28 (m, 1H, –SCH_aH_b–), 3.35–3.42 (m, 1H, –SCH_aH_b–), 4.63–4.69 (m, 1H, –CH_aH_bONO₂), 4.86–4.91 (m, 1H, –CH_aH_bONO₂), 5.28–5.36 (m, 1H, –CHONO₂–), 6.19 (s, 2H, –OCH₂O–), 7.12 (d, 1H, C₆H₄), 7.33 (t, 1H, C₆H₄), 7.42 (d, 2H, C₆H₄), 7.60 (t, 1H, C₆H₄), 8.03–8.11 (m, 3H, C₆H₄). ¹³C NMR (CDCl₃) δ 21.0, 31.4, 69.3, 77.2, 79.9, 122.0, 124.0, 126.2, 127.4, 128.3, 131.0, 132.3, 134.7, 141.1, 151.1, 163.0, 164.5, 169.7. MS (CI) *m/z* 511 (M + 1)⁺.

[2-(Acetyloxy)benzoyl]oxy)methyl 6-(Hydroxymethyl)pyridine-2-carboxylate (**40**). Eluent (CH₂Cl₂/EtOAc 9/1 v/v); pale-yellow oil; yield 17%. ¹H NMR (CDCl₃) δ 2.26 (s, 3H,

CH₃COO–), 3.55 (br s, 1H, –CH₂OH), 4.75 (s, 2H, –CH₂OH), 6.14 (s, 2H, –OCH₂O–), 7.02 (d, 1H, C₆H₄), 7.22 (t, 1H, C₆H₄), 7.48–7.52 (m, 2H, C₆H₄ + C₅H₃N), 7.74 (t, 1H, C₆H₄), 7.90–8.00 (m, 2H, C₅H₃N). ¹³C NMR (CDCl₃) δ 19.9, 63.5, 79.4, 120.8, 123.0, 123.4, 123.8, 125.2, 131.2, 133.8, 136.9, 144.6, 150.1, 160.0, 161.9, 162.6, 168.8. MS (CI) *m/z* 346 (M + 1)⁺.

(**4**-[3-(Nitrooxy)propyl]sulfinyl]benzoyl]oxy)methyl 2-(Acetyloxy)benzoate (**41**). A solution of 70% mCPBA (0.25 g, 1.0 mmol) in dry CH₂Cl₂ (7 mL) was slowly added to a solution of **38** (0.45 g, 1.0 mmol) in dry CH₂Cl₂ (7 mL) and stirred at –78 °C. At the end of the addition, the reaction was completed. The mixture was poured in 10% Na₂SO₃ (50 mL), the layers separated, and the aqueous layer was extracted twice with Et₂O (50 mL). The organic layers were dried, filtered, and concentrated under reduced pressure. The crude product so obtained was purified by flash chromatography (PE/EtOAc 6/4 v/v) to give the title compound as a colorless oil. Yield 73%. ¹H NMR (CDCl₃) δ 1.97–2.05 (m, 1H, –SOCH₂CH_aH_b–), 2.23–2.33 (m, 1H, –SOCH₂CH_aH_b–), 2.37 (s, 3H, CH₃COO–), 2.76–2.85 (m, 1H, –SOCH_aH_b–), 2.98–3.07 (m, 1H, –SOCH_aH_b–), 4.52–4.58 (m, 2H, –CH₂ONO₂), 6.21 (s, 2H, –OCH₂O–), 7.13 (d, 1H, C₆H₄), 7.33 (t, 1H, C₆H₄), 7.61 (t, 1H, C₆H₄), 7.70 (d, 2H, C₆H₄), 8.10 (d, 1H, C₆H₄), 8.26 (d, 2H, C₆H₄). ¹³C NMR (CDCl₃) δ 19.7, 21.0, 52.3, 71.1, 79.9, 121.8, 124.1, 124.1, 126.2, 131.0, 131.6, 132.2, 134.9, 149.1, 151.2, 162.9, 164.2, 169.7. MS (CI) *m/z* 466 (M + 1)⁺.

