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Searching for New NO-Donor Aspirin-like Molecules: A New Class of Nitrooxy-acyl Derivatives of Salicylic Acid†

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A new class of products in which the phenol group of salicylic acid is linked to alkanoyl moieties bearing nitrooxy functions has been synthesized and studied for their polyvalent actions. The products were stable in acid and neutral media, while they were hydrolyzed in human serum. Their half-lives were dependent upon the structure of alkanoyl moieties. The products showed anti-inflammatory activities similar to aspirin when tested in the carrageenan-induced paw edema assay in the rat. Interestingly, unlike aspirin, they showed reduced or no gastrotoxicity in a lesion model in rats at equimolar doses. A number of them were able to inhibit platelet aggregation induced by collagen in human platelet-rich plasma. All of the products were capable of relaxing rat aortic strips precontracted with phenylephrine in a concentration-dependent manner. Selected members of this new class of nonsteroidal anti-inflammatory drugs might represent possible safer alternatives to aspirin in different clinical settings.

Introduction

Aspirin (**1**) (Chart 1) is a well-established drug, belonging to the class of nonsteroidal anti-inflammatory drugs (NSAIDs). These products are widely used because of their anti-inflammatory, analgesic, antipyretic, and platelet anti-aggregatory properties. The pharmacological basis of these beneficial effects are related, at least in part, to their ability to inhibit two cyclooxygenase isoenzymes, COX-1 and COX-2, which are involved in the production of prostanoids from arachidonic acid.[1,2] Aspirin is unique among NSAIDs because it modifies both isoforms covalently by acetylating a serine residue (Ser^{530}) positioned in the arachidonic acid-binding channel of the enzyme.[3] It is considered a nonselective COX inhibitor, although it displays a somewhat higher inhibitor potency in COX-1 assays. The major drawback of the drug is a significant gastrotoxicity that is responsible for gastric ulceration, exacerbation of peptic ulcer symptoms, gastrointestinal hemorrhage, erosive gastritis, and in some cases, death.[1,2,4] Nitric oxide (NO)-releasing aspirins are an interesting class of products that were originally designed to reduce gastrotoxicity of the parent aspirin.[5,6] They are hybrid drugs that combine aspirin properties with gastroprotection exerted by NO, which they are able to release. NO-induced gastroprotection occurs through a number of mechanisms, including an increase of gastric microcirculation and mucous and bicarbonate secretion, as well as the inhibition of neutrophil adhesion to vascular endothelium of gastric microcirculation.[7,8] These products were originally obtained by joining, through a simple ester bridge, the carboxylic group of aspirin with moieties containing NO-donor nitrooxy groups ($-ONO_2$). Prototypes of such structures **2** (NCX4040), **3** (NCX4016), and **4** (NCX4060) are reported in Chart 1.

Subsequently, a second family of NO-donor aspirins **5** (Chart 1) was developed,[9] which, despite a close similarity with the first-generation molecules, contains furoxan derivatives as NO-donor groups. Unlike the previous NO-donor aspirins that seem to require enzymatic metabolism for NO release,[10–12] these products proved to release NO under the action of thiols.[13] Intracellular release induced by glutathione is potentiated by ascorbic acid.[14] Recently, an additional class of NO-donor NSAIDs, including aspirins **6** (Chart 1), has been proposed. They have moieties, containing an ONN(O)N substructure, linked to the carboxy group through a methylene bridge.[15] In serum, these compounds are metabolized to a mixture of products, including *N*-diazeniumdiolate anions, which are able to release NO spontaneously.

It is becoming more and more evident that NO-donor aspirins display a number of effects exceeding their original intended use. They display a variety of actions, including anti-inflammatory and analgesic effects, antiplatelet and vasodilator properties, and beneficial effects in the treatment of restenosis and myocardial ischemia. In addition, a number of them proved to be more potent than aspirin in inhibiting the proliferation of cancer cell lines, including prostate cancer cells.[16–19] Thus, **3**, the prototype of NO-donor aspirins, was found to enhance the preventive and therapeutic effectiveness of the antitumor immunity elicited by cancer vaccination.[20] Finally, a possible use of NO aspirins in the treatment of type-2 diabetes has been claimed.[18] The mechanism of action of NO aspirins is still under investigation, also taking into account that the simple esters of aspirin and, in particular, **3** are often not true aspirin prodrugs. Some of them are rapidly metabolized, with little or no formation of aspirin, when incubated in serum, plasma, and rat liver subcellular fractions.[9,12] This behavior is in keeping with the knowledge that the loss of the negative charge by the aspirin molecule, because of the esterification of the COOH group ($pK_a = 3.5$), makes the acetoxy moiety extremely susceptible to enzymatic cleavage.[21] Interestingly, it was recently found that

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Chart 1. Examples of NO-Donor Aspirin

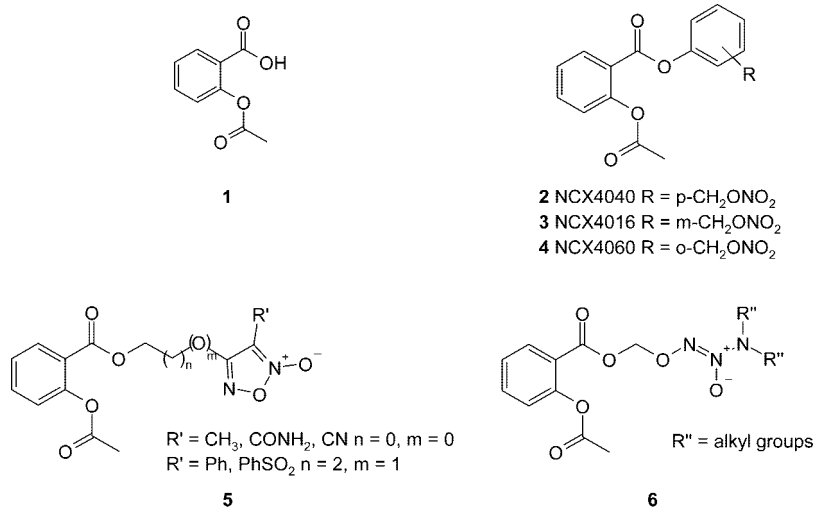
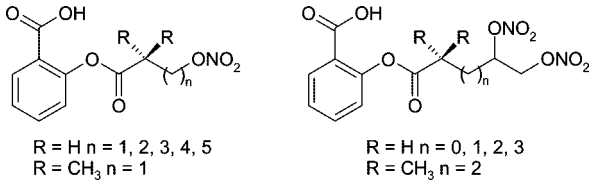


Chart 2. NO-Donor "Aspirin-like" Compounds



neither aspirin nor NO contributes to the antitumor effect of **2**, **4**, which, in contrast, is due to the quinone methides formed after carboxylic ester hydrolysis.[22,23] As a development of our work in this area, we designed a new class of NO-donor "aspirin-like" compounds (Chart 2). These products are formally derived from aspirin by substituting acyl groups containing nitrooxy NO-donor moieties for the acetyl group. They are not prodrugs of aspirin but true aspirin/NO-donor hybrids. All of the NO-donor aspirins known thus far were designed by merging NO-donor moieties at the carboxylic site of aspirin. In this paper, we describe the synthesis, stability in different buffer solutions and human serum of these products, as well as preliminary pharmacological characterization including in vivo anti-inflammatory, gastrosparing properties, in vitro anti-aggregatory activity, and cGMP-dependent vasodilation.

