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(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 liability 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 gastrosparing 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*-diazeniumdiolate 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 $AgNO_3$ in acetonitrile solution afforded the corresponding 3-nitrooxypropoxy analogues **8**−**10**. These latter intermediates treated with $KMnO_4$ 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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[a] Abbreviations: NSAIDs, nonsteroidal anti-inflammatory drugs; PRP, platelet rich plasma; NO, nitric oxide; sGC, soluble guanylate cyclase; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one.

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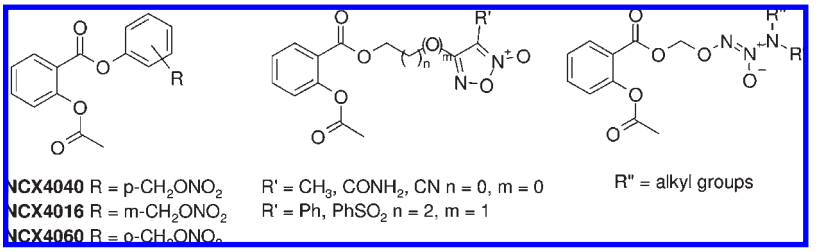
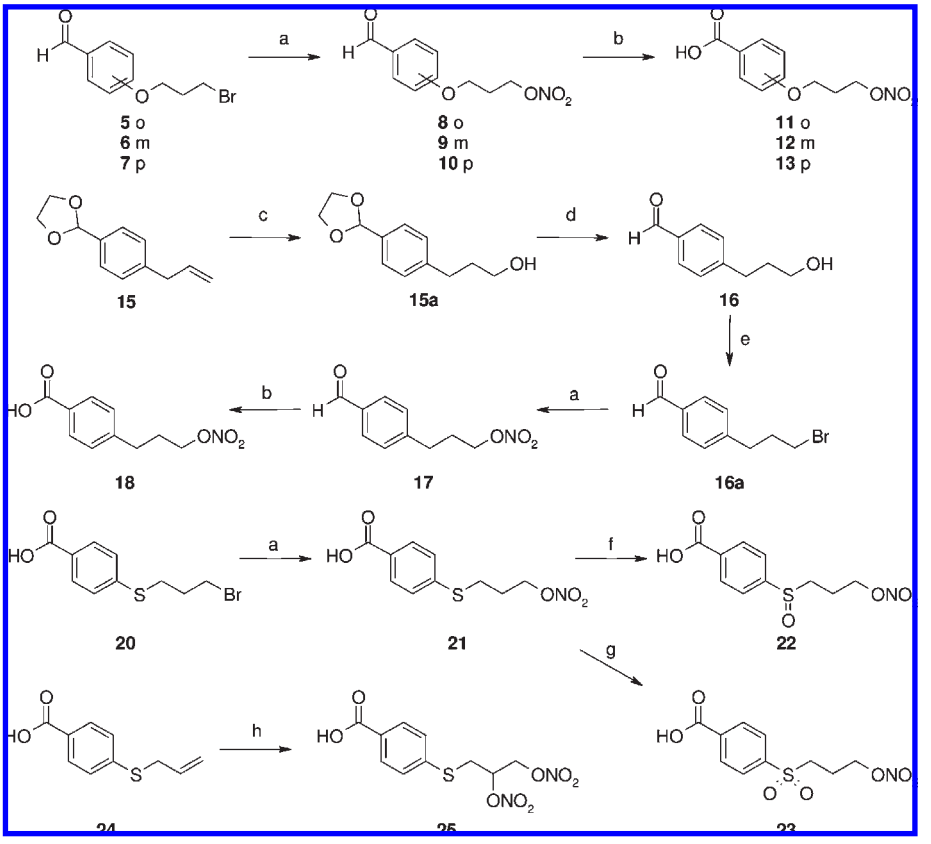
Chart 1. Examples of NO-donors Aspirin



Scheme 1[a]



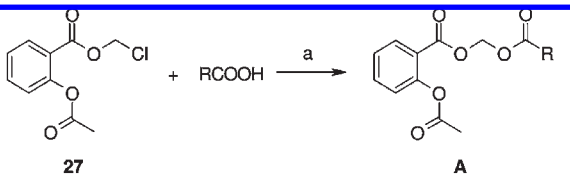
[a] Reagents and conditions: (a) $AgNO_3$, CH_3CN, 70 °C; (b) $KMnO_4$, acetone; (c) $(Sia)_2BH$, dry THF then NaOH, H_2O_2; (d) H_2O/MeOH, 4 M HCl, 60 °C; (e) Ph_3P, NBS, CH_3CN; (f) mCPBA, CH_2Cl_2, −78 °C; (g) 85%H_2O_2, CF_3COOH; (h) I_2, $AgNO_3$, CH_3CN rt then $AgNO_3$, CH_3CN reflux.

which was transformed into alcohol **15a** by action of disiamylborane ($(Sia)_2BH$) and then of H_2O_2 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 (Ph_3P) to give **16a** that was immediately treated with $AgNO_3$, 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 $AgNO_3$ in acetonitrile solution. Acids **22** and **23** were prepared from **21** for *m*-chloroperbenzoic acid and H_2O_2 oxidation, respectively. Acid **25** was prepared by action of I_2 and $AgNO_3$ in acetonitrile solution on 4-allylthiobenzoic acid (**24**).

