Second-Generation Aspirin and Indomethacin Prodrugs Possessing an O^2-(Acetoxymethyl)-1-(2-carboxypyrrolidin-1-yl)diazenium-1,2-diolate Nitric Oxide Donor Moiety: Design, Synthesis, Biological Evaluation, and Nitric Oxide Release Studies

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The carboxylic acid group of the anti-inflammatory (AI) drugs aspirin and indomethacin was covalently linked to the 1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate ion via a one-carbon methylene spacer to obtain two new hybrid prodrugs. The aspirin prodrug (**23**) was a 2.2-fold more potent AI agent than aspirin, whereas the indomethacin prodrug (**26**) was about 1.6-fold less potent than indomethacin. Prodrugs **23** and **26** slowly released nitric oxide (NO) upon dissolution in phosphate buffer at pH 7.4 (1.1 mol of NO/mol of compound after 43 h), but the rate and the extent of NO release were higher (1.9 mol of NO/mol of compound in 3 min or less) when the compounds were incubated in the presence of porcine liver esterase. In vivo ulcer index (UI) studies showed that the aspirin prodrug **23** (UI = 0.7) and indomethacin prodrug **26** (UI = 0) were substantially less ulcerogenic than the parent drugs aspirin (UI = 51) and indomethacin (UI = 64).

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs)[a] are one of the most frequently used medications worldwide to treat pain, fever, and inflammation. NSAIDs exert their therapeutic activity by inhibiting cyclooxygenase-derived prostaglandin synthesis, but this mechanism of action is inherently responsible for the gastrointestinal (GI),[1–5] renal,[6–8] and hepatic[9] side effects observed in patients undergoing a long-term treatment. The most common side effects associated with NSAID therapy are upper GI irritation, ulceration, dyspepsia, bleeding, and in some cases death.[10] There are numerous reports describing the incidence of NSAID-induced GI side effects, but the estimates vary greatly depending upon the study design, patient population, drug(s) used, dosage, and duration of treatment; nevertheless, it is clear that NSAID-induced toxicity is a serious public-health problem contributing significantly to morbidity and mortality.

Three different strategies have emerged to improve the safety profile of NSAIDs: (a) the development of selective cyclooxygenase-2 (COX-2) inhibitors; (b) the coadministration of a proton pump inhibitor with the NSAID; and (c) the linkage of a nitrate-based nitric oxide (NO)-releasing moiety to classical NSAIDs (NO-NSAIDs). Each strategy presents a different set of advantages and limitations. For example, despite the relatively safe profile of COX-2 inhibitors in the GI tract, their role with respect to the adverse cardiovascular effects reported in some patients undergoing chronic treatment has attracted considerable attention.[11] In this regard, the adverse hypertensive effect induced by rofecoxib (**1**) was the primary factor that prompted its withdrawal from the market.[12] Organic nitrate-based NO-NSAIDs (Figure 1) such as NCX-4016 (**2**),[13] AZD-3835 (**3**),[14] NCX-2216 (**4**),[15,16] and NO-diclofenac (**5**)[17] suppress prostaglandin synthesis as effectively as the parent NSAIDs[18–20] but have been shown to spare the GI tract in animals and humans. However, an important drawback to this design is the fact that production of NO from nitrate esters requires a three-electron

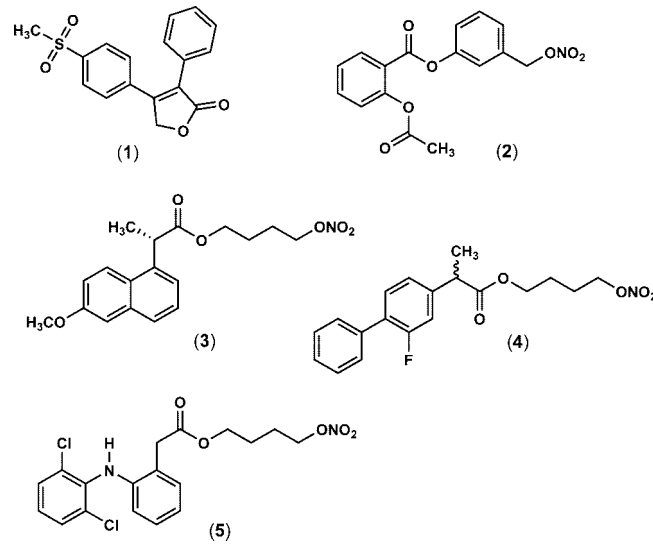

Figure 1. Chemical structures of **1** (rofecoxib) and some representative NO-NSAIDs (organic nitrates): **2–4** and **5** (NO-diclofenac).

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[a] Abbreviations: AUC, area under the curve; COX-2, cyclooxygenase-2; DCM, dichloromethane; DMA/NO, 1-(*N*,*N*-dimethylamino)diazen-1-ium-1,2-diolate nitric oxide donor; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; GI, gastrointestinal; cGMP, cyclic guanosine monophosphate; 2-HEMA/NO, 1-[2-(hydroxyethyl)methylamino]diazen-1-ium-1,2-diolate ntric oxide donor; NO, nitric oxide; NOA, nitric oxide analyzer; NONO-aspirin, O^2-acetoxymethyl 1-[2-(acetylsalicyloyloxymethyloxycarbonyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate; NONOate, *N*-diazen-1-ium-1,2-diolate moiety that can theoretically release two molecules of nitric oxide; NONO-indomethacin, O^2-acetoxymethyl 1-{2-[2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetoxy methoxycarbonyl]pyrrolidin-1-yl}diazen-1-ium-1,2-diolate; NO-NSAIDs and NONO-NSAIDs, nonsteroidal anti-inflammatory drugs that can theoretically release one and two molecules of nitric oxide, respectively; NSAIDs, nonsteroidal anti-inflammatory drugs; PLE, porcine liver esterase; PROLI/NO, 1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate ntric oxide donor; PYRRO/NO, 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate nitric oxide donor; UI, ulcer index.