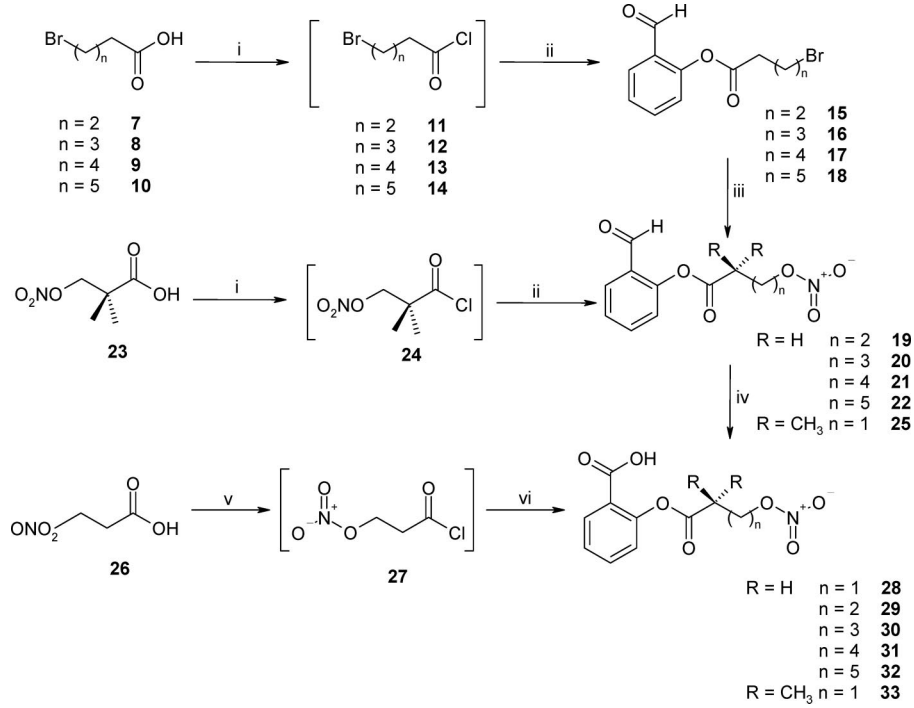
Chemistry

Mononitrooxy-substituted compounds were synthesized according to the pathways reported in Scheme 1. The preparation of the products **29**–**32** was carried out starting from the bromo-substituted alkanoic acids **7**–**10**. These starting materials were transformed into the corresponding acyl chlorides **11**–**14** by $SOCl_2$ in dry CH_2Cl_2, in the presence of a few drops of dry *N*,*N*-dimethylformamide (DMF). The acyl chlorides were conjugated to salicylaldehyde in dry CH_2Cl_2, in the presence of dry pyridine to give **15**–**18**. The action of $AgNO_3$ on these intermediates in refluxing acetonitrile afforded the corresponding mononitrooxy-substituted aldehydes **19**–**22** that gave rise to the final compounds **29**–**31**, under the action of $KMnO_4$. For the final compound **32**, mild oxidative reagents ($NaClO_2$ and H_2O_2) were used, because better yields were obtained. The preparation of the target products **28** and **33** was carried out starting from the nitrooxy-substituted alkanoic acids **23** and **26**. These acids were transformed into the corresponding acyl chlorides **24** and **27** by the action of $SOCl_2$. Coupling of **24** with salicylaldehyde under the same conditions used to prepare the aldehydes **15**–**18** gave **25** that yielded the target model **33** after subsequent oxidation with $KMnO_4$. The remaining mononitrooxy derivative **28** was prepared by direct coupling of **27** with salicylic acid in dry tetrahydrofuran (THF), in the presence of dry pyridine. The preparation of the dinitrooxy-substituted final products **51**–**55** is reported in Scheme 2. Two of them required the dinitrooxy-substituted alkanoic acids **35** and **36** as starting materials. The former is a product known in the literature,[24] while the latter was prepared by action of I_2 and $AgNO_3$ in acetonitrile solution on hept-6-enoic acid (**34**). These products were transformed into the corresponding acyl chlorides **37** and **38** and then coupled with salicylaldehyde to give **48** and **49**. Oxidation of these intermediates afforded the final target compounds **53** and **54**. All of these reactions were carried out under conditions similar to those described for the preparation of the mononitrooxy-substituted product **33** from **23**. To prepare **51**, **52**, and **55**, the unsaturated acids **39**–**41** were used as starting materials. Acids **39** and **41** were transformed in the corresponding active intermediates through the reaction with *N*,*N*′-dicyclohexylcarbodiimide (DCC); these intermediates were then coupled to salicylaldehyde in dry CH_2Cl_2 solution in the presence of 4-*N*,*N*′-dimethylaminopyridine (DMAP) to give **43** and **45**. To prepare aldehyde **44**, the best results were obtained transforming **40** into the corresponding acyl chloride **42** and then coupling **42** with salicylaldehyde, under the conditions already described. The unsaturated aldehydes **43**–**45** were transformed into the corresponding dinitrooxy derivatives **46**, **47**, and **50** by action of I_2 and $AgNO_3$ in CH_3CN solution. The aldehydes **47** and **50** afforded the desired final products by usual oxidation with $KMnO_4$, while aldehyde **46** was oxidated using mild conditions ($NaClO_2$ and H_2O_2).

Results and Discussion

Stability in Aqueous Buffer Solutions and Human Serum. The stability of all of the final products was studied by high-performance liquid chromatography (HPLC) in aqueous buffer solutions of pH 1 and 7.4 as well as in human serum (Table 1). Salicylic acid (**56**) and the nitrooxyalkanoic acids were the only transformation products. In an acidic medium, the stability, after 3 h of incubation, ranged from 85 to 97%. At pH 7.4, most of the products showed a stability >90%, with the only exception of products **28** and **51**, for which only 55 and 20% remained unchanged over the same time period. A different situation occurred when the compounds were incubated

Scheme 1[a]



[a] Reagents and conditions: (i) $SOCl_2$, dry CH_2Cl_2, dry DMF, (ii) salicylaldehyde, dry CH_2Cl_2, dry Py, (iii) $AgNO_3$, CH_3CN, 70 °C, (iv) $KMnO_4$, acetone for $n = 1$–4 and R = H and CH_3; 80% $NaClO_2$, 30% H_2O_2, CH_3CN for $n = 5$ and R = H, (v) $SOCl_2$, dry THF, dry DMF, and (vi) salicylic acid, dry THF, dry Py.

in serum, in which they can undergo enzymatic metabolism. All of them were hydrolyzed, following a first-order kinetic mechanism. In Figure 1, the hydrolysis in serum of **31** is reported as an example. The observed pseudo-first-order rate constants (k_{obs}) for the hydrolysis were calculated from the slopes of linear plots of the logarithm of the remaining ester against time; the corresponding half-lives ($t_{1/2}$) were obtained from eq 1 (Table 1).

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

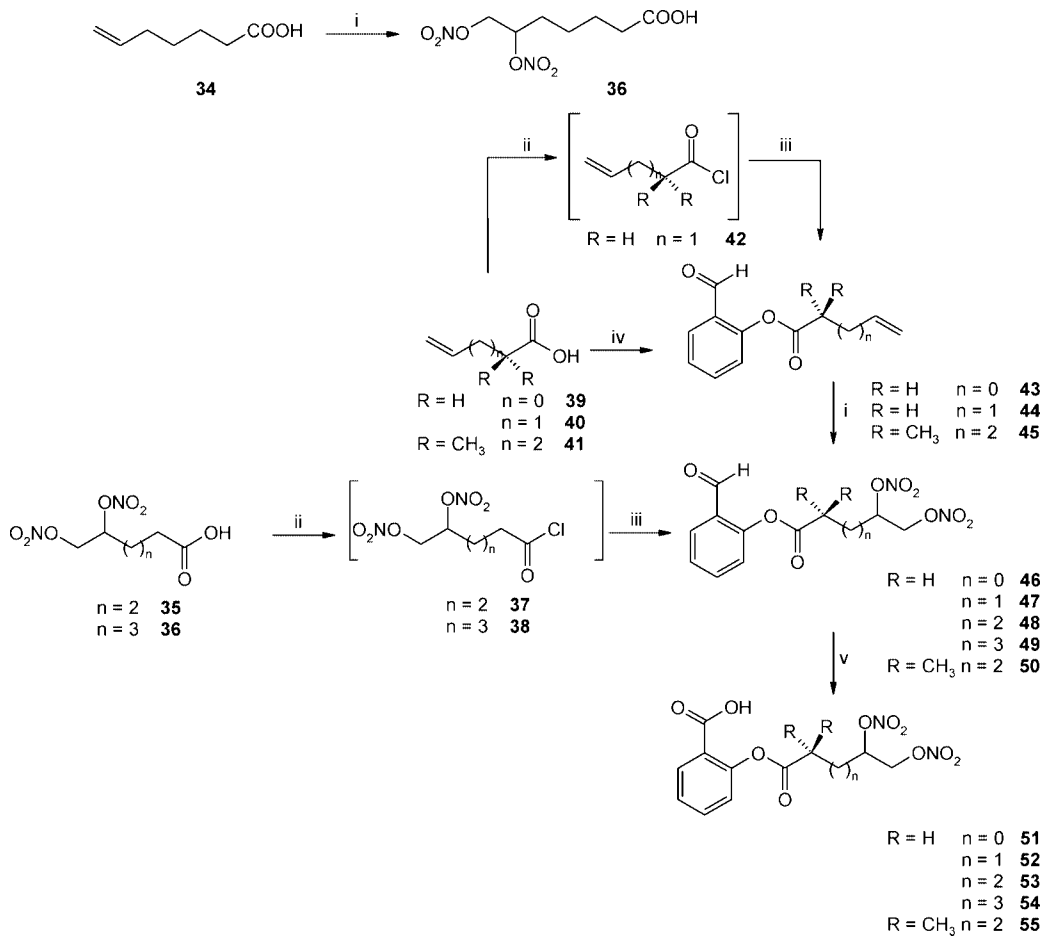
Analysis of these parameters shows that they are influenced by the structure of the ester chain. In the mononitrooxy series, some products are less stable than aspirin, while others as stable as or more stable than it. The stability increases with the length of the chain, but when the number of carbons become >6 (compound **32**), it decreases. The presence of two methyl groups at the α position of the ester function (compound **33**) induces strong stabilization. A similar situation occurs in the dinitrooxy series, but all of these compounds are definitively more stable than the related monosubstituted analogues. This picture might be justified by a different ability of the models to interact with the esterase enzyme and/or bind with plasma proteins, following their different lipophilicity and stereoelectronic properties.[25,26]