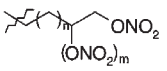
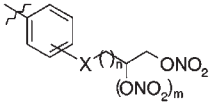
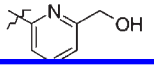
Most of the NO-aspirins described in the present work, namely products **28**−**39**, and the intermediate **40** were prepared by action of the cesium salt of appropriate acid prepared in situ on chloromethyl 2-(acetyloxy)benzoate (**27**) in DMF solution (Scheme 2). The remaining products were obtained 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 Cs_2CO_3 to produce the final compound **45**. Nitration of intermediate **40** with 65% HNO_3 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]



27 A

R	X	Position	n	m	RCOOH number	Compound A number
	-	-	2	0	**1**[25]	**28**
	-	-	2	1	**2**[26]	**29**
	-	-	3	0	**3**[27]	**30**
	-	-	3	1	**4**[28]	**31**
	O	Orto	1	0	**11**	**32**
	O	Meta	1	0	**12**	**33**
	O	Para	1	0	**13**	**34**
	O	Para	1	1	**14**[29]	**35**
	CH_2	Para	0	0	**18**	**36**
	CH_2	Para	0	1	**19**[29]	**37**
	S	Para	1	0	**21**	**38**
	S	Para	1	1	**25**	**39**
	-	-	-	-	**26**[30]	**40**

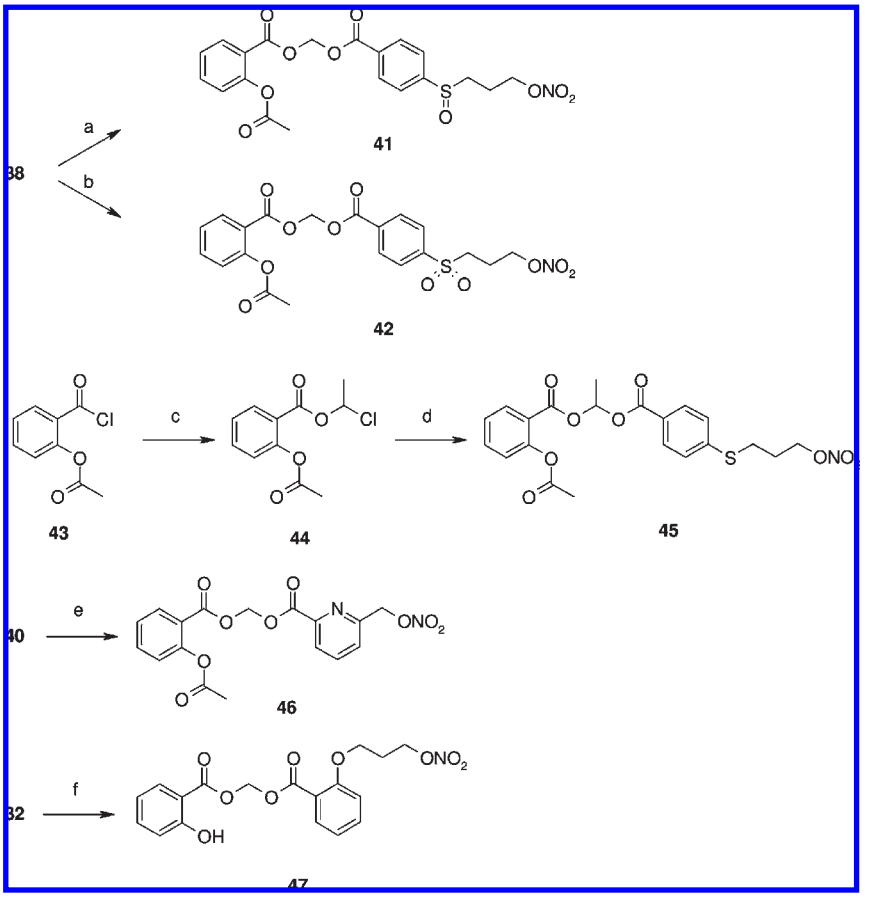
[a] Reagents and conditions: (a) Cs_2CO_3, DMF.

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 SO_2 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.