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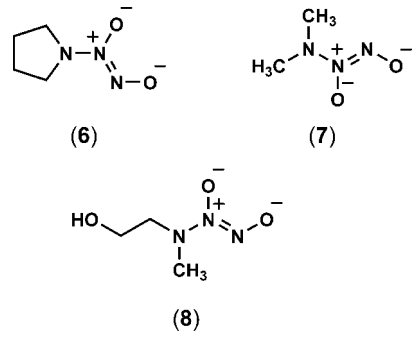


Figure 2. Chemical structures of the 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (PYRRO/NO, **6**), 1-(*N*,*N*-dimethylamino)diazen-1-ium-1,2-diolate (DMA/NO, **7**), and 1-[2-(hydroxyethyl)methylamino]diazen-1-ium-1,2-diolate (2-HEMA/NO, **8**).

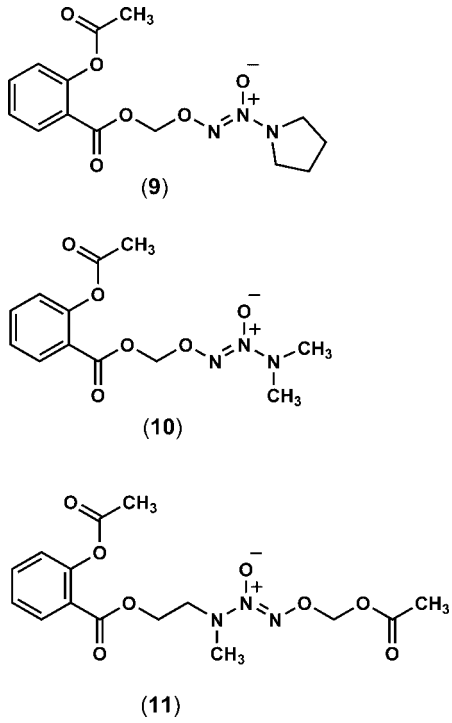


Figure 3. Chemical structures of representative NONO-NSAIDs (**9**−**11**).

reduction, and this metabolic activation can decrease in efficiency on continued use of the drugs, contributing to nitrate tolerance.[21–23]

To address safety and efficacy issues related to classical NSAIDs, COX-2 selective inhibitors, and organic nitrate-based NO-NSAIDs, our group has introduced and developed the concept of NONO-NSAIDs, which is based on the linkage of a *N*-diazen-1-ium-1,2-diolate (NONOate, ions **6**−**8**, Figure 2) functional group to the structure of a classical NSAID.[24,25] Diazen-1-ium-1,2-diolate ions release up to 2 equiv of NO without metabolic activation (first-order kinetics), they are structurally diverse, and they possess a rich derivatization chemistry that facilitates delivery of NO to specific organ and/or tissue sites.[26] These features distinguish NONO-NSAIDs **9**−**11** (Figure 3) from currently available nitrate-based NO-NSAIDs which require redox activation before NO is released.

The spontaneous decomposition reaction of *N*-diazeniumdiolates in physiological medium releases up to 2 equiv of NO (for secondary amine-based NONOates) or different ratios of NO/nitroxyl (for primary amine-based NONOates);[27–29] therefore, NONO-NSAIDs possessing a *N*-diazeniumdiolate would release (upon esterase-mediated hydrolysis) not only the active components (NSAID, NO, and/or HNO) but also 1 equiv of the corresponding amine. Considering the toxicity of *N*-nitrosopyrrolidine (**12**), *N*-nitrosodimethylamine (**13**), and *N*-nitroso-*N*-(2-hydroxyethyl)methylamine (**14**, possible decomposition products of the NONO-NSAIDs shown in Figure 4),[30] we wanted to address this issue by developing strategies to minimize or eliminate the risk of exposure to toxic nitrosamines in patients who may be treated with NONO-NSAIDs. One way to deal with this concern is to use a diazeniumdiolate ion obtained from an amine, the *N*-nitroso derivative of which is considered to be nontoxic. One such secondary amine is L-proline. Its *N*-nitroso derivative (**15**) has been the subject of numerous reports examining the effects of its long-term administration to animals, but none of these studies showed it to be tumorigenic.[31–36]

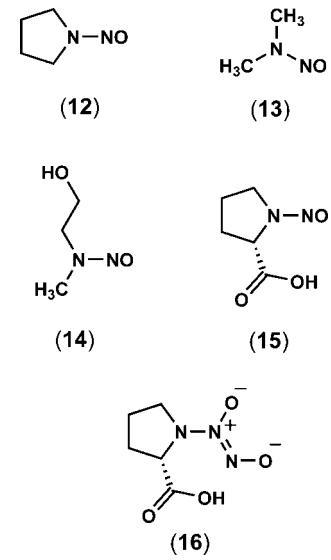
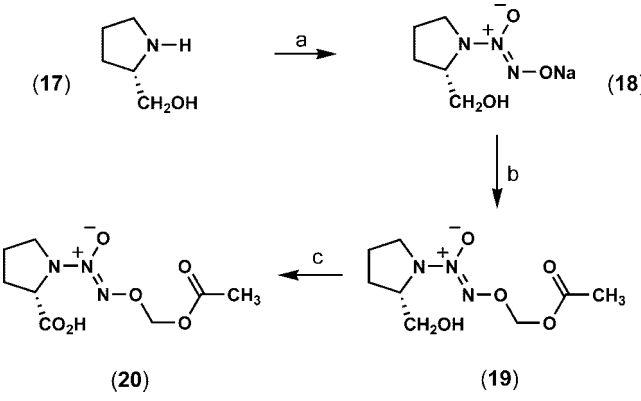


Figure 4. Chemical structures of *N*-nitrosopyrrolidine (**12**), *N*-nitrosodimethylamine (**13**), *N*-nitroso-*N*-(2-hydroxyethyl)methylamine (**14**), *N*-nitrosoproline (**15**), and 1-[2-(hydroxycarbonyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (PROLI/NO, **16**).