Antiinflammatory Activity. All of the products, including aspirin (**1**) and salicylic acid (**56**) as references, were tested on carrageenan-induced paw edema in conscious rats. The injection of carrageenan into rat hind paw produced an immediate paw swelling, which reached a peak at 4–5 h. Aspirin, administered by intragastric route at 120 mg/kg, just prior to carrageenan injection, significantly reduced (48.1 ± 2.9%) paw edema at 3 h, when compared to vehicle-treated animals (Figure 2). Also, a number of the new NO-donor products were able to reduce paw edema in a significant or highly significant manner, when administered intragastrically at a dose equimolar to aspirin (120 mg/kg; Figure 2). The most active compounds, which induce anti-inflammatory effects comparable to those caused by aspirin, belong to both the class of mononitrooxy derivatives (compounds **30**, **31**, and **33**) and that of dinitrooxy derivatives (compounds **52**–**54**).

As aforementioned, the anti-inflammatory effects of NSAIDs can be partly explained by the inhibition of prostanoid production following the inhibition of COX enzymes. More recently, it was found that the irreversible acetylation of COX-2 isoform by aspirin may contribute to its anti-inflammatory activity, through the synthesis of aspirin-triggered lipoxins (ATLs).[27] Because the drug is rapidly deacetylated under the action of esterases present in blood, intestinal mucosa, and tissues, such as liver and kidney,[28,29] it has been proposed that salicylic acid may also contribute to its anti-inflammatory action.[30] It is known that rodents (rats and guinea pigs) tend to metabolize ester-containing drugs, including aspirin, much faster than humans.[25,28] Furthermore, NO may display anti-inflammatory actions of its own when produced in the appropriate amounts.[31–33] In particular, both NO and salicylate are able to inhibit NF-κB, a transcription factor involved in the synthesis of inflammatory cytokines, cytokine receptors, and adhesion molecules in a variety of cells.[30,34–37] Actually, **3** was shown to inhibit this factor and suppress the processing of IL-1β and IL-18, two inflammatory cytokines, by inhibiting caspase-1 activity.[17,38] Studies dedicated to this question are necessary to clarify whether the mechanisms underlying the in vivo anti-inflammatory properties of this new class of NSAIDs involve combinations of COX effects of the native products, the metabolite salicylic acid, and possibly NO release.

Acute Gastric Mucosal Damage. All of the compounds, including aspirin (**1**) and salicylic acid (**56**) as references, were assessed for their ulcerogenic properties in conscious rats. The development of gastric lesions was assessed 3 h after intragastric administration of the compounds, and lesions were quantified by determining the "lesion index" on the basis of their greatest

Scheme 2[a]



[a] Reagents and conditions: (i) I_2, $AgNO_3$, CH_3CN room temperature and then $AgNO_3$, CH_3CN reflux, (ii) $SOCl_2$, dry CH_2Cl_2, dry DMF, (iii) salicylaldehyde, dry CH_2Cl_2, dry Py, (iv) DCC, dry CH_2Cl_2 and then salicylaldehyde, dry CH_2Cl_2, DMAP, and (v) $KMnO_4$, acetone for $n = 1$–3 and R = H and CH_3; 80% $NaClO_2$, 30% H_2O_2, CH_3CN for $n = 0$ and R = H.

Table 1. Stability in Aqueous Buffers and Human Serum, Anti-aggregatory, and Vasodilator Activity of the Products **28**–**33** and **51**–**55**

	stability			anti-aggregatory activity		vasodilator activity
	aqueous buffers percent unchanged at 3 h (%)					
compound	pH 1.0	pH 7.4	human serum $t_{1/2}$ (h) ± SD	IC_{50} (CL 95%) (μM) [+50 μM ODQ]	percent inhibition ± SEM at 300 μM[a]	EC_{50} (μM) ± SEM
1	>90	>90	1.06 ± 0.08	54 (49–60)		
28	>90	55	0.26 ± 0.01	162 (129–204) [186 (172–200)]		37 ± 2[b]
29	>97	>95	0.47 ± 0.01	30 (24–37) [24 (17–34)]		23 ± 6[b]
30	>97	>95	0.76 ± 0.01	97 (85–110) [91 (76–110)]		21 ± 2[b]
31	>97	>95	2.03 ± 0.11	*a*	27 ± 10	14 ± 1[b]
32	>97	>95	1.19 ± 0.08	inactive		8.1 ± 1.4[b]
33	>97	>95	9.03 ± 0.22	*a*	26 ± 6	6.2 ± 0.9[b]
51	>85	20	0.28 ± 0.01	126 (98–160) [119 (105–134)]		9.2 ± 0.9[b]
52	>85	>95	4.09 ± 0.08	*a*	15 ± 8	8.2 ± 1.2[b]
53	>85	>95	4.87 ± 0.07	*a*	6.7 ± 5.8	5.8 ± 0.7[b]
54	>85	>95	6.25 ± 0.17	inactive		3.1 ± 0.7[b]
55	>97	>95	15.2 ± 0.40	inactive		5.3 ± 0.6[b]

[a] Because of the low activity of the compound, IC_{50} could not be calculated. In this case, the percent of inhibition is reported at 300 μM. [b] In the presence of 1 μM ODQ, EC_{50} values were >100 μM.

length in millimeters (Figure 3). Aspirin displays strong gastrotoxicity despite its ability to generate ATLs, which are endowed with gastroprotective effects as well.[39] In the present study, aspirin, administered at 120 mg/kg, produced macroscopically detectable gastric damage, characterized by mucosal necrosis and hemorrhage (lesion index = 53.5 ± 5.2%). All of the NO-donor products described here displayed greatly reduced gastrotoxicity when administered at a dose equimolar to aspirin. There are two effects that contribute to gastrotoxicity of conventional acidic NSAIDs: the systemic and local irritant

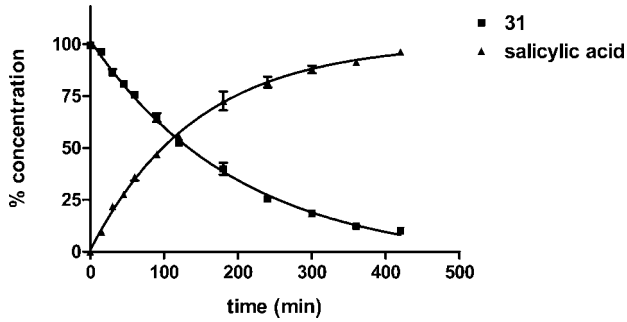


Figure 1. Time courses for compound **31** and salicylic acid 56 in human serum at 37 °C. Values are mean ± SEM (SEM ≤ 2; number of determinations ≥ 4).

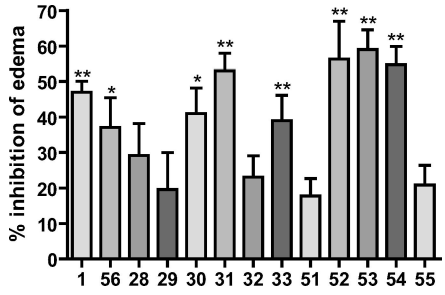


Figure 2. Anti-inflammatory effects of aspirin (**1**), salicylic acid (**56**) and NO-donor aspirin-like molecules (**28**–**33** and **51**–**55**) on carrageenan-induced paw edema in conscious rats. The aspirin-like compounds were administered by intragastric route at a dose equimolar to aspirin, 120 mg/kg, at the same time as carrageenan, and their effects were evaluated 3 h later. Results are expressed as a percentage of inhibition of edema observed in the vehicle-treated group. This edema was considered arbitrarily as 100. (∗) $p < 0.05$. (∗∗) $p < 0.01$ versus vehicle (ANOVA, followed by Dunnett test). Values are mean ± SEM (n = 8–10 rats per group).