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

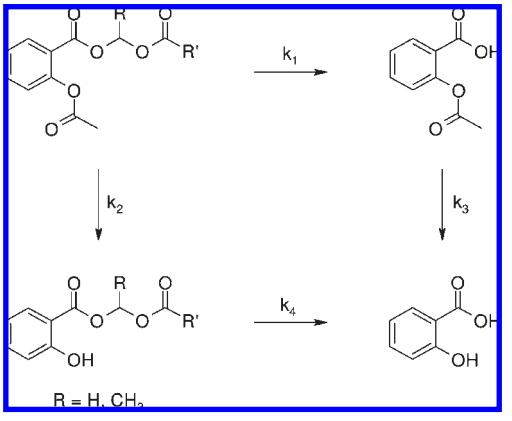
In Table 1, the maximal amounts of aspirin detected for each product, expressed as % of the initial ester concentration, are also collected. Analyses of the data show that all the compounds are very quickly hydrolyzed in serum. A number of products display $t_{1/2} < 1$ min, and this figure does not 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, CH_2Cl_2, −78 °C; (b) oxone, H_2O/MeOH; (c) CH_3CHO, $ZnCl_2$, CH_2Cl_2; (d) **21**, Cs_2CO_3, DMF; (e) $(CH_3CO)_2O$, 65% HNO_3, CH_2Cl_2; (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 IC_{50}, 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 μM 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 IC_{50} 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 IC_{50} 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).

$$IC_{50} = -0.27(\pm 0.03)AUC + 174(\pm 12) \qquad (2)$$

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

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

	stability in buffered solutions % unchanged at 3 h[a]		stability in human serum			platelet aggregation	
compd	pH 1.0	pH 7.4	$t_{1/2}$ (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, IC_{50} could not be calculated: in this case the percent of inhibition is reported at 300 μM.

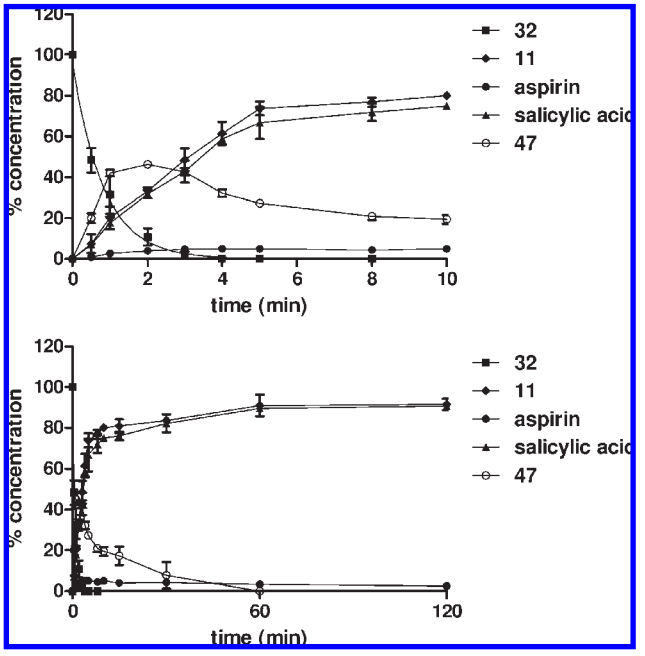


Figure 1. Time course of the metabolites of product **32** at 10 min and at 2 h incubation time in human serum: values are mean ± SEM (SEM ≤ 5; number of determinations = 3).

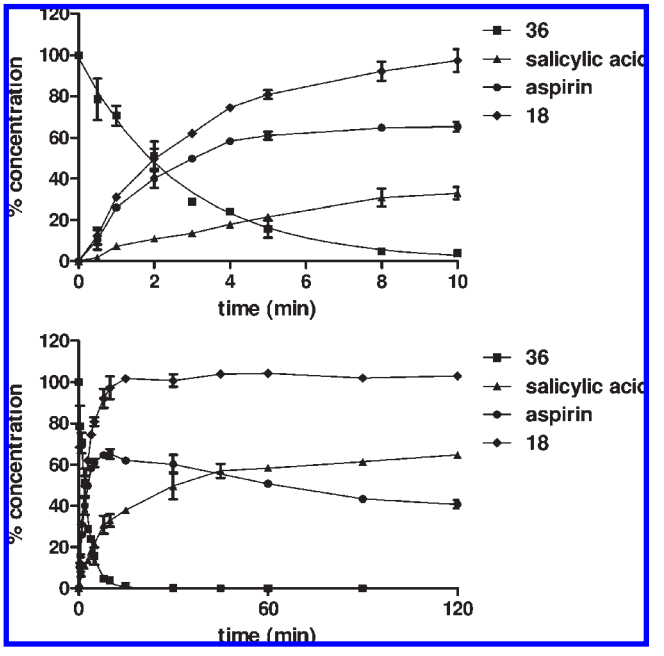


Figure 2. Time course of the metabolites of product **36** at 10 min and at 2 h incubation time in human serum: values are mean ± SEM (SEM ≤ 5; number of determinations = 3).