(**4**-[3-(Nitrooxy)propyl]sulfonyl]benzoyl]oxy)methyl 2-(Acetyloxy)benzoate (**42**). Oxone (0.40 g, 0.55 mmol) was added to a stirred solution of **38** (0.10 g, 0.22 mmol) in MeOH (3 mL) and H₂O (1 mL). Two hours later, the reaction was completed, and the mixture was diluted with H₂O (10 mL) and extracted with CH₂Cl₂ (3 × 10 mL). The combined organic layers were dried, filtered, and concentrated under reduced pressure. The crude product so obtained was purified by flash chromatography (PE/EtOAc 8/2 v/v) to give the title compound as a colorless oil. Yield 88%. ¹H NMR (CDCl₃) δ 2.20 (qi, 2H, –CH₂CH₂ONO₂), 2.36 (s, 3H, CH₃COO–), 3.21 (t, 2H, –SO₂CH₂CH₂–, ³J_{HH} = 7.2 Hz), 4.56 (t, 2H, –CH₂ONO₂, ³J_{HH} = 6.3 Hz), 6.22 (s, 2H, –OCH₂O–), 7.13 (d, 1H, C₆H₄), 7.34 (t, 1H, C₆H₄), 7.61 (t, 1H, C₆H₄), 8.01 (d, 2H, C₆H₄), 8.10 (d, 1H, C₆H₄), 8.29 (d, 2H, C₆H₄). ¹³C NMR (CDCl₃) δ 20.6, 21.0, 52.3, 70.2, 80.1, 121.7, 124.1, 126.2, 128.3, 131.1, 132.2, 134.0, 135.0, 143.1, 151.2, 162.9, 163.6, 169.7. MS (CI) *m/z* 482 (M + 1)⁺.

1-Chloroethyl 2-(Acetyloxy)benzoate (44). To a solution of **43** (10.0 g, 50.35 mmol) in dry CH₂Cl₂ (100 mL) stirred under N₂ at room temperature dry ZnCl₂ (0.14 g) was added. After 15 min, the reaction mixture was cooled at –15 °C and a solution of CH₃CHO (2.80 mL, 50.35 mmol) in dry CH₂Cl₂ (30 mL) was slowly added. The reaction mixture was allowed to reach room temperature and was stirred for 48 h. Then it was washed with H₂O (100 mL) and a saturated solution of NaHCO₃ (100 mL), dried, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE/EtOAc 90/10 v/v) to give the title compound as a colorless oil that became solid on standing. Yield 64%; mp 42.5–45.5 °C. ¹H NMR (CDCl₃) δ 1.89 (d, 3H, CH₃CH–, ³J_{HH} = 6.0 Hz), 2.36 (s, 3H, CH₃COO–), 6.73 (q, 1H, CH₃CH–, ³J_{HH} = 6.0 Hz), 7.13 (d, 1H, C₆H₄), 7.33 (t, 1H, C₆H₄), 7.59 (t, 1H, C₆H₄), 8.03 (d, 1H, C₆H₄). ¹³C NMR (CDCl₃) δ 21.0, 25.3, 81.1, 122.0, 124.0, 126.1, 131.9, 134.7, 151.0, 162.0, 169.5. MS (CI) *m/z* 243/245 (M + 1)⁺.

1-[(4-[3-(Nitrooxy)propyl]thio]benzoyl]oxy]ethyl 2-(Acetyloxy)benzoate (45). To a solution of **21** (0.50 g, 2.06 mmol) in DMF (5 mL) Cs₂CO₃ (0.34 g, 1.03 mmol) was added and after 10 min **44** (0.50 g, 2.06 mmol). The mixture was stirred for 4 days and then poured in H₂O (30 mL) and extracted with Et₂O (3 × 20 mL). The combined organic layers were washed with a saturated solution of NaHCO₃ (30 mL), dried, filtered, and concentrated under reduced pressure. The crude product was

purified by flash chromatography (PE/EtOAc 9/1 v/v) to give the title compound as a colorless oil. Yield 25%. ^1H NMR (CDCl_3) δ 1.72 (d, 3H, $\text{CH}_3\text{CH}-$, $^3J_{\text{HH}} = 5.4$ Hz), 2.10 (q, 2H, $-\text{CH}_2\text{CH}_2\text{ONO}_2$), 2.30 (s, 3H, $\text{CH}_3\text{COO}-$), 3.10 (t, 2H, $-\text{SCH}_2-$, $^3J_{\text{HH}} = 6.9$ Hz), 4.58 (t, 2H, $-\text{CH}_2\text{ONO}_2$, $^3J_{\text{HH}} = 6.0$ Hz), 7.11 (d, 1H, C_6H_4), 7.27–7.34 (m, 4H, $\text{C}_6\text{H}_4 + \text{CH}_3\text{CH}-$), 7.58 (t, 1H, C_6H_4), 7.97 (d, 2H, C_6H_4), 8.04 (d, 1H, C_6H_4). ^{13}C NMR (CDCl_3) δ 19.8, 21.0, 26.1, 28.2, 71.0, 89.6, 122.5, 123.9, 126.1, 126.4, 127.0, 130.5, 132.0, 134.4, 143.3, 150.9, 162.4, 164.0, 169.6. MS (CI) m/z 463 ($\text{M} + 1$) $^+$.