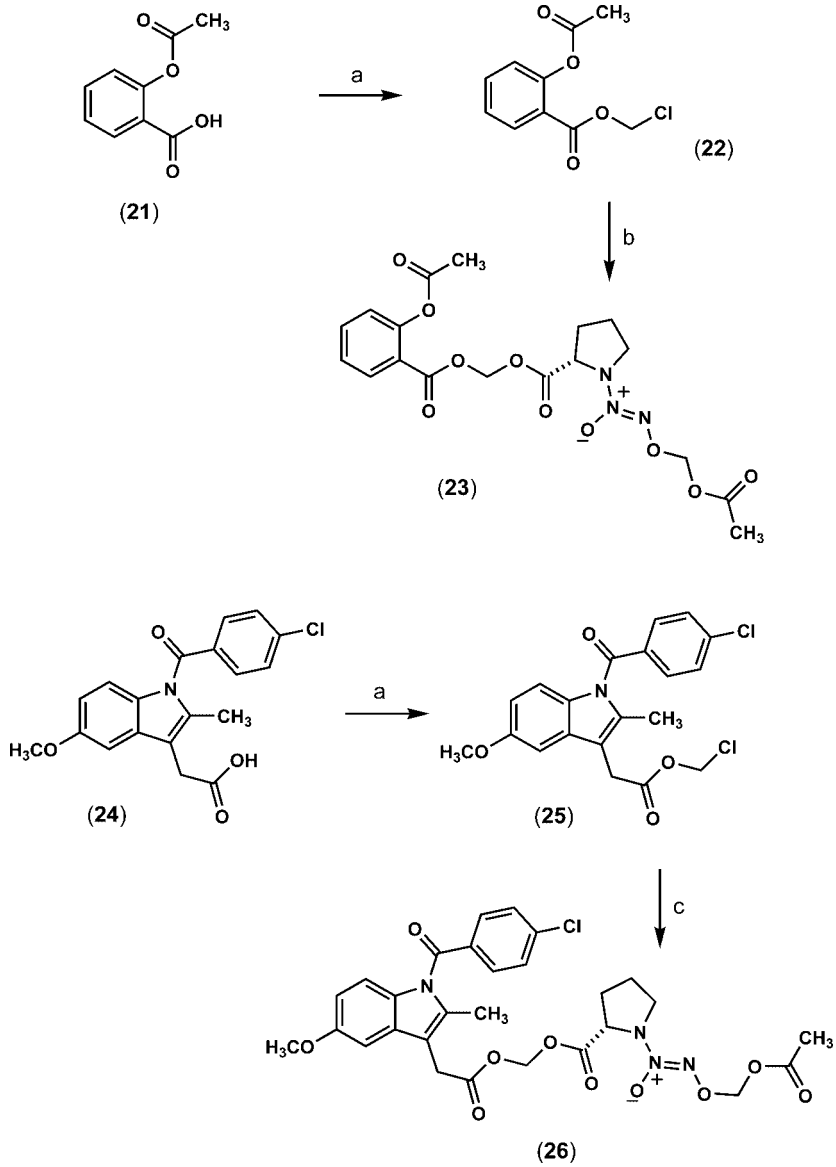
Scheme 1. Synthesis of **20**[a]



[a] Reagents and conditions: (a) nitric oxide (40 psi), $NaOCH_3/CH_3OH$, THF/ether, 25 °C, 24 h; (b) $CH_3CO_2CH_2Br$, K_2CO_3, DMSO, 25 °C, 15 h; and (c) $NaIO_4$, $RuCl_3 \cdot H_2O$, MeCN, EtOAc, H_2O, 25 °C, 2 h.

As part of our ongoing research program targeted toward the development of improved anti-inflammatory agents with a greater safety profile, we now report the synthesis, in vivo analgesic and anti-inflammatory activities, nitric oxide release data, and results from ulcerogenicity studies for two new

Scheme 2. Synthesis of **23** and **26**[a]



[a] Reagents and conditions: (a) $ClSO_3CH_2Cl$, CH_2Cl_2, H_2O, $NaHCO_3$, $(Bu)_4NSO_4H$, 25 °C, 30 min; (b) TEA, DMSO, **20**, 25 °C, 24 h; and (c) TEA, DMF, **17**, 40−45 °C, 17 h.

compounds, O^2-acetoxymethyl 1-[2-(acetylsalicyloyloxymethyloxycarbonyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**23**, NONO-aspirin) and O^2-acetoxymethyl 1-{2-[2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetoxy methoxycarbonyl]-pyrrolidin-1-yl}diazen-1-ium-1,2-diolate (**26**, NONO-indomethacin) possessing a 1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate (PROLI/NO) moiety (**16**). Hydrolysis of PROLI/NO under aerobic conditions yields NO, L-proline (a naturally occurring amino acid), nitrite/nitrate ions, and *N*-nitrosoproline (**15**).[37]

Chemistry

The synthesis of O^2-(acetoxymethyl) 1-[2-(hydroxycarbonyl)-pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**20**) was carried out by a procedure patterned after that of Chakrapani et al.,[38] as illustrated in Scheme 1. Thus, reaction of L-prolinol (**17**) with nitric oxide gas (40 psi) at room temperature in the presence of sodium methoxide afforded sodium 1-[2-(hydroxymethyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**18**) in 86% yield. The sodium salt was alkylated with bromomethyl acetate in dimethyl sulfoxide (DMSO) to afford O^2-acetoxymethyl 1-[2-(hydroxymethyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**19**), which was subsequently oxidized with sodium periodate and ruthenium chloride in a mixture of water, ethyl acetate, and acetonitrile to afford compound **20** in 83% yield.