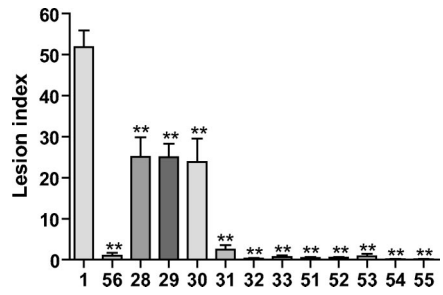


Figure 3. Gastric ulcerogenic effects of aspirin (**1**), salicylic acid (**56**), and NO-donor aspirin-like molecules (**28**–**33** and **51**–**55**) in conscious rats. The aspirin-like compounds were administered by intragastric route at a dose equimolar to aspirin, 120 mg/kg, and the stomachs were examined 3 h later. Gastric lesions were measured along the greatest length, and the cumulative length in millimeters was designated as the "lesion index" for each stomach. All of the compounds tested produced significantly less gastric damage than aspirin (∗∗) $p < 0.01$; (ANOVA and Newman−Keuls test). Values are mean ± SEM (n = 8–10 rats per group).

effects.[40,41] The former is dependent upon COX inhibition, in particular, the COX-1 isoform in gastric epithelial cells. The latter is the result of a number of events, including perturbation of physicochemical properties of phospholipids and "ion trapping" of the drugs into surface epithelial cells. These two events are tightly linked to the pK_a and the lipophilicity of acid NSAIDs.[42] From this point of view, the new products developed here show acid properties (pK_a = 3.85–3.69) close to that of aspirin and greater lipophilicity (log P = 1.60–3.22; log $P_{aspirin}$ = 1.14) (unpublished data). While it is plausible to hypothesize a role of NO release in the reduced gastrotoxicity, it must be remembered that these products are metabolized to salicylic acid, which is unable to induce gastric mucosal damage (see Figure 3 and ref 30).

Platelet Anti-aggregatory Activity. Anti-aggregatory effects of the compounds were studied on collagen-induced platelet aggregation of human platelet-rich plasma (PRP). It is known that collagen-induced aggregation occurs through a pathway dependent upon the arachidonic acid cascade.[43] The results expressed as IC_{50} or, when IC_{50} could not be calculated, as a percentage of inhibition at the maximal concentration tested are reported in Table 1. Analysis of the data show that the most active compounds were **29** > **30** > **28**, belonging to the mononitrooxy series and the dinitrooxy-substituted model **51**. To determine whether NO-mediated stimulation of soluble guanylate cyclase (sGC) was involved in their anti-aggregatory action, these products were also tested in parallel in the presence of 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), a well-known inhibitor of sGC. The ineffectiveness of ODQ on the anti-aggregatory activities of compounds **28**–**30** and **51** excludes any involvement of NO-mediated stimulation of sGC in keeping with the known inability of platelets to effect NO release from organic nitrates.[14,44] This strengthens the possibility that platelet COX-1 inhibition is the main underlying mechanism.

Vasodilator Activity. It is known that organic nitrates display vasodilator activities. The generally accepted mechanism of this action involves their conversion in vascular smooth-muscle cells into NO with consequent activation of the sGC. In turn, sGC increases the intracellular levels of cyclic guanosine-3,5-monophosphate (cGMP), with consequent activation of cGMP-dependent protein kinase (cGK-I) and vasodilation.[45] To highlight the NO-releasing activity of the compounds described in the present work, their vasodilatory effects were evaluated on denuded rat aorta strips precontracted with phenylephrine. All of the products were capable of relaxing the contracted tissue in a concentration-dependent manner, in keeping with the presence in the structures of NO-donor nitrooxy functions. The vasodilator potencies, expressed as EC_{50}, are shown in Table 1. In the mononitrooxy series, the potency increases with the length of the linear lateral chain. The most active term was the branched product **33**, which bears two methyl groups at the α carbon to ester function. Similar behavior is shown by the dinitrooxy-substituted compounds, but in this series, the branched compound **55** is less active than **54**, containing the longest linear lateral chain, and as active as **53**, which is homologous, immediately inferior to **54**. With the length of the chain being equal, the dinitrooxy-substituted compounds are always more active than the corresponding mononitroxy analogues, in keeping with the statement that the number of nitrate groups determines reactivity and potency of organic nitrates.[46] When the vasodilator experiments were repeated in the presence of 1 μM ODQ, a decrease in the potencies was observed, in keeping with NO-induced activation of the sGS.

Conclusions

We have been able to successfully prepare a new class of "aspirin-like" products obtained through a novel approach, which implies the merging of nitrooxy-acyl moieties at the phenol site of salicylic acid. Generally speaking, these compounds are stable in acidic media and at physiological pH, but they are hydrolyzed when incubated in serum. This new class of products displays anti-inflammatory activity in the carrageenan-induced paw edema test, with several compounds being as potent as aspirin; nevertheless, they show reduced or no

gastrotoxicity when compared to this lead. Several components may contribute to their in vivo behavior, including salicylic acid formation, NO release, and variable linkers. Furthermore, some of them are able to block platelet aggregation induced by collagen in human plasma-rich platelets. All of the products trigger cGMP-dependent vasodilator actions when tested on precontracted rat aorta strips, according to the presence in their structures of nitrooxy NO-donor moieties. Additional pharmacological and biochemical studies, mainly addressing COX-1/COX-2 inhibition, are necessary to highlight action mechanisms of these products and understand whether selected members of this new class of NSAIDs might represent possible safer alternatives to aspirin in different clinical settings.

Experimental Section

Synthesis. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 at 300 and 75 MHz, respectively, using $SiMe_4$ as the internal standard. Low-resolution mass spectra were recorded with a Finnigan-Mat TSQ-700. Melting points were determined with a capillary apparatus (Büchi 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 × 20 cm plates, with a layer thickness of 0.2 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 × 25 mm, 10 μm) (Merck Darmstadt, Germany) with a Varian ProStar mod-210 with Varian UV detector mod-325. Elemental analyses (C, H, and N) were performed by REDOX (Monza), and the results are within ± 0.4% of the theoretical values. Compounds **8**,[47] **10**,[48] **23**,[49] **26**,[50] **35**,[24] and **41**[51] were synthesized according to the literature.

General Procedure for the Preparation of 15, 16, 17, 18, 25, 44, 48, and 49. $SOCl_2$ (6.55 mmol) and a few drops of dry DMF were added to a solution of the appropriate carboxilic acid **7**, **8**, **9**, **10**, **23**, **35**, **36**, and **40** (5.46 mmol) in dry CH_2Cl_2 (15 mL), stirred under N_2 at room temperature. The stirring was continued for 2 h at room temperature. The solution of the acyl chloride thus obtained was slowly added to a stirred solution of salicylaldehyde (4.37 mmol) and dry Py (8.19 mmol) in dry CH_2Cl_2 (10 mL) kept under N_2 at 0 °C. The reaction was allowed to reach room temperature and then stirred for 2.5 h. Then, the mixture was washed with 2 M HCl (3 × 10 mL). The combined organic layers were dried, filtered, and concentrated under reduced pressure. The crude product thus obtained was purified by flash chromatography. Chromatographic eluents and yields of the products were as follows.

2-Formylphenyl 4-Bromobutanoate (15). Eluent [90:10 PE/EtOAc (v/v)]. Pale yellow oil. Yield 80%. ^{1}H NMR ($CDCl_3$) δ: 2.34–2.36 (m, 2H, $-CH_2CH_2Br$), 2.89 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.1 Hz), 3.57 (t, 2H, $-CH_2Br$, $^3J_{HH}$ = 6.4 Hz), 7.18 (d, 1H, C_6H_4), 7.43 (t, 1H, C_6H_4), 7.66 (t, 1H, C_6H_4), 7.88 (d, 1H, C_6H_4), 10.1 (s br, 1H, C*H*O). ^{13}C NMR ($CDCl_3$) δ: 27.4, 32.3, 32.5, 123.5, 126.5, 128.0, 132.0, 134.8, 151.0, 171.0, 188.9. MS (CI) *m*/*z*: 271/273 $(M + 1)^+$.

2-Formylphenyl 5-Bromopentanoate (16). Eluent [90:10 PE/EtOAc (v/v)]. Pale yellow oil. Yield 57%. ^{1}H NMR ($CDCl_3$) δ: 1.91–2.05 (m, 4H, $-CH_2CH_2CH_2Br$), 2.70 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 6.8 Hz), 3.48 (t, 2H, $-CH_2Br$, $^3J_{HH}$ = 6.3 Hz), 7.18 (d, 1H, C_6H_4), 7.40 (t, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 7.88 (d, 1H, C_6H_4), 10.1 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 23.2, 31.9, 32.9, 33.1, 123.5, 126.5, 128.2, 131.7, 135.3, 151.3, 171.4, 188.8. MS (CI) *m*/*z*: 285/287 $(M + 1)^+$.