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 EC_{50}, 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 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

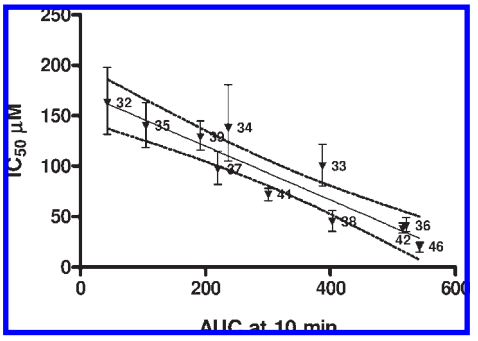


Figure 3. Linear correlation between AUC values of aspirin released in human serum from the indicated compounds and the corresponding antiaggregatory IC_{50} values.

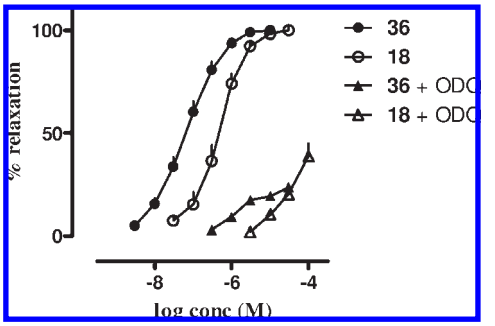


Figure 4. Concentration response curves for vasodilator activity of the compound **36** and of its corresponding nitrooxy-substituted acid **18** in the absence and in the presence of ODQ.

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 $SiMe_4$ 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 Kieselgel 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 C_{18} 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 method[39] 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 $AgNO_3$ (3.10 g, 18.10 mmol) in CH_3CN (25 mL) was stirred at 70 °C for 1 h. Then brine was added to precipitate the excess of $AgNO_3$, the mixture was filtered through celite and concentrated under reduced pressure. The residue was treated with CH_2Cl_2 (50 mL) and H_2O (50 mL). After separation, the aqueous layer was extracted twice with CH_2Cl_2 (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 ($CDCl_3$) δ 2.31 (qi, 2H, $-CH_2CH_2ONO_2$), 4.20 (t, 2H, $-OCH_2-$, $^3J_{HH}$ = 6.0 Hz), 4.71 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.0 Hz), 6.98 (d, 1H, C_6H_4), 7.06 (t, 1H, C_6H_4), 7.53−7.59 (m, 1H, C_6H_4), 7.82−7.85 (m, 1H, C_6H_4), 10.48 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) 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 $(M + 1)^+$.

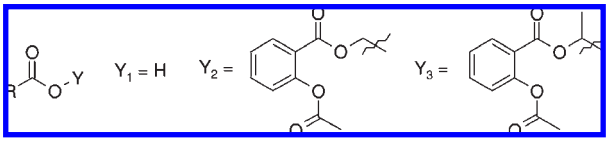
3-(3-Formylphenoxy)propyl Nitrate (9). Yield 90%. ^1H NMR ($CDCl_3$) δ 2.25 (qi, 2H, $-CH_2CH_2ONO_2$), 4.14 (t, 2H, $-OCH_2$, $^3J_{HH}$ = 6.0 Hz), 4.69 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.3 Hz), 7.13−7.21 (m, 1H, C_6H_4), 7.38−7.50 (m, 3H, C_6H_4), 9.97 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ 26.9, 63.9, 69.8, 112.6, 121.8, 124.0, 130.2, 137.8, 159.0, 192.0. MS (CI) m/z 226 $(M + 1)^+$.