Aspirin (**21**) and indomethacin (**24**) were reacted with chloromethyl chlorosulfate in dichloromethane at 25 °C to afford chloromethyl 2-(acetoxy)benzoate (**22**) and chloromethyl 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetate (**25**), respectively, on the basis of a procedure reported by Binderup et al.[39] The target ester prodrugs **23** and **26** were synthesized in moderate to good yields (90 and 56%, respectively) by condensation of chloromethyl esters **22** or **25** with intermediate **20** by using polar aprotic solvents such as DMSO or dimethylformamide (DMF) (Scheme 2).

Results and Discussion

Two novel diazeniumdiolate-based nonsteroidal anti-inflammatory prodrugs (NONO-NSAIDs) possessing an O^2-acetoxymethyl-protected PROLI/NO moiety were obtained. When

Table 1. Anti-inflammatory and Analgesic Activities of Prodrugs 23, 26, and the Reference Compounds Acetylsalicylic Acid (21) and Indomethacin (24)

		analgesic activity[b]		
compound	AI activity ED_{50} (μmol/kg)[a]	dose (μmol/kg)	% inhibition at 30 min	% inhibition at 60 min
23	314	68	24.6 ± 3.8	65.8 ± 5.5
aspirin (**21**)	710	277	56.5 ± 9.8	64.3 ± 13.7
26	18.5	48	36.3 ± 8.7	61.5 ± 13.7
indomethacin (**24**)	11.8	84	51.8 ± 3.5	67.6 ± 9.4

[a] Anti-inflammatory (AI) activity in a carrageenan-induced rat paw edema assay. The results are expressed as the effective dose of the test compound necessary to decrease by 50% the inflammatory response in the group of animals ($n = 4$). [b] Inhibitory activity in the 4% NaCl-induced rat abdominal constriction assay. The results are expressed as mean ± SEM ($n = 4$).

administered orally to rats, both prodrugs **23** and **26** produced a significant anti-inflammatory effect in vivo. The carrageenan-induced rat paw edema assay data (Table 1) showed a 2.2-fold increase in potency for prodrug **23** ($ED_{50} = 314$ μmol/kg) compared with the reference drug aspirin ($ED_{50} = 710$ μmol/kg), whereas NONO-indomethacin **26** ($ED_{50} = 18.5$ μmol/kg) was about 1.6-fold less potent relative to indomethacin ($ED_{50} = 11.8$ μmol/kg). Plausible explanations for the observed difference between the potency ratio for the prodrugs **23** and **26** relative to the respective aspirin and indomethacin parent compounds include different absorption, bioconversion to the parent compound, and biodistribution and/or pharmacodynamic profiles. These results are consistent with previous observations reported by our group, describing the anti-inflammatory properties of diazeniumdiolate-based NO-NSAIDs (NONO-NSAIDs) possessing a PYRRO/NO (**6**, Figure 2), a DMA/NO (**7**, moieties attached via a one-carbon methylene spacer to the carboxylic acid group of traditional NSAIDs),[24] or an O^2-acetoxymethyl-protected 2-HEMA/NO (**8**, moiety attached via a two-carbon ethylene spacer to the carboxylic acid group of the NSAID).[25]

In vivo analgesic activity was determined by using a 4% NaCl-induced rat abdominal constriction assay, and the results are shown in Table 1. Prodrugs **23** and **26** exhibited moderate analgesic activities (24.6 and 36.3% inhibition of writhing, respectively) 30 min after oral administration of a 30 mg/kg dose, compared to the reference drugs aspirin (56.5% inhibition, 50 mg/kg) and indomethacin (51.8% inhibition, 30 mg/kg); however, at 60 min post-administration, the analgesic activity observed for both prodrugs (**23**, 65.8% inhibition and **26**, 61.5% inhibition) was equipotent to that of the parent compounds (aspirin, 64.3% inhibition and indomethacin, 67.6% inhibition). Nevertheless, when we analyzed the correspondent doses on a molar basis, it was evident that NONO-aspirin (68 μmol/kg dose) and NONO-indomethacin (48 μmol/kg dose) showed an increased potency relative to the parent NSAIDs aspirin (277 μmol/kg dose) and indomethacin (84 μmol/kg dose). On the basis of these results, NONO-aspirin was 4-fold more potent than aspirin, and NONO-indomethacin was about 1.7-fold more potent than indomethacin.