2-Formylphenyl 6-Bromohexanoate (17). Eluent [90:10 PE/EtOAc (v/v)]. Pale yellow oil. Yield 68%. ^{1}H NMR ($CDCl_3$) δ: 1.57–1.65 (m, 2H), 1.78–1.83 (m, 2H), 1.89–1.97 (m, 2H, $-CH_2CH_2CH_2CH_2Br$), 2.70 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.5 Hz), 3.44 (t, 2H, $-CH_2Br$, $^3J_{HH}$ = 6.7 Hz), 7.19 (d, 1H, C_6H_4), 7.39 (t, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 7.88 (d, 1H, C_6H_4), 10.10 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 23.8, 27.6, 32.4, 33.5, 34.0, 123.5, 126.4, 128.1, 131.0, 135.3, 151.5, 171.7, 188.8. MS (CI) *m*/*z*: 299/301 $(M + 1)^+$.

2-Formylphenyl 7-Bromoheptanoate (18). Eluent [95:5 PE/EtOAc (v/v)]. Pale yellow oil. Yield 62%. ^{1}H NMR ($CDCl_3$) δ: 1.47–1.55 (m, 4H), 1.78–1.96 (m, 4H, $-CH_2CH_2CH_2CH_2CH_2Br$), 2.70 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.3 Hz), 3.45 (t, 2H, $-CH_2Br$, $^3J_{HH}$ = 6.7 Hz), 7.19 (d, 1H, C_6H_4), 7.42 (t, 1H, C_6H_4), 7.65 (t, 1H, C_6H_4), 7.90 (d, 1H, C_6H_4), 10.12 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 24.4, 27.8, 28.2, 32.5, 33.8, 33.9, 123.5, 126.4, 128.1, 131.2, 135.3, 151.6, 171.9, 188.7. MS (CI) *m*/*z*: 313/315 $(M + 1)^+$.

2-Formylphenyl 2,2-Dimethyl-3-(nitrooxy)propanoate (25). In this case, the formation of acyl chloride and the next synthetic step are very slow, and 1 week of stirring was required. Eluent [90:10 PE/EtOAc (v/v)]. Pale yellow oil. Yield 45%. ^{1}H NMR ($CDCl_3$) δ: 1.51 (s, 6H, $-CH_3$), 4.69 (s, 2H, $-CH_2ONO_2$), 7.15 (d, 1H, C_6H_4), 7.43 (t, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 7.88 (d, 1H, C_6H_4), 10.07 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 22.4, 42.7, 77.5, 123.3, 126.8, 128.2, 131.8, 135.3, 151.0, 172.8, 188.5. MS (CI) *m*/*z*: 268 $(M + 1)^+$.

2-Formylphenyl Pent-4-enoate (44). Eluent [95:5 PE/EtOAc (v/v)]. Pale yellow oil, immediately used in the next synthetic step. Yield 65%.

2-Formylphenyl 5,6-Bis(nitrooxy)hexanoate (48). Eluent [90:10 → 80:20 PE/EtOAc (v/v)]. Pale yellow oil. Yield 56%. ^{1}H NMR ($CDCl_3$) δ: 1.89–1.98 (m, 4H, $-CH_2CH_2CH_2CH-$), 2.74–2.78 (m, 2H, $-OCOCH_2-$), 4.49–4.55 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.73–4.83 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.36–5.39 (m, 1H, AMX-like system, $-CHONO_2$), 7.17 (d, 1H, C_6H_4), 7.44 (t, 1H, C_6H_4), 7.66 (t, 1H, C_6H_4), 7.86 (d, 1H, C_6H_4), 10.02 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 19.9, 28.5, 33.0, 71.1, 78.8, 123.5, 126.7, 128.0, 133.0, 135.4, 150.6, 171.1, 189.3. MS (CI) *m*/*z*: 343 $(M + 1)^+$.

2-Formylphenyl Hept-6-enoate (49). Eluent [85:15 PE/EtOAc (v/v)]. Pale yellow oil. Yield 58%. ^{1}H NMR ($CDCl_3$) δ: 1.55–1.67 (m, 2H), 1.80–1.90 (m, 4H, $-CH_2CH_2CH_2CH_2CH-$), 2.71 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.2 Hz), 4.50 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.78 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.29–5.37 (m, 1H, AMX-like system, $-CHONO_2$), 7.17 (d, 1H, C_6H_4), 7.43 (t, 1H, C_6H_4), 7.65 (t, 1H, C_6H_4), 7.87 (d, 1H, C_6H_4), 10.1 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 24.0, 24.3, 29.0, 33.4, 71.2, 79.0, 119.9, 123.5, 128.1, 132.2, 135.4, 151.0, 171.4, 189.0. MS (CI) *m*/*z*: 357 $(M + 1)^+$.

General Procedure for the Preparation of 19, 20, 21, and 22. A solution of the appropriate bromo derivative **15**, **16**, **17**, and **18** (18.4 mmol) and $AgNO_3$ (46.0 mmol) in CH_3CN (150 mL) was stirred at 70 °C for 7 h. 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 thus obtained was purified by flash chromatography. Chromatographic eluents and yields of the products were as follows.

2-Formylphenyl 4-(Nitrooxy)butanoate (19). Eluent [90:10 PE/EtOAc (v/v)]. Pale yellow oil. Yield 84%. ^{1}H NMR ($CDCl_3$) δ: 2.17–2.27 (m, 2H, $-CH_2CH_2ONO_2$), 2.82 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.1 Hz), 4.61 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.2 Hz), 7.18 (d, 1H, C_6H_4), 7.44 (t, 1H, C_6H_4), 7.65 (t, 1H, C_6H_4), 7.87 (d, 1H, C_6H_4), 10.0 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 22.1, 30.1, 71.8, 123.5, 126.7, 127.9, 132.7, 134.9, 150.6, 170.8, 189.1. MS (CI) *m*/*z*: 254 $(M + 1)^+$.

2-Formylphenyl 5-(Nitrooxy)pentanoate (20). Eluent [90:10 PE/EtOAc (v/v)]. Pale yellow oil. Yield 64%. ^{1}H NMR ($CDCl_3$) δ: 1.88–1.95 (m, 4H, $-CH_2CH_2CH_2ONO_2$), 2.75 (m, 2H, $-OCOCH_2-$), 4.55 (m, 2H, $-CH_2ONO_2$), 7.19 (d, 1H, C_6H_4), 7.43 (t, 1H, C_6H_4), 7.66 (t, 1H, C_6H_4), 7.89 (d, 1H, C_6H_4), 10.1 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 20.9, 26.2, 33.3, 72.7, 123.5, 126.5, 128.0, 132.2, 135.4, 151.0, 171.2, 188.9. MS (CI) *m*/*z*: 268 $(M + 1)^+$.

2-Formylphenyl 6-(Nitrooxy)hexanoate (21). Eluent [90:10 PE/EtOAc (v/v)]. Pale yellow oil. Yield 80%. ^{1}H NMR ($CDCl_3$) δ: 1.52–1.62 (m, 2H), 1.77–1.89 (m, 4H, $-CH_2CH_2$-$CH_2CH_2ONO_2$), 2.68 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.4 Hz), 4.49 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.5 Hz), 7.17 (d, 1H, C_6H_4), 7.41 (t, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 7.88 (d, 1H, C_6H_4), 10.10 (s, 1H, -CHO). ^{13}C NMR ($CDCl_3$) δ: 24.1, 25.1, 26.5, 33.7, 73.0, 123.5, 126.5, 128.1, 131.7, 135.3, 151.3, 171.6, 188.9. MS (CI) *m/z*: 282 $(M + 1)^+$.

2-Formylphenyl 7-(Nitrooxy)heptanoate (22). Eluent [90:10 PE/EtOAc (v/v)]. Pale yellow oil. Yield 79%. ^{1}H NMR ($CDCl_3$) δ: 1.47–1.52 (m, 4H), 1.75–1.83 (m, 4H, $-CH_2CH_2$-$CH_2CH_2CH_2ONO_2$), 2.69 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.4 Hz), 4.47 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.6 Hz), 7.17 (d, 1H, C_6H_4), 7.41 (t, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 7.88 (d, 1H, C_6H_4), 10.10 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 24.4, 25.4, 26.6, 28.4, 33.9, 73.2, 123.5, 126.4, 128.1, 131.4, 135.4, 151.5, 171.8, 188.9. MS (CI) *m/z*: 296 $(M + 1)^+$.