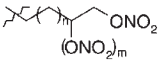
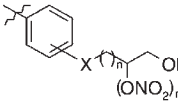
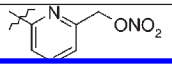
3-(4-Formylphenoxy)propyl Nitrate (10). Yield 93%. ^1H NMR ($CDCl_3$) δ 2.26 (qi, 2H, $-CH_2CH_2ONO_2$), 4.16 (t, 2H, $-OCH_2-$, $^3J_{HH}$ = 6.0 Hz), 4.69 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.3 Hz), 7.00 (d, 2H, C_6H_4), 7.84 (d, 2H, C_6H_4), 9.89 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ 26.9, 64.0, 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 $NaBH_4$ (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 $BF_3 \cdot Et_2O$ (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, H_2O (140 mL), NaOH 3 M (140 mL), and H_2O_2 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 H_2O (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/H_2O 1/1 (90 mL). After 2 h, the reaction was completed; the mixture was concentrated under reduced pressure and extracted twice with CH_2Cl_2 (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 Ph_3P (6.06 g, 23.11 mmol) in dry CH_3CN (50 mL), stirred at 0 °C. After 30 min, the reaction was completed, the mixture was diluted with CH_2Cl_2 (50 mL), washed twice with H_2O (50 mL), dried, filtered, and concentrated under reduced pressure. $AgNO_3$ (7.14 g; 42.02 mmol) was added to a solution of the crude in CH_3CN (50 mL), and the mixture was heated at 70 °C for 1 h. Then brine was added to precipitate the excess of $AgNO_3$, the mixture was filtered through celite, and the residue was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (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 ODQ
	-	-	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%. ^{1}H NMR ($CDCl_3$) δ 2.04−2.14 (m, 2H, $-CH_2CH_2ONO_2$), 2.83 (t, 2H, $-CH_2-$, $^3J_{HH}$ = 7.5 Hz), 4.47 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.6 Hz), 7.36 (d, 2H, C_6H_4), 7.83 (d, 2H, C_6H_4), 9.99 (s, 1H, −CHO). ^{13}C NMR ($CDCl_3$) 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. $KMnO_4$ (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 CH_2Cl_2 (50 mL). The organic layer was washed with H_2O (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%. ^{1}H NMR ($CDCl_3$) δ 2.34 (qi, 2H, $-CH_2CH_2ONO_2$), 4.30 (t, 2H, $-OCH_2-$, $^3J_{HH}$ = 6.0 Hz), 4.73 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.0 Hz), 7.03 (d, 1H, C_6H_4), 7.11 (t, 1H, C_6H_4), 7.53−7.59 (m, 1H, C_6H_4), 8.09−8.12 (m, 1H, C_6H_4), 11.0 (br s, 1H, −COOH). ^{13}C NMR ($CDCl_3$) 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 iPr_2O); white solid; yield 75%. ^{1}H NMR (DMSO-d_6) δ 2.16 (qi, 2H, $-CH_2CH_2ONO_2$), 4.12 (t, 2H, $-OCH_2-$), 4.70 (t, 2H, $-CH_2ONO_2$), 7.19−7.21 (m, 1H, C_6H_4), 7.40−7.71 (m, 3H, C_6H_4), 13.0 (br s, 1H, −COOH). ^{13}C 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 iPr_2O/iPrOH); white solid; yield 81%. ^{1}H NMR (DMSO-d_6) δ 2.20 (qi, 2H, $-CH_2CH_2ONO_2$), 4.18 (t, 2H, $-OCH_2-$, $^3J_{HH}$ = 6.0 Hz), 4.73 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.3 Hz), 7.05 (d, 2H, C_6H_4), 7.93 (d, 2H, C_6H_4), 12.7 (s, 1H, −COOH). ^{13}C 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 iPr_2O); white solid; yield 89%. ^{1}H NMR ($CDCl_3$) δ 2.09 (qi, 2H, $-CH_2CH_2ONO_2$), 2.82 (t, 2H, $C_6H_4CH_2-$, $^3J_{HH}$ = 7.5 Hz), 4.46 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.3 Hz), 7.30 (d, 2H, C_6H_4), 8.06 (d, 2H, C_6H_4), 11.7 (br s, 1H, −COOH). ^{13}C NMR ($CDCl_3$) 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 $AgNO_3$ (3.40 g, 20.0 mmol) in CH_3CN (50 mL) was stirred at 70 °C for 5 h. Then brine was added to precipitate the excess of $AgNO_3$, and the mixture was filtered through celite and concentrated under reduced pressure. The residue was treated with CH_2Cl_2 (50 mL) and H_2O (50 mL). After separation, the aqueous layer was extracted twice with CH_2Cl_2 (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 *i*Pr_2O/*i*PrOH). ^{1}H NMR (CD_3OD) δ 2.07 (qi, 2H, $-CH_2CH_2ONO_2$), 3.13 (t, 2H, $-SCH_2-$, $^3J_{HH}$ = 7.2 Hz), 4.60 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.3 Hz), 7.38 (d, 2H, C_6H_4), 7.93 (d, 2H, C_6H_4). ^{13}C NMR (CD_3OD) 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 CH_2Cl_2 (10 mL) was slowly added to a solution of **21** (0.26 g, 1.0 mmol) in dry CH_2Cl_2 (15 mL), stirred at −78 °C. At the end of the addition, the reaction was completed. The mixture was poured in 10% Na_2SO_3 (50 mL), the layers separated, and the aqueous