Previously reported O^2-acetoxymethyl diazen-1-ium-1,2-diolates are relatively stable compounds that hydrolyze slowly at pH 7.4.[40] Consistent with these observations, when prodrugs **23** and **26** were incubated in phosphate buffer at pH 7.4, the time required to detect about 1 mol of NO/mol of test compound (50% of the total amount of NO typically obtained from diazeniumdiolate ions) was 43−45 h, which is indicative of slow NO release under these conditions. However, both ester prodrugs are hydrolyzed more extensively (1.9 mol of NO/mol of prodrug) in the presence of porcine liver esterase (PLE) with a considerable increase in their corresponding rates of hydrolysis (95% of NO released in 2.4−3.2 min, see Table 2). NONO-NSAID ester prodrugs **23** and **26** were designed to possess three different ester groups (four in the case of NONO-aspirin). Two carboxyl groups (the one from NSAID and the one from PROLI/NO) are linked to each other by a one-carbon methylene spacer, forming two ester groups which bind the active components of the prodrug (the NSAID and the NO-releasing group). The third ester group protects the PROLI/NO moiety from releasing NO spontaneously because of the presence of a one-carbon methylene spacer between the acetoxy group of the acetoxymethyl protecting group and the diazen-1-ium-1,2-diolate O^2-atom. Following enzymatic hydrolysis of the acetate moiety, the O^2-(hydroxymethyl)diazen-1-ium-1,2-diolate moieties of **27** or **28** thus produced would spontaneously eliminate formaldehyde to form the free NONOate moiety (**16** or **29**), which would subsequently fragment to release two molecules of NO (Scheme 3A or B).

Table 2. Nitric Oxide from NONO-NSAIDs 23, 26, and PROLI/NO (16)

	nitric oxide released[a]			
compound	buffer pH 7.4[b]	t (h)[c]	PLE[d]	t (min)[e]
23	1.1[f]	43	1.9[g]	2.4
26	1.1[h]	45	1.8[i]	3.2
PROLI/NO (**16**)	2.0[j]			0.03

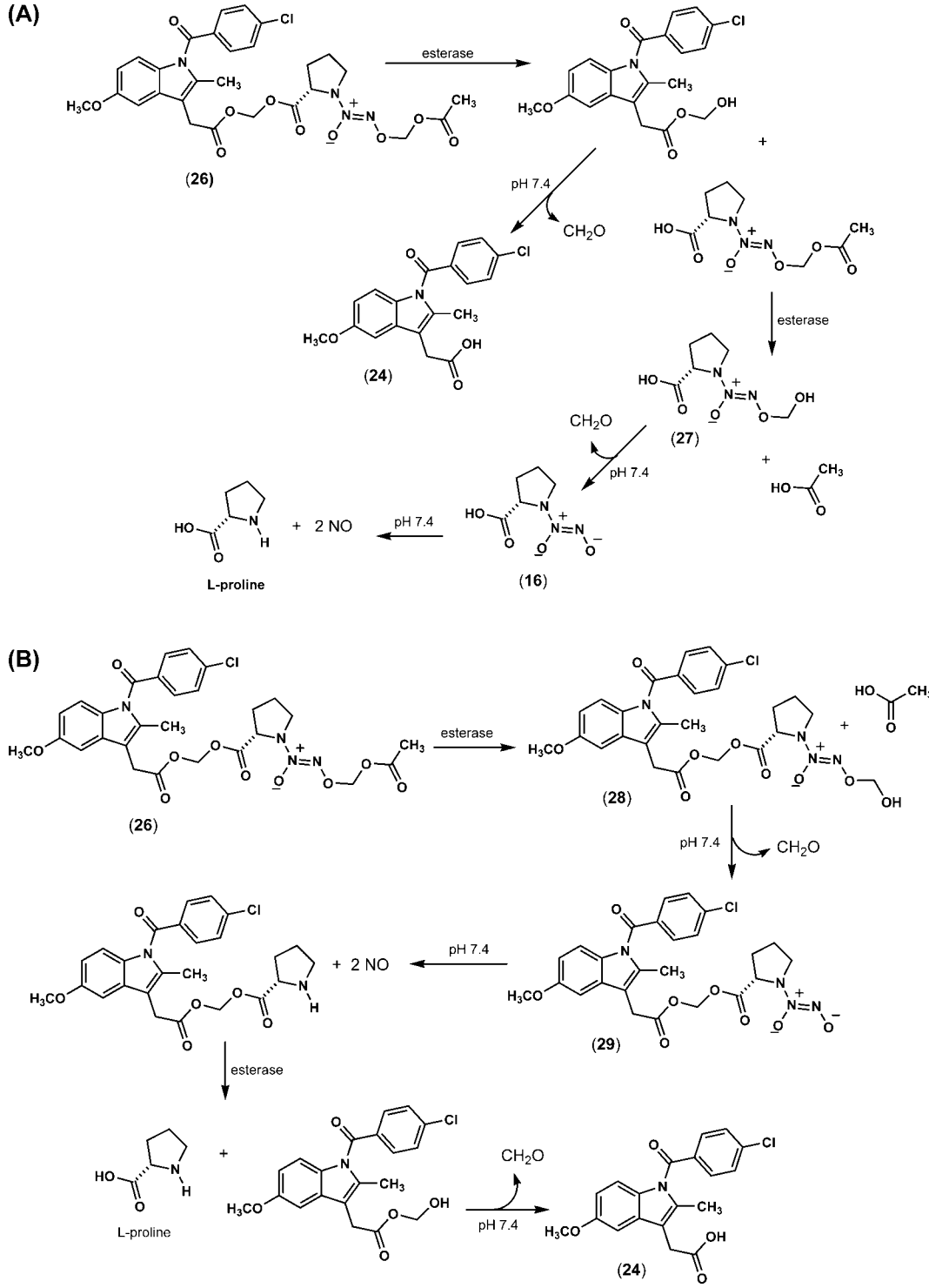
[a] Moles of NO/mol of test compound. [b] Incubated in 0.1 M phosphate-buffered solution at pH 7.4 and 37 °C. [c] Time required to detect about 50% of the theoretical total amount of NO/mol of test compound. Calculated graphically from the corresponding time vs mol NO/min curves. [d] Moles of NO released at 37 °C in the presence of porcine liver esterase (PLE, 29 units, 10 μL of a suspension in 3.2 M $(NH_4)_2SO_4$, Sigma). [e] Time required to detect more than 90% of the theoretical total amount of NO/mol of test compound. Calculated graphically from the corresponding time vs mol NO/min curves. [f] 10 μL of a 37 μM solution (in DMSO) injected into a flask containing 3 mL of phosphate buffer pH 7.4. [g] 10 μL of a 37 μM solution (in DMSO) injected into a flask containing 3 mL of phosphate buffer pH 8.0. [h] 10 μL of a 46 μM solution (in DMSO) injected into a flask containing 3 mL of phosphate buffer pH 7.4. [i] 10 μL of a 46 μM solution (in DMSO) injected into a flask containing 3 mL of phosphate buffer pH 8.0. [j] Moles of NO released, with a calculated half-life of 1.8 s (0.03 min) reported by Saavedra et al.[49]