2-Formylphenyl But-3-enoate (43). To a solution of vinyl acetic acid (3.0 mL, 35.3 mmol) in dry CH_2Cl_2 (50 mL), stirred under inert atmosphere, DCC (2.18 g, 31.7 mmol) was added. After 1 h, salicylaldehyde (3.0 mL, 28.2 mmol) and DMAP (0.43 g, 3.53 mmol) were added. The reaction was completed after 2 h. The mixture was filtered, and the filtrate was washed with H_2O (20 mL) and brine (20 mL). The organic layer was dried, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography [95:5 PE/EtOAc (v/v)] to give the title compound as a yellow oil. Yield 67%. ^{1}H NMR ($CDCl_3$) δ: 3.46 (d, 2H, $-CH_2CH{=}CH_2$), 5.28–5.36 (m, 2H, $-CH_2CH{=}CH_2$), 6.02–6.11 (m, 1H, $-CH_2CH{=}CH_2$), 7.20 (d, 1H, C_6H_4), 7.41 (t, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 7.89 (d, 1H, C_6H_4), 10.11 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 38.9, 119.7, 123.4, 126.5, 128.0, 129.2, 131.2, 135.3, 151.5 169.8, 188.7. MS (CI) *m/z*: 191 $(M + 1)^+$.

2-Formylphenyl (2,2-Dimethyl)-hex-5-enoate (45). The title compound was prepared in the same manner as **43**. Eluent [98:2 PE/EtOAc (v/v)]. Yellow oil. Yield 47%. ^{1}H NMR ($CDCl_3$) δ: 1.40 (s, 6H, $-CH_3$) 1.80–1.86 (m, 2H, $-CH_2CH_2CH-$), 2.12–2.20 (m, 2H, $-CH_2CH_2CH-$), 5.10 (dd, 1H, AMX-like system, $-CH{=}CH_aH_b$), 4.86 (dd, 1H, AMX-like system, $-CH{=}CH_aH_b$), 5.85 (m, 1H, AMX-like system, $-CH{=}CH_2$), 7.14 (d, 1H, C_6H_4), 7.38 (t, 1H, C_6H_4), 7.63 (t, 1H, C_6H_4), 7.92 (d, 1H, C_6H_4), 10.15 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 24.8, 29.4, 39.6, 42.8, 115.0, 123.3, 126.2, 128.3, 129.9, 135.3, 138.0, 152.5, 175.9, 188.3. MS (CI) *m/z*: 247 $(M + 1)^+$.

General Procedure for the Preparation of 36, 46, 47, and 50. Iodine (9.79 mmol) was added portion-wise to a stirred solution of the appropriate unsatured compounds **34**, **43**, **44**, and **45** (9.79 mmol) and $AgNO_3$ (1.66 g, 9.79 mmol) in CH_3CN (100 mL) kept at −15 °C. At the end of the addition, the stirring was continued for 1 h. Then, $AgNO_3$ (19.6 mmol) was added, and the mixture was heated at 70 °C until the disappearance of the starting material, as checked by TLC. After cooling, the mixture was filtered through Celite. The filtrate was concentrated under reduced pressure, dissolved in water (50 mL), and extracted with EtOAc (4 × 50 mL). The combined organic layers were dried, filtered, and concentrated under reduced pressure. The crude product thus obtained, when necessary, was purified by flash chromatography. Chromatographic eluents and yields of the products were as follows.

6,7-Dinitrooxyheptanoic Acid (36). Yellow oil. Yield 88%. ^{1}H NMR ($CDCl_3$) δ: 1.50–1.55 (m, 2H), 1.67–1.80 (m, 4H, $-CH_2CH_2CH_2CH_2OCO-$), 2.41 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.1 Hz), 4.48 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.76 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.26–5.33 (m, 1H, AMX-like system, $-CHONO_2$), 9.66 (s br, 1H, $-COOH$). ^{13}C NMR ($CDCl_3$) δ: 24.0, 24.3, 27.4, 33.5, 71.2, 78.6, 179.5. MS (CI) *m/z*: 253 $(M + 1)^+$.

2-Formylphenyl 3,4-Bis(nitrooxy)butanoate (46). Eluent [90:10 PE/EtOAc (v/v)]. The orange solid was treated with *i*Pr_2O to obtain the title compound as a pale yellow solid. Yield 15%. ^{1}H NMR ($CDCl_3$) δ: 3.18 (d, 2H, $-OCOCH_2-$), 4.71–4.77 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.95–5.00 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.81–5.84 (m, 1H, AMX-like system, $-CHONO_2$), 7.18 (d, 1H, C_6H_4), 7.49 (t, 1H, C_6H_4), 7.67 (t, 1H, C_6H_4), 7.86 (d, 1H, C_6H_4), 9.96 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 34.0, 70.3, 74.9, 123.4, 127.2, 127.6, 134.3, 135.6, 149.4, 167.1, 189.6. MS (CI) *m/z*: 315 $(M + 1)^+$.

2-Formylphenyl 4,5-Bis(nitrooxy)pentanoate (47). Eluent [90:10 PE/EtOAc (v/v)]. Pale yellow oil. Yield 53%. ^{1}H NMR ($CDCl_3$) δ: 2.10–2.27 (m, 2H, $-CH_2CH_2CH-$), 2.87 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 6.9 Hz), 4.57 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.86 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.52 (m, 1H, AMX-like system, $-CHONO_2$), 7.17 (d, 1H, C_6H_4), 7.46 (t, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 7.85 (d, 1H, C_6H_4), 9.98 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 24.2, 29.2, 71.1, 78.0, 123.5, 126.8, 127.8, 133.7, 135.5, 150.1, 170.6, 189.5. MS (CI) *m/z*: 329 $(M + 1)^+$.

2-(2,2-Dimethyl)-formylphenyl 5,6-Bis(nitrooxy)hexanoate (50). Eluent [90:10 PE/EtOAc (v/v)]. Pale yellow oil. Yield 55%. ^{1}H NMR ($CDCl_3$) δ: 1.37 (s, 6H, $-CH_3$), 1.82–1.95 (m, 4H, $-CH_2CH_2CH-$), 4.48–4.54 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.77–4.82 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.30–5.37 (m, 1H, AMX-like system, $-CHONO_2$), 7.11 (d, 1H, C_6H_4), 7.41 (t, 1H, C_6H_4), 7.65 (t, 1H, C_6H_4), 7.87 (d, 1H, C_6H_4), 10.0 (s, 1H, $-CHO$). ^{13}C NMR ($CDCl_3$) δ: 24.6, 25.1, 35.2, 42.4, 71.2, 79.6, 123.4, 126.6, 128.3, 132.2, 135.3, 151.1, 175.4, 188.8. MS (CI) *m/z*: 371 $(M + 1)^+$.

2-{[3-(Nitrooxy)propanoyl]oxy)}benzoic Acid (28). $SOCl_2$ (2.43 mL, 33.3 mmol) and a few drops of dry DMF were added to a solution of 3-(nitrooxy)propionic acid (3.0 g, 22.2 mmol) in dry THF (20 mL), stirred under N_2 at room temperature; the stirring was continued for 3 h at room temperature. The solution of the acyl chloride thus obtained was slowly added to a stirred solution of salycilic acid (3.07 g, 22.2 mmol) and dry Py (2.7 mL, 33.3 mmol) in dry THF (40 mL) kept under N_2 at 0 °C. The mixture was allowed to reach room temperature, and the stirring was continued overnight. The mixture was diluted with Et_2O (90 mL) and washed twice with 2 M HCl (60 mL). The organic layer was dried, filtered, and concentrated under reduced pressure. The crude product was partially purified by flash chromatography [97:3 CH_2Cl_2/MeOH (v/v)]. The crude solid thus obtained was crystallized by toluene. Yield 46%. mp 86–88 °C (from toluene). ^{1}H NMR ($CDCl_3$) δ: 3.09 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 6.4 Hz), 4.87 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.4 Hz), 7.16 (d, 1H, C_6H_4), 7.39 (t, 1H, C_6H_4), 7.65 (t, 1H, C_6H_4), 8.16 (d, 1H, C_6H_4), 10.0 (s vvbr, 1H, $-COOH$). ^{13}C NMR ($CDCl_3$) δ: 32.2, 67.6, 121.8, 123.9, 126.6, 132.7, 135.2, 150.8, 168.3, 169.8. MS (CI) *m/z*: 256 $(M + 1)^+$.