layer extracted twice with Et_2O (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. ^{1}H NMR (DMSO-d_6) δ 1.70−1.90 (m, 1H, $-CH_aH_bCH_2ONO_2$), 2.00−2.14 (m, 1H, $-CH_aH_bCH_2ONO_2$), 2.86−2.95 (m, 1H, $SOCH_aH_b-$), 3.15−3.35 (m, 1H, $SOCH_aH_b-$), 4.58 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.3 Hz), 7.80 (d, 2H, C_6H_4), 8.12 (d, 2H, C_6H_4), 13.32 (br s, 1H, COO*H*). ^{13}C 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% H_2O_2 (0.31 g, 7.76 mmol) in CF_3COOH (2 mL) was slowly added to a suspension of **21** (0.50 g, 1.94 mmol) in CF_3COOH (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 (CH_2Cl_2/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. ^{1}H NMR (DMSO-d_6) δ 1.97 (qi, 2H, $-CH_2CH_2ONO_2$), 3.51 (t, 2H, $SO_2CH_2CH_2-$, $^3J_{HH}$ = 7.5 Hz), 4.56 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.3 Hz), 8.04 (d, 2H, C_6H_4), 8.19 (d, 2H, C_6H_4), 13.6 (br s, 1H, COO*H*). ^{13}C 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 $AgNO_3$ (5.50 g, 32.38 mmol) in CH_3CN (100 mL) kept at −15 °C. At the end of the addition, the stirring was continued for 1 h. Then $AgNO_3$ (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 CH_2Cl_2 (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). ^{1}H NMR (DMSO-d_6) δ 3.50−3.63 (*m*, 2H, $-SCH_2-$), 4.77−4.86 (m, 1H, $-CH_aH_bONO_2$), 5.00−5.05 (*m*, 1H, $-CH_aH_bONO_2$), 5.52−5.58 (m, 1H, $-CH(ONO_2)-$), 7.53 (d, 2H, C_6H_4), 7.87 (d, 2H, C_6H_4), 13.00 (br s, 1H, −COO*H*). ^{13}C 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 Cs_2CO_3 (0.50 mmol) and after 10 min **27** (1.00 mmol). The mixture was stirred for 24 h and then poured in H_2O (10 mL) and extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with a saturated solution of $NaHCO_3$ (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%. ^{1}H NMR ($CDCl_3$) δ 1.41−1.49 (m, 2H), 1.64−1.78 (m, 4H) ($-CH_2CH_2CH_2CH_2ONO_2$), 2.36 (s, 3H, CH_3COO-), 2.41 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.2 Hz), 4.41 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.6 Hz), 5.95 (s, 2H, $-OCH_2O-$), 7.12 (d, 1H, C_6H_4), 7.33 (t, 1H, C_6H_4), 7.61 (t, 1H, C_6H_4), 8.07 (d, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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%. ^{1}H NMR ($CDCl_3$) δ 1.74−1.83 (m, 4H, $-CH_2CH_2CH-$), 2.36 (s, 3H, CH_3COO-), 2.45−2.48 (m, 2H, $-OCOCH_2-$), 4.39−4.46 (m, 1H, $-CH_aH_bONO_2$), 4.68−4.74 (*m*, 1H, $-CH_aH_bONO_2$), 5.25−5.28 (m, 1H, $-CHONO_2-$), 5.95 (s, 2H, $-OCH_2O-$), 7.12 (d, 1H, C_6H_4), 7.34 (t, 1H, C_6H_4), 7.61 (t, 1H, C_6H_4), 8.08 (d, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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%. ^{1}H NMR ($CDCl_3$) δ 1.34−1.43 (m, 4H), 1.61−1.71 (m, 4H) ($-CH_2CH_2CH_2CH_2CH_2ONO_2$), 2.36−2.42 (m, 5H, CH_3-COO− + $-OCOCH_2-$), 4.41 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.6 Hz), 5.94 (s, 2H, $-OCH_2O-$), 7.12 (d, 1H, C_6H_4), 7.33 (t, 1H, C_6H_4), 7.61 (t, 1H, C_6H_4), 8.07 (d, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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, CH_3CN/H_2O 65/35 v/v, injection 2 mL, solution 100 mg/mL); colorless oil; yield 30%. ^{1}H NMR ($CDCl_3$) δ 1.45−1.51 (m, 2H), 1.65−1.76 (m, 4H) ($-CH_2CH_2CH_2CH-$), 2.35 (s, 3H, CH_3COO-), 2.42 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 6.9 Hz), 4.38−4.45 (m, 1H, $-CH_aH_bONO_2$), 4.67−4.72 (m, 1H, $-CH_aH_bONO_2$), 5.15−5.28 (m, 1H, $-CHONO_2-$), 5.95 (s, 2H, $-OCH_2O-$), 7.12 (d, 1H, C_6H_4), 7.34 (t, 1H, C_6H_4), 7.60 (t, 1H, C_6H_4), 8.08 (d, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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%. ^{1}H NMR ($CDCl_3$) δ 2.21 (qi, 2H, $-CH_2CH_2ONO_2$), 2.36 (s, 3H, CH_3COO-), 4.12 (t, 2H, $-OCH_2CH_2-$, $^3J_{HH}$ = 5.7 Hz), 4.72 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.3 Hz), 6.16 (s, 2H, $-OCH_2O-$), 6.94 (d, 1H, C_6H_4), 7.01 (t, 1H, C_6H_4), 7.12 (d, 1H, C_6H_4), 7.33 (t, 1H, C_6H_4), 7.50 (t, 1H, C_6H_4), 7.60 (t, 1H, C_6H_4),7.91 (d, 1H, C_6H_4), 8.11 (d, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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%. ^{1}H NMR ($CDCl_3$) δ 2.23 (qi, 2H, $-CH_2CH_2ONO_2$), 2.36 (s, 3H, CH_3COO-), 4.12 (t, 2H, $-OCH_2CH_2-$, $^3J_{HH}$ = 6.0 Hz), 4.67 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.3 Hz), 6.19 (s, 2H, $-OCH_2O-$), 7.12 (d, 2H, C_6H_4), 7.30−7.40 (m, 2H, C_6H_4),