The most common side effects associated with the long-term administration of NSAIDs are gastric erosions, ulcer formation, and sometimes severe bleeding. Therefore, the potential ulcerogenic side effects of prodrugs **23** and **26** were determined and compared to those produced by the parent NSAIDs (Table 3). Both NONO-NSAIDs showed an improved safety profile relative to their NSAID counterparts. Unlike acetylsalicylic acid (ulcer index (UI) = 51), the NONO-aspirin **23** (UI = 0.75) produced only a few detectable lesions on the gastric mucosa in the group of animals examined, and NONO-indomethacin **26** showed no visible lesions. This represents a remarkable improvement considering that indomethacin (UI = 64) was the most irritant compound on a molar basis. The gastric-sparing effect observed for NONO-NSAIDs is believed to be due (at least in part) to the nitric oxide-releasing properties of these compounds. Future studies will be conducted to measure COX-derived prostaglandin levels at target tissues, cyclic guanosine monophosphate (cGMP) concentration (guanylate cyclase activity), leukocyte recruitment, and other biochemical mechanisms associated with cytoprotective effects.

Conclusions

We demonstrated that design of *N*-diazeniumdiolate-based nitric oxide-releasing nonsteroidal anti-inflammatory prodrugs (NONO-NSAIDs) represents a rational approach to improved anti-inflammatory and analgesic agents, with markedly reduced

Scheme 3. (A and B) Theoretical Metabolic Activation Mechanisms (Esterase-Based Hydrolysis) of Prodrug **26** (NO-indomethacin, Shown as a Representative Example)



GI side effects. NONO-NSAIDs invoke metabolic activation by esterase-mediated hydrolysis. The use of a second-generation 1-[2-(hydroxycarbonyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate group (PROLI/NO) derived from a naturally occurring amino acid (L-proline) proved to be an advantageous alternative to the use of previously reported *N*-diazeniumdiolates. Finally, NONO-aspirins obtained with the use of this technology could represent a suitable alternative to the use of aspirin for the prophylactic prevention of a wide variety of disorders where the pharmacological inhibition of cyclooxygenase enzymes is recommended or required, including (but not limited to) inflammation, stroke, myocardial infarction, or colon cancer prevention.

Experimental Section

General. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were acquired by using a Varian Unity Inova spectrometer. UV spectra were recorded by using an Agilent 8453 spectrophotometer (Agilent Technologies). IR spectra were acquired by using a Buck Scientific M-500 spectrometer. Optical rotations were measured by using a JASCO P-1010 polarimeter. Microanalyses were performed

Table 3. Ulcer Index Assay for Compounds 23, 26, Aspirin (21), and Indomethacin (24)

compound	dose (mmol/kg)	ulcer index[a]
23	1.38	0.75 ± 0.87
aspirin (**21**)	1.38	51.4 ± 9.0
26	0.08	0
indomethacin (**24**)	0.08	64.5 ± 10.5
control group[b]		0

[a] Calculated by adding the total length (in mm) of individual ulcers in each stomach and averaging over the number of animals ($n = 4$) in each group. Data are presented as mean total length ± SEM at 6 h after oral administration of the test compound. [b] 1.0% methylcellulose solution.

by Midwest Analytic (Indianapolis, IN) and were within ±0.4% of theoretical values for all elements listed. Flash column chromatography was performed by using Versapak 23 × 53 mm or 23 × 110 mm cartridges (silica gel 20−45 μm). Nitric oxide gas was purchased from Matheson Gas Products (Montgomeryville, PA). Quantification of NO by chemiluminescence was determined by using a Sievers nitric oxide analyzer (NOA) model 280i, as previously described.[41] Sodium 1-[2-(hydroxymethyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**18**),[38] chloromethyl chlorosulfate,[39] and bromomethyl acetate[42] were prepared according to reported procedures, but all other reagents (including the PLE, suspended in 3.2 M ammonium sulfate) were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. The in vivo anti-inflammatory,[43] analgesic,[44] and ulcer index assays[45–48] were carried out by using protocols approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

Sodium 1-[2-(Hydroxymethyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (18). (S)-(+)-2-(Hydroxymethyl)pyrrolidine (**17**, 10.0 g, 98 mmol) was dissolved in a 1:1 mixture of THF/diethyl ether (200 mL) and mixed with sodium methoxide (118 mmol, 22 mL of a 30% w/v solution in methanol) while being stirred at 25 °C for 5 min. This mixture was flushed with dry nitrogen for 5 min, and the reaction was allowed to proceed under an atmosphere of nitric oxide (40 psi internal pressure) with stirring at 25 °C for 24 h. The product, which precipitated as a fine white powder, was isolated by filtration and then suspended in diethyl ether (100 mL) while being stirred for 5 min. The suspension was filtered, and the collected solid was dried at 25 °C under reduced pressure for 4 h to afford **18** as a fine white powder (15.5 g, 86%). mp 122−125 °C (dec.), UV (2-propanol) λ_{max} (ϵ) 232 nm (23.0 mM^{-1} cm^{-1}); ^{1}H NMR (NaOD-D_2O) δ 1.67−2.10 (m, 4H, pyrrolidin-1-yl H-3, H-4), 3.15−3.62 (m, 5H, CH_2OH, pyrrolidin-1-yl H-2, H-5). Intermediate **18** was used immediately after drying without further purification.