General Procedure for the Preparation of 29, 30, 31, 33, 52, 53, 54, and 55. $KMnO_4$ (4.38 mmol) was added to a stirred solution of the appropriate aldehyde **19**, **20**, **21**, **25**, **47**, **48**, **49** and **50** (2.92 mmol) in acetone (20 mL) kept at 0 °C. The reaction was allowed to reach room temperature and was completed after 3 h. Oxalic acid was added. The mixture was filtered, and the filtrate was diluted with CH_2Cl_2 (20 mL). The organic layer was washed with H_2O (20 mL) and then dried, filtered, and concentrated under reduced pressure. The crude product was purified by crystallization.

2-{[4-(Nitrooxy)butanoyl]oxy}benzoic Acid (29). mp 70.5–71.5 °C [from 50:50 PE/toluene (v/v)]. White solid. Yield 52%. ^{1}H NMR (DMSO-d_6) δ: 2.05 (qi, 2H, $-CH_2CH_2ONO_2$), 2.71 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 6.0 Hz), 4.63 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.0 Hz), 7.21 (d, 1H, C_6H_4), 7.39 (t, 1H, C_6H_4), 7.65 (t, 1H, C_6H_4), 7.94 (d, 1H, C_6H_4), 13.13 (s, 1H, $-COOH$). ^{13}C NMR (DMSO-d_6) δ: 21.4, 29.7, 72.6, 123.7, 123.8, 126.1, 131.3, 133.8, 150.0, 165.5, 170.9. MS (CI) *m/z*: 270 $(M + 1)^+$.

2-{[5-(Nitrooxy)pentanoyl]oxy}benzoic Acid (30). mp 48.5–50.5 °C [from 70:30 PE/toluene (v/v)]. White solid. Yield 56%. ^{1}H NMR ($CDCl_3$) δ: 1.89 (m, 4H, $-CH_2CH_2CH_2ONO_2$), 2.66 (m, 2H, $-OCOCH_2-$), 4.47 (m, 2H, $-CH_2ONO_2$), 7.12 (d, 1H, C_6H_4), 7.37 (t, 1H, C_6H_4), 7.63 (t, 1H, C_6H_4), 8.12 (d, 1H, C_6H_4), 12.1 (s br, 1H, $-COOH$). ^{13}C NMR ($CDCl_3$) δ: 20.9, 26.3, 33.5, 73.0, 122.2, 124.1, 126.4, 132.7, 135.2, 151.3, 170.5, 171.7. MS (CI) *m/z*: 284 $(M + 1)^+$.

2-{[6-(Nitrooxy)hexanoyl]oxy}benzoic Acid (31). mp 68.0–70.0 °C [from 75:25 PE/toluene (v/v)]. White solid. Yield 82%. ^{1}H NMR (DMSO-d_6) δ: 1.41–1.77 (m, 6H, $-CH_2CH_2CH_2CH_2ONO_2$), 2.60 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.3 Hz), 4.55 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.0 Hz), 7.20 (d, 1H, C_6H_4), 7.39 (t, 1H, C_6H_4), 7.65 (t, 1H, C_6H_4), 7.94 (d, 1H, C_6H_4), 13.10 (s, 1H, $-COOH$). ^{13}C NMR (DMSO-d_6) δ: 23.5, 24.4, 25.7, 33.1, 73.6, 123.7, 124.1, 126.0, 131.3, 133.7, 150.0, 165.6, 171.5. MS (CI) *m/z*: 298 $(M + 1)^+$.

2-{[2,2-Dimethyl-3-(nitrooxy)propanoyl]oxy}benzoic Acid (33). mp 95.0–96 °C [from 75:25 PE/toluene (v/v)]. White solid. Yield 61%. ^{1}H NMR ($CDCl_3$) δ: 1.47 (s, 6H, $-CH_3$), 4.67 (s, 2H, $-CH_2-$), 7.10 (d, 1H, C_6H_4), 7.37 (t, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 8.13 (d, 1H, C_6H_4), 12.1 (s vvbr, 1H, $-COOH$). ^{13}C NMR ($CDCl_3$) δ: 22.3, 42.5, 77.5, 122.2, 123.8, 126.5, 132.6, 135.1, 150.9, 170.2, 172.8. MS (CI) *m/z*: 284 $(M + 1)^+$.

2-{[4,5-Bis(nitrooxy)pentanoyl]oxy}benzoic Acid (52). mp 92.5–93.0 °C [from 45:55 PE/toluene (v/v)]. White solid. Yield 89%. ^{1}H NMR ($CDCl_3$) δ: 2.13–2.25 (m, 2H, $-CH_2CH_2CH-$), 2.83 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 6.0 Hz), 4.54 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.84 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.50 (m, 1H, AMX-like system, $-CHONO_2$), 7.13 (d, 1H, C_6H_4), 7.40 (t, 1H, C_6H_4), 7.66 (t, 1H, C_6H_4), 8.14 (d, 1H, C_6H_4), 11.0 (s vvbr, 1H, $COOH$). ^{13}C NMR ($CDCl_3$) δ: 24.6, 29.8, 71.4, 78.2, 122.0, 124.2, 126.9, 133.0, 135.6, 151.3, 169.9, 171.2. MS (CI) *m/z*: 345 $(M + 1)^+$.

2-{[5,6-Bis(nitrooxy)hexanoyl]oxy}benzoic Acid (53). mp 101.5–102.5 °C [from 50:50 PE/toluene (v/v)]. White solid. Yield 72%. ^{1}H NMR (DMSO-d_6) δ: 1.73–1.86 (m, 4H, $-CH_2CH_2CH-$), 2.64 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 6.0 Hz), 4.73 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.96 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.46 (m, 1H, AMX-like system, $-CHONO_2$), 7.19 (d, 1H, C_6H_4), 7.39 (t, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 7.93 (d, 1H, C_6H_4), 13.3 (s br, 1H, $-COOH$). ^{13}C NMR (DMSO-d_6) δ: 19.5, 27.5, 32.8, 71.7, 80.0, 123.7, 123.9, 126.0, 131.3, 133.7, 150.0, 165.5, 171.2. MS (CI) *m/z*: 359 $(M + 1)^+$.

2-{[6,7-Bis(nitrooxy)heptanoyl]oxy}benzoic Acid (54). The crude product was purified by preparative HPLC (Lichrospher 250-25 C_{18}, 60:40:0.1 CH_3CN/H_2O/TFA, flow of 39 mL/min, λ of 224 nm, injection of 2 mL, solution at 100 mg/mL) to give the title compound as a white solid. Yield 89%. mp 92.5–93.0 °C [from 45:55 PE/toluene (v/v)]. ^{1}H NMR ($CDCl_3$) δ: 1.51–1.65 (m, 2H), 1.74–1.86 (m, 4H, $-CH_2CH_2CH_2CH-$), 2.67 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 6.0 Hz), 4.47 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.74 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.30 (m, 1H, AMX like system, $-CHONO_2$), 7.13 (d, 1H, C_6H_4), 7.38 (t, 1H, C_6H_4), 7.65 (t, 1H, C_6H_4), 8.11 (d, 1H, C_6H_4), 8.49 (s br, 1H, $-COOH$). ^{13}C NMR ($CDCl_3$) δ: 23.9, 24.3, 29.0, 33.6, 71.1, 78.9, 121.8, 124.0, 126.4, 132.5, 135.3, 151.1, 170.0, 172.2. MS (CI) *m/z*: 373 $(M + 1)^+$.

2-{[2,2-Dimethyl-5,6-bis(nitrooxy)hexanoyl]oxy}benzoic Acid (55). mp 72.0–73.0 °C [from 70:30 PE/toluene (v/v)]. White solid. Yield 49%. ^{1}H NMR ($CDCl_3$) δ: 1.40 (s, 6H, $-CH_3$), 1.78–1.93 (m, 4H, $-CH_2CH_2CH-$), 4.45–4.52 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.73–4.79 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.27–5.34 (m, 1H, AMX-like system, $-CHONO_2$), 7.07 (d, 1H, C_6H_4), 7.37 (t, 1H, C_6H_4), 7.64 (t, 1H, C_6H_4), 8.11 (d, 1H, C_6H_4), 11.75 (s vvbr, 1H, $-COOH$). ^{13}C NMR ($CDCl_3$) δ: 24.6, 25.0, 25.6, 35.2, 42.1, 71.2, 79.4, 122.3, 123.7, 126.3, 132.4, 135.0, 151.2, 170.2, 175.4. MS (CI) *m/z*: 387 $(M + 1)^+$.