[(2-(Acetyloxy)benzoyl)oxy]methyl 6-[(Nitrooxy)methyl]pyridine-2-carboxylate (46). A solution of **40** (0.10 g, 0.29 mmol) in $(\text{CH}_3\text{CO})_2\text{O}$ (0.30 mL) was slowly added to a mixture of 65% HNO_3 (0.10 mL) and $(\text{CH}_3\text{CO})_2\text{O}$ (0.20 mL) and stirred at 0 °C. Then the reaction mixture was allowed to reach room temperature, and the stirring was continued for 2 h. The mixture was poured into H_2O (10 mL) and extracted with CH_2Cl_2 (5 \times 5 mL). The organic layers were dried, filtered, and concentrated under reduced pressure. The crude product so obtained was purified by flash chromatography ($\text{CH}_2\text{Cl}_2/\text{EtOAc}$ 95/5 v/v) to give the title compound as a colorless oil that became solid on standing; mp 73–78 °C; yield 50%. ^1H NMR (CDCl_3) δ 2.37 (s, 3H, $\text{CH}_3\text{COO}-$), 5.67 (s, 2H, $-\text{CH}_2\text{ONO}_2$), 6.26 (s, 2H, $-\text{OCH}_2\text{O}-$), 7.13 (d, 1H, C_6H_4), 7.33 (t, 1H, C_6H_4), 7.58–7.64 (m, 2H, $\text{C}_5\text{H}_3\text{N}$), 7.93 (t, 1H, C_6H_4), 8.09–8.17 (m, 2H, $\text{C}_6\text{H}_4 + \text{C}_5\text{H}_3\text{N}$). ^{13}C NMR (CDCl_3) δ 21.0, 73.9, 80.5, 121.8, 124.0, 125.4, 125.6, 126.2, 132.3, 134.8, 138.4, 147.0, 151.2, 153.8, 162.8, 163.2, 169.7. MS (CI) m/z 391 ($\text{M} + 1$) $^+$.

[(2-[3-(Nitrooxy)propoxy]benzoyl)oxy]methyl 2-(Hydroxybenzoate (47). 6 M HCl (4 mL) was added to a solution of **32** (0.20 g, 0.46 mmol) in 1,4-dioxane (4 mL) and the mixture was heated at 70 °C for 6 h. Then it was poured in H_2O (20 mL) and extracted with CH_2Cl_2 (3 \times 30 mL). The combined organic layers were dried, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (PE/EtOAc 90/10 v/v) to give the title compound as a colorless oil; yield 78%. ^1H NMR (CDCl_3) δ 2.22 (q, 2H, $-\text{CH}_2\text{CH}_2\text{ONO}_2$), 4.13 (t, 2H, $-\text{OCH}_2\text{CH}_2-$, $^3J_{\text{HH}} = 5.7$ Hz), 4.72 (t, 2H, $-\text{CH}_2\text{ONO}_2$, $^3J_{\text{HH}} = 6.0$ Hz), 6.23 (s, 2H, $-\text{OCH}_2\text{O}-$), 6.87–7.04 (m, 4H, C_6H_4), 7.46–7.54 (m, 2H, C_6H_4), 7.89–7.93 (m, 2H, C_6H_4), 10.46 (s, 1H, OH). ^{13}C NMR (CDCl_3) δ 26.9, 64.2, 69.8, 79.5, 111.4, 113.1, 117.7, 118.3, 119.5, 120.7, 130.3, 132.6, 134.8, 136.6, 158.8, 162.0, 164.2, 169.0. MS (CI) m/z 392 ($\text{M} + 1$) $^+$.

Evaluation of Stability in Buffered Solutions and in Human Serum. Hydrolysis in Acidic Medium (pH 1.0) and in Phosphate Buffer (pH 7.4). A 2 mL aliquot of 0.5 mM solution of each compound in acetonitrile was diluted to 10 mL using HCl to reach pH 1.0 or phosphate buffer 50 mM to obtain pH 7.4. The resulting solution was maintained at 37 ± 0.5 °C and at appropriate time intervals a 20 μL aliquot of reaction solution was analyzed by RP-HPLC. All experiments were performed in triplicate.