***O*^{2}-(Acetoxymethyl) 1-[2-(Hydroxymethyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (19).** The sodium diazeniumdiolate **18** (5.0 g, 27 mmol) was added to a suspension of potassium carbonate (1.8 g, 13 mmol) in DMSO (70 mL) at 25 °C while being stirred for 5 min. Freshly distilled bromomethyl acetate (4.1 g, 27 mmol) was added dropwise, and the reaction was allowed to proceed at 25 °C for 15 h with stirring. Ethyl acetate (200 mL) was added to dilute the reaction, the solids were filtered off, and the organic phase was washed with water (5 × 80 mL) and dried (Na_2SO_4). The solvent was removed in vacuo to give a liquid residue which was purified by flash column chromatography by using EtOAc/hexane (1:1, v/v) as eluent. Compound **19** (1.1 g, 18%) was obtained as a pale yellow liquid. UV (2-propanol) λ_{max} (ϵ) 220 nm (16.7 mM^{-1} cm^{-1}), ^{1}H NMR ($CDCl_3$) δ 1.80−2.11 (m, 4H, pyrrolidin-1-yl H-3, H-4), 2.12 (s, 3H, $OCOCH_3$), 3.57−3.72 (m, 3H, HOC*H*H′, pyrrolidin-1-yl H-5), 3.79 (dd, J = 11.3, 3.8 Hz, 1H, HOCH*H*′), 4.11−4.17 (m, 1H, pyrrolidin-1-yl H-2), 5.77 (s, 2H, OCH_2O); ^{13}C NMR ($CDCl_3$) δ 20.8, 23.0, 26.9, 52.7, 63.9, 65.2, 87.24, 169.5.

***O*^{2}-(Acetoxymethyl) 1-[2-(Hydroxycarbonyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (20).** A solution of compound **19** (1.0 g, 4.2 mmol) in acetonitrile (8 mL), water (12 mL), and ethyl acetate (8 mL) was stirred with sodium periodate (3.76 g, 17.6 mmol) and ruthenium chloride monohydrate (cat. amount) at 25 °C for 2 h. The white solid suspended in the reaction media was removed by filtration, and the clear solution was diluted with ethyl acetate (30 mL) and water (30 mL). After shaking, the water layer was separated and acidified to pH 2 with conc. HCl solution. One additional extraction with ethyl acetate (20 mL) was carried out. Combined organic phases were dried (Na_2SO_4), and the solvent was evaporated. The residue (dark oil) was purified by flash chromatography by using DCM/MeOH (95:5, v/v) as eluent to furnish **20** (0.8 g, 83%). UV (2-propanol) λ_{max} (ϵ) 247 nm (7.9 mM^{-1} cm^{-1}); ^{1}H NMR ($CDCl_3$) δ 2.02−2.17 (m, 2H, pryrrolidin-1-yl H-4), 2.12 (s, 3H, $COCH_3$), 2.21−2.38 (m, 2H, pyrrolidin-1-yl H-3), 3.63−3.77 (m, 1H, pyrrolidin-1-yl H-5), 3.82−3.88 (m, 1H, pyrrolidin-1-yl H′-5), 4.61 (dd, J = 8.9, 4.1 Hz, 1H, pyrrolidin-1-yl H-2), 5.75 (d, J = 7.2 Hz, 1H, -OC*H*H′O-), 5.78 (d, J = 7.2, 1H, -OCH*H*′O-); ^{13}C NMR ($CDCl_3$) δ 20.8, 22.5, 27.5, 51.1, 61.8, 87.2, 169.5, 175.0.

Chloromethyl 2-(Acetoxy)benzoate (22). Acetylsalicylic acid (**21**, 2.0 g, 11 mmol), dichloromethane (DCM, 10 mL), water (10 mL), sodium bicarbonate (3.5 g, 42 mmol), and tetrabutylammonium hydrogen sulfate (0.4 g, 1.1 mmol) were stirred at 25 °C for 2 min. A solution of chloromethyl chlorosulfate (2.14 g, 13 mmol) in DCM (3 mL) was added dropwise. This biphasic system was stirred vigorously at 25 °C for 30 min; the organic phase was separated and dried ($MgSO_4$). After evaporation of the solvent, the residue (2.2 g of a colorless oil) was purified by flash chromatography by using EtOAc/hexane (1:4, v/v) as eluent to give **22** (2.1 g, 86%). ^{1}H NMR ($CDCl_3$) δ 3.37 (s, 3H, -CH_3), 5.89 (s, 2H, -OCH_2Cl), 7.14 (dd, J = 8.1, 1.1 Hz, phenyl H-3), 7.34 (td, J = 7.8, 1.1 Hz, 1H, phenyl H-5), 7.61 (td, J = 7.8, 1.4 Hz, 1H, phenyl H-4), 8.04 (dd, J = 7.8, 1.4 Hz, 1H, phenyl H-6); ^{13}C NMR ($CDCl_3$) δ 20.9, 69.0, 121.7, 124.0, 126.1, 131.9, 134.8, 151.1, 162.2, 169.5. Anal. ($C_{10}H_9ClO_4$) C, H.