2-{[7-(Nitrooxy)heptanoyl]oxy}benzoic Acid (32). To a solution of **22** (2.6 g, 8.80 mmol) in CH_3CN (20 mL) kept at 0 °C were added a solution of KH_2PO_4 (0.80 g) in H_2O (10 mL) and 30% H_2O_2 (1.1 mL, 9.68 mmol) and dropwise a solution of 80% $NaClO_2$ (1.40 g, 12.3 mmol) in H_2O (12 mL). After 2 h, the reaction was completed. Na_2SO_3 was added to destroy the excess of H_2O_2. After acidification with 6 M HCl, the mixture was diluted with H_2O (100 mL) and extracted twice with CH_2Cl_2 (100 mL). The organic layer was dried, filtered, and concentrated under reduced pressure. The crude product was crystallized from 70:30 PE/toluene (v/v) to give the title compound as a white solid. Yield 71%. mp 47.0–49.0 °C [from 70:30 PE/toluene (v/v)]. ^{1}H NMR ($CDCl_3$) δ: 1.36–1.54 (m, 4H), 1.74–1.81 (m, 4H, $-CH_2CH_2CH_2CH_2CH_2ONO_2$) 2.64 (t, 2H, $-OCOCH_2-$, $^3J_{HH}$ = 7.3 Hz), 4.44 (t, 2H, $-CH_2ONO_2$, $^3J_{HH}$ = 6.6 Hz), 7.12 (d, 1H, C_6H_4), 7.36 (t, 1H, C_6H_4), 7.63 (t, 1H, C_6H_4), 8.10 (d, 1H, C_6H_4), 11.53 (s, 1H, $-COOH$). ^{13}C NMR ($CDCl_3$) δ: 24.2, 25.4, 26.6, 28.6, 33.9, 73.3, 122.3, 124.0, 126.2, 132.4, 135.0, 151.2, 170.3, 172.1. MS (CI) *m/z*: 312 $(M + 1)^+$.

2-{[3,4-Bis(nitrooxy)butanoyl]oxy}benzoic Acid (51). The title compound was prepared in the same manner as **32**. mp 128–129 °C (from toluene). Yield 68%. ^{1}H NMR ($CDCl_3$) δ: 3.15 (d, 2H, $-OCOCH_2-$), 4.69–4.76 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 4.92–4.98 (dd, 1H, AMX-like system, $-CH_aH_bONO_2$), 5.77–5.84 (m, 1H, AMX-like system, $-CHONO_2$), 7.16 (d, 1H, C_6H_4), 7.41 (t, 1H, C_6H_4), 7.67 (t, 1H, C_6H_4), 8.15 (d, 1H, C_6H_4), 10.90 (s vvbr, 1H, $-COOH$). ^{13}C NMR ($CDCl_3$) δ: 34.0, 70.2, 74.8, 121.5, 123.7, 126.9, 132.8, 135.3, 138.4, 150.5, 167.4. MS (CI) *m/z*: 331 $(M + 1)^+$.

Evaluation of Stability in Aqueous Buffer Solutions and Human Serum. Hydrolysis in Acidic Medium (pH 1) and Phosphate Buffer (pH 7.4). A solution of each compound (10 mM) in acetonitrile was added to 0.1 M HCl or phosphate buffer at pH 7.4 (50 mM) preheated at 37 °C. The final concentration of the compound was 250 μM. 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.

Hydrolysis in Human Serum. A solution of each compound (10 mM) in acetonitrile was added to human serum (Sigma) 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, 500 μL of the reaction mixture was withdrawn and added to 750 μL of acetonitrile containing 0.1% trifluoroacetic acid to deproteinize the serum. The sample was sonicated, vortexed, and then centrifuged for 10 min at 2150*g*. The clear supernatant was filtered by 0.45 μm PTFE filters (Alltech) and analyzed by RP-HPLC.

The reverse-phase HPLC procedure allowed for the separation and quantitation of the remaining compound and salicylic 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 performed using a HP ChemStation system (Agilent Technologies). The analytical column was a Nucleosil 100-5C18 Nautilus (250 × 4.6 mm, 5 μm particle size) (Macherey−Nagel). The mobile phase consisted of acetonitrile/water (55:45) with 0.1% trifluoroacetic acid, and the flow rate was 1.2 mL/min. The injection volume was 20 μL (Rheodyne, Cotati, CA). The column effluent was monitored at 226 nm (for compounds) and 240 nm (for salicylic acid) referenced against a 600 nm wavelength. Quantitation was performed by a comparison of peak areas with standards chromatographed under the same conditions.

Anti-inflammatory Activity. Male Wistar rats, weighing 180–200 g (Harlan, S. Pietro al Natisone, Italy) were individually housed in hanging stainless-steel cages with grid floors, at constant room temperature (25 ± 1 °C) and humidity (60 ± 5%), with an artificial 12:12 h light/dark cycle. Edema was induced in conscious rats by intraplantar injection into the right hindpaw of 0.1 mL of 1% carrageenan, suspended in 1% carboxymethylcellulose (CMC). Immediately after carrageenan injection, compounds or vehicle (1% CMC) were administered intragastrically to different groups of rats in a volume of 10 mL/kg. Salicylic acid and NO aspirin derivatives were administered at a dose equimolar to 120 mg/kg aspirin. Groups of 6–8 animals were used. The paw volume was measured with a water plethysmometer (Basile, Comerio, Italy) immediately before carrageenan injection and 3 h afterward. The edema reduction in treated animals was expressed as percent inhibition of the edema observed in vehicle-treated animals, considered as 100. The results obtained are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed with analysis of variation (ANOVA) followed by the Dunnett test.

Gastrotoxicity. Male Wistar rats, weighing 180–200 g (Harlan, S. Pietro al Natisone, Italy) were individually housed in hanging stainless-steel cages with grid floors, at constant room temperature (25 ± 1 °C) and humidity (60 ± 5%), with an artificial 12:12 h light/dark cycle. They were deprived of food but not water 24 h before the experiments. Groups of rats (n = 8–10) were given 120 mg/kg aspirin by intragastric route or equimolar doses of the compounds under study (1% vehicle CMC). Rats were sacrificed 3 h after the administration of the compounds. Immediately after the sacrifice, the stomachs were removed, opened along the lesser curvature, and examined for the assessment of mucosal lesions. The stomachs were laid on a flat surface under a stereomicroscope. The glandular mucosa was examined, and each individual hemorrhagic lesion was measured along its greatest length (<1 mm, rating = 1; 1–2 mm, rating = 2; >2 mm, rating according to their greatest length). The lengths of the lesions were summed to give an overall total, designated as the lesion index, for each stomach. The results obtained are presented as mean ± SEM. Statistical analysis was performed with ANOVA followed by the Newman−Keuls test.

Inhibition of Human Platelet Aggregation in Vitro. Venous blood samples were obtained from healthy volunteers who had not taken any drugs for at least 2 weeks. Volunteers, who were treated according to the Helsinki protocol for biomedical experimentation, gave their informed consent to the use of blood samples for research purposes. PRP was prepared by centrifugation of citrated blood at 200 g 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 the addition of the stimulus. Collagen at submaximal concentrations (0.8–1.5 μg/mL) was used as the platelet activator in PRP. Compounds under study were preincubated with PRP 10 min before the addition of the stimulus (collagen). Vehicle alone (0.5% DMSO) added to PRP did not affect platelet function in control samples. The role of NO and sGC in the inhibitory effect was investigated using the sGC inhibitor, ODQ (50 μM). At least five experiments for each compound were performed.

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

Vasodilator Activity. Thoracic aortas were isolated from male Wistar rats weighing 180–200 g (Harlan, S. Pietro al Natisone, Italy). As few animals as possible were used. The purposes and the protocols of our studies have been approved by Ministero della Salute, Rome, Italy. The endothelium was removed, and the vessels were helically cut. Three strips were obtained from each aorta. The tissues were mounted under 1.0 g of tension in organ baths containing 30 mL of Krebs-bicarbonate buffer with the following composition: 111.2 mM NaCl, 5.0 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.0 mM KH_2PO_4, 12.0 mM $NaHCO_3$, and 11.1 mM glucose, maintained at 37 °C and gassed with 95:5% O_2/CO_2 (pH 7.4). The aortic strips were allowed to equilibrate for 120 min 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 mean EC_{50} ± standard error of the mean (SEM) (in micromolars). The effects of 1 μM ODQ upon relaxation were evaluated in a separate series of experiments, in which it was added to the organ bath 5 min before the contraction. Responses were recorded by an isometric transducer connected to the MacLab System PowerLab. The addition of the drug vehicle (DMSO) had no appreciable effect on the contraction level.

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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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