7.58−7.73 (m, 2H, C_6H_4), 7.71 (d, 1H, C_6H_4), 8.09 (d, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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%. ^{1}H NMR ($CDCl_3$) δ 2.23 (qi, 2H, $-CH_2CH_2ONO_2$), 2.35 (s, 3H, CH_3COO-), 4.12 (t, 2H, $-OCH_2CH_2-$, $^3J_{HH} = 5.7$ Hz), 4.66 (t, 2H, $-CH_2ONO_2$,), 6.17 (s, 2H, $-OCH_2O-$), 6.91 (d, 2H, C_6H_4), 7.11 (d, 1H, C_6H_4), 7.32 (t, 1H, C_6H_4), 7.59 (t, 1H, C_6H_4), 8.03−8.10 (m, 3H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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%. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3COO-), 4.31 (d, 2H, $-OCH_2CH-$ $^3J_{HH} = 5.4$ Hz), 4.75−4.81 (m, 1H, $-CH_aH_bONO_2$), 4.90−4.96 (m, 1H, $-CH_aH_bONO_2$), 5.61−5.64 (m, 1H, $-CHONO_2-$), 6.17 (s, 2H, $-OCH_2O-$), 6.94 (d, 2H, C_6H_4), 7.11 (d, 1H, C_6H_4), 7.32 (t, 1H, C_6H_4), 7.59 (t, 1H, C_6H_4), 8.05−8.09 (m, 3H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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%. ^{1}H NMR ($CDCl_3$) δ 2.07 (qi, 2H, $-CH_2CH_2ONO_2$), 2.36 (s, 3H, CH_3COO-), 2.80 (t, 2H, $-CH_2CH_2CH_2-$, $^3J_{HH} = 7.2$ Hz), 4.44 (t, 2H, $-CH_2ONO_2$, $^3J_{HH} = 6.3$ Hz), 6.19 (s, 2H, $-OCH_2O-$), 7.12 (d, 1H, C_6H_4), 7.26−7.35 (m, 3H, C_6H_4), 7.59 (t, 1H, C_6H_4), 8.02−8.11 (m, 3H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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%. ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H, CH_3COO-), 3.03−3.18 (m, 2H, $-CH_2CH-$), 4.40−4.46 (m, 1H, $-CH_aH_bONO_2$), 4.70−4.75 (m, 1H, $-CH_aH_bONO_2$), 5.42−5.50 (m, 1H, $-CHONO_2-$), 6.19 (s, 2H, $-OCH_2O-$), 7.12 (d, 1H, C_6H_4), 7.26−7.35 (m, 3H, C_6H_4), 7.59 (t, 1H, C_6H_4), 8.06−8.10 (m, 3H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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%. ^{1}H NMR ($CDCl_3$) δ 2.10 (qi, 2H, $-CH_2CH_2ONO_2$), 2.35 (s, 3H, CH_3COO-), 3.09 (t, 2H, $-SCH_2CH_2-$, $^3J_{HH} = 7.9$ Hz), 4.57 (t, 2H, $-CH_2ONO_2$, $^3J_{HH} = 6.3$ Hz), 6.18 (s, 2H, $-OCH_2O-$), 7.12 (d, 1H, C_6H_4), 7.30−7.34 (m, 3H, C_6H_4), 7.59 (t, 1H, C_6H_4), 7.99 (d, 2H, C_6H_4), 8.08 (d, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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%. ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3COO-), 3.20−3.28 (m, 1H, $-SCH_aH_b-$), 3.35−3.42 (m, 1H, $-SCH_aH_b-$), 4.63−4.69 (m, 1H, $-CH_aH_bONO_2$), 4.86−4.91 (m, 1H, $-CH_aH_bONO_2$), 5.28−5.36 (m, 1H, $-CHONO_2-$), 6.19 (s, 2H, $-OCH_2O-$), 7.12 (d, 1H, C_6H_4), 7.33 (t, 1H, C_6H_4), 7.42 (d, 2H, C_6H_4), 7.60 (t, 1H, C_6H_4), 8.03−8.11 (m, 3H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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 (CH_2Cl_2/EtOAc 9/1 v/v); pale-yellow oil; yield 17%. ^{1}H NMR ($CDCl_3$) δ 2.26 (s, 3H, CH_3COO-), 3.55 (br s, 1H, $-CH_2OH$), 4.75 (s, 2H, $-CH_2OH$), 6.14 (s, 2H, $-OCH_2O-$), 7.02 (d, 1H, C_6H_4), 7.22 (t, 1H, C_6H_4), 7.48−7.52 (m, 2H, C_6H_4 + C_5H_3N), 7.74 (t, 1H, C_6H_4), 7.90−8.00 (m, 2H, C_5H_3N). ^{13}C NMR ($CDCl_3$) 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 CH_2Cl_2 (7 mL) was slowly added to a solution of **38** (0.45 g, 1.0 mmol) in dry CH_2Cl_2 (7 mL) and stirred at −78 °C. At the end of the addition, the reaction was completed. The mixture was poured in 10% Na_2SO_3 (50 mL), the layers separated, and the acqueous layer was extracted twice with Et_2O (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%. ^{1}H NMR ($CDCl_3$) δ 1.97−2.05 (m, 1H, $-SOCH_2CH_aH_b-$), 2.23−2.33 (m, 1H, $-SOCH_2CH_aH_b-$), 2.37 (s, 3H, CH_3COO-), 