Hydrolysis in Human Serum. A solution of each compound (10 mM) in acetonitrile was added to human serum (sterile-filtered from human male AB plasma, Sigma-Aldrich) preheated at 37 °C; the final concentration of the compound was 250 μM . The resulting solution was incubated at 37 ± 0.5 °C and at appropriate time intervals 300 μL of the reaction mixture was withdrawn and added to 450 μL of acetonitrile containing 0.1% trifluoroacetic acid in order to deproteinize the serum. The sample was sonicated, vortexed, and then centrifuged for 10 min at 2150g, and the clear supernatant was filtered by 0.45 μm PTFE filters (Alltech) and analyzed by RP-HPLC. All experiments were performed at least in triplicate.

The reverse-phase HPLC procedure allowed separation and quantitation of the remaining compound and of the products of hydrolysis (aspirin, salicylic acid, salicylate, and nitrooxy-substituted carboxylic acid). HPLC analyses were performed with a HP 1100 chromatograph system (Agilent Technologies, Palo

Alto, CA) equipped with a quaternary pump (model G1311A), a membrane degasser (G1379A), and a diode-array detector (DAD) (model G1315B) integrated in the HP1100 system. Data analysis was done using a HP ChemStation system (Agilent Technologies). The injection volume was 20 μL (Rheodyne, Cotati, CA). The analytical column was a Nucleosil 100-5C18 Nautilus (250 mm \times 4.6 mm, 5 μm particle size) (Macherey-Nagel) eluting with a flow rate of 1.2 mL/min. The samples were analyzed using a gradient method employing a mobile phase consisting of acetonitrile/water with 0.1% trifluoroacetic acid 55/45 over the first 4 min, grading to 70/30 to 6 min, keeping 70/30 until 15 min and then back to 55/45 to 20 min. The column effluent was monitored at 226 nm (for compounds, aspirin, and nitrooxy-substituted carboxylic acids) and at 240 nm (for salicylic acid and salicylates) referenced against a 600 nm wavelength. Quantitation was done by comparison of peak areas with standards chromatographed under the same conditions.

Inhibition of Platelet Aggregation in Vitro. Venous blood samples were obtained from healthy volunteers who had not taken any drug for at least two weeks. Volunteers, who were treated according to Helsinki protocol for biomedical experimentation, gave their informed consent to the use of blood samples for research purposes. Platelet-rich plasma (PRP) was prepared by centrifugation of citrated blood at 210g for 20 min. Aliquots (500 μL) of PRP were added into aggregometer (Chrono-log 4902D) cuvettes, and aggregation was recorded as increased light transmission under continuous stirring (1000 rpm) at 37 °C for 10 min after addition of the stimulus. Collagen at submaximal concentration (0.8–1.5 $\mu\text{g}/\text{mL}$) was used as a platelet activator in PRP. Compounds under study were preincubated with PRP 10 min before addition of the stimulus (collagen). Vehicle alone (0.5% DMSO) added to PRP did not affect platelet function in control samples. At least five experiments for each compound were performed.

The antiaggregatory activity of tested compounds is evaluated as % inhibition of platelet aggregation compared to control samples. For most active compounds, IC_{50} values could be calculated by nonlinear regression analysis, otherwise % inhibition at maximal concentration tested (300 μM) is reported.

Vasodilator Activities. Thoracic aortas were isolated from male Wistar rats weighing 180–200 g. As few animals as possible were used. The purposes and the protocols of our studies have been approved by the Ministero della Salute, Rome, Italy. The endothelium was removed, the vessels were cut helically, and three strips were obtained from each aorta. The tissues were mounted under 1.0 g tension in organ baths containing 30 mL of Krebs-bicarbonate buffer with the following composition (mM): NaCl 111.2, KCl 5.0, CaCl_2 2.5, MgSO_4 1.2, KH_2PO_4 1.0, NaHCO_3 12.0, glucose 11.1, maintained at 37 °C and gassed with 95% O_2 – 5% CO_2 (pH = 7.4). The aortic strips were allowed to equilibrate for 1.5 h and then contracted with 1 μM L-phenylephrine. When the response to the agonist reached a plateau, cumulative concentrations of the vasodilating agent were added. Results are expressed as $\text{EC}_{50} \pm \text{SEM}$ (μM). The effects of 1 μM ODQ on relaxation were evaluated in a separate series of experiments in which it was added 5 min before the contraction. With this protocol, the inhibitor is preincubated for at least 30 min before the addition of the vasodilator compound. Responses were recorded by an isometric transducer connected to the MacLab System PowerLab. Addition of the drug vehicle, DMSO, had no appreciable effect on contraction level. At least five experiments for each compound were performed.

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Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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