2.76−2.85 (m, 1H, $-SOCH_aH_b-$), 2.98−3.07 (m, 1H, $-SOCH_aH_b-$), 4.52−4.58 (m, 2H, $-CH_2ONO_2$), 6.21 (s, 2H, $-OCH_2O-$), 7.13 (d, 1H, C_6H_4), 7.33 (t, 1H, C_6H_4), 7.61 (t, 1H, C_6H_4), 7.70 (d, 2H, C_6H_4), 8.10 (d, 1H, C_6H_4), 8.26 (d, 2H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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 H_2O (1 mL). Two hours later, the reaction was completed, and the mixture was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (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%. ^{1}H NMR ($CDCl_3$) δ 2.20 (qi, 2H, $-CH_2CH_2ONO_2$), 2.36 (s, 3H, CH_3COO-), 3.21 (t, 2H, $-SO_2CH_2CH_2-$, $^3J_{HH} = 7.2$ Hz), 4.56 (t, 2H, $-CH_2ONO_2$, $^3J_{HH} = 6.3$ Hz), 6.22 (s, 2H, $-OCH_2O-$), 7.13 (d, 1H, C_6H_4), 7.34 (t, 1H, C_6H_4), 7.61 (t, 1H, C_6H_4), 8.01 (d, 2H, C_6H_4), 8.10 (d, 1H, C_6H_4), 8.29 (d, 2H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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 CH_2Cl_2 (100 mL) stirred under N_2 at room temperature dry $ZnCl_2$ (0.14 g) was added. After 15 min, the reaction mixture was cooled at −15 °C and a solution of CH_3CHO (2.80 mL, 50.35 mmol) in dry CH_2Cl_2 (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 H_2O (100 mL) and a saturated solution of $NaHCO_3$ (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. ^{1}H NMR ($CDCl_3$) δ 1.89 (d, 3H, CH_3CH-, $^3J_{HH} = 6.0$ Hz), 2.36 (s, 3H, CH_3COO-), 6.73 (q, 1H, CH_3CH-, $^3J_{HH} = 6.0$ Hz), 7.13 (d, 1H, C_6H_4), 7.33 (t, 1H, C_6H_4), 7.59 (t, 1H, C_6H_4), 8.03 (d, 1H, C_6H_4). ^{13}C NMR ($CDCl_3$) 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) Cs_2CO_3 (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 H_2O (30 mL) and extracted with Et_2O (3 × 20 mL). The combined organic layers were washed with a saturated solution of $NaHCO_3$ (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%. ^{1}H NMR ($CDCl_3$) δ 1.72 (d, 3H, CH_3CH−, $^3J_{HH}$ = 5.4 Hz), 2.10 (qi, 2H, −CH_2CH$_2$ONO$_2$), 2.30 (s, 3H, CH_3COO−), 3.10 (t, 2H, −SCH_2−, $^3J_{HH}$ = 6.9 Hz), 4.58 (t, 2H, −CH_2ONO$_2$, $^3J_{HH}$ = 6.0 Hz), 7.11 (d, 1H, C_6H_4), 7.27−7.34 (m, 4H, C_6H_4 + CH$_3$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 (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 $(CH_3CO)_2O$ (0.30 mL) was slowly added to a mixture of 65% HNO_3 (0.10 mL) and $(CH_3CO)_2O$ (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 (5×5 mL). The organic layers were dried, filtered, and concentrated under reduced pressure. The crude product so obtained was purified by flash chromatography (CH_2Cl_2/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%. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3COO−), 5.67 (s, 2H, −CH_2ONO$_2$), 6.26 (s, 2H, −OCH_2O−), 7.13 (d, 1H, C_6H_4), 7.33 (t, 1H, C_6H_4), 7.58−7.64 (m, 2H, C_5H_3N), 7.93 (t, 1H, C_6H_4), 8.09−8.17 (m, 2H, C_6H_4 + C_5H_3N). ^{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 (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 × 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%. ^{1}H NMR ($CDCl_3$) δ 2.22 (qi, 2H, −CH_2CH$_2$ONO$_2$), 4.13 (t, 2H, −OCH_2CH$_2$−, $^3J_{HH}$ = 5.7 Hz), 4.72 (t, 2H, −CH_2ONO$_2$, $^3J_{HH}$ = 6.0 Hz), 6.23 (s, 2H, −OCH_2O−), 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 (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 × 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 μg/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 EC_{50} ± 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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