Chloromethyl 2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetate (25). Indomethacin (**24**, 2.0 g, 5.6 mmol), DCM (5 mL), water (5 mL), sodium bicarbonate (1.8 g, 21.0 mmol), and tetrabutylammonium hydrogen sulfate (0.2 g, 0.5 mmol) were stirred at 25 °C for 2 min. A solution of chloromethyl chlorosulfate (1.7 g, 10.3 mmol) in DCM (3 mL) was added dropwise. This biphasic system was stirred vigorously at 25 °C for 3 h; the organic phase was separated and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by flash chromatography by using EtOAc/hexane (1:4, v/v) as eluent to give **25** (1.7 g, 74%). ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H, CH_3), 3.74 (s, 2H, CH_2CO_2), 3.83 (s, 3H, OCH_3), 5.71 (s, 2H, OCH_2Cl), 6.68 (dd, J = 8.9, 2.5 Hz, 1H, indolyl H-6), 6.87 (d, J = 8.9 Hz, 1H, indolyl H-7), 6.92 (d, J = 2.5 Hz, 1H, indolyl H-4), 7.47 (ddd, J = 8.6, 2.2, 1.9 Hz, 2H, benzoyl H-3, H-5), 7.65 (ddd, J = 8.6, 2.2, 1.9 Hz, 2H, benzoyl H-2, H-6); ^{13}C NMR ($CDCl_3$) δ 13.3, 30.0, 55.7, 69.0, 101.0, 111.2, 111.8, 114.9, 129.1, 130.2, 130.7, 131.1, 133.7, 136.2, 139.3, 156.0, 168.2, 168.8. Anal. ($C_{20}H_{17}Cl_2NO_4$) C, H, N.

***O*^{2}-Acetoxymethyl 1-[2-(Acetylsalicyloyloxymethyloxycarbonyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (23).** Compound **20** (0.8 g, 3.2 mmol) was dissolved in DMSO (3 mL) and stirred with triethylamine (0.4 mL, 3.2 mmol) at 25 °C for 5 min. Then, it was added to a solution of compound **22** (0.7 g, 3.2 mmol) in DMSO (3 mL). After the solution was stirred for 24 h at 25 °C, ethyl acetate (100 mL) was added to dilute the reaction. The organic phase was washed with water (5 × 30 mL), dried ($MgSO_4$), and evaporated under vacuum. The residue (pale yellow oil) was purified by flash chromatography by using EtOAc/hexane (1:2, v/v) as eluent to furnish **23** (1.2 g, 90%). UV (2-propanol) λ_{max} (ϵ) 208 nm (17.2 mM^{-1} cm^{-1}); $[\alpha]^{18.8}_D$ = −39.7 (0.0433, $CHCl_3$); IR (neat) 2982 (C-H aliphatic), 1760 (*C*═O); ^{1}H NMR ($CDCl_3$) δ 1.96−2.15 (m, 3H, pyrrolidin-1-yl H-3, H-4, H′-4), 2.09 (s, 3H, $COCH_3$), 2.27−2.34 (m, 1H, pyrrolidin-1-yl H′-3), 2.35 (s, 3H, $COCH_3$), 3.66−3.72 (m, 1H, pyrrolidin-1-yl H-5), 3.85−3.91 (m, 1H, pyrrolidin-1-yl H′-5), 4.61 (dd, J = 8.8, 4.0 Hz, 1H, pyrrolidin-1-yl H-2), 5.68 (d, J = 7.2 Hz, 1H, -OC*H*H′OAc), 5.72 (d, J = 7.2, 1H, OCH*H*′OAc), 5.94 (d, J = 5.6 Hz, 1H, -CO_2C*H*H′O_2C-), 6.05 (d, J = 5.6 Hz, 1H, -CO_2CH*H*′O_2C-), 7.12 (dd, J = 8.0, 1.2 Hz, 1H, phenyl H-3), 7.34 (td, J = 7.6, 1.2 Hz, 1H, phenyl H-5), 7.61

(td, $J = 7.6$, 1.6 Hz, 1H, phenyl H-4), 8.07 (dd, $J = 8.0$, 1.6 Hz, 1H, phenyl H-6); ^{13}C NMR ($CDCl_3$) δ 20.8, 20.97, 22.2, 27.7, 50.7, 61.5, 79.6, 87.2, 121.8, 124.0, 126.1, 132.2, 134.8, 151.1, 162.9, 169.4, 169.6, 169.9. Anal. ($C_{18}H_{21}N_3O_{10}$) C, H, N.

***O*^{2}-Acetoxymethyl 1-{2-[2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)acetoxy methoxycarbonyl]pyrrolidin-1-yl}diazen-1-ium-1,2-diolate (26).** A solution of compound **20** (1.0 g, 4.0 mmol) in DMF (4 mL) and triethylamine (0.6 mL, 4.4 mmol) was stirred at 25 °C for 10 min, and a solution of compound **25** (1.6 g, 4.0 mmol) in DMSO (3 mL) was added. After the solution was stirred for 17 h at 40−45 °C, the reaction mixture was diluted with ethyl acetate (100 mL). The organic phase was washed with water (5 × 30 mL), dried ($MgSO_4$), and evaporated under vacuum. The residue (yellow oil) was purified by column chromatography by using EtOAc/hexane (1:1, v/v) as eluent to furnish **26** (1.4 g, 56%) as a clear yellow oil. UV (2-propanol) λ_{max} (ϵ) 247 nm (14.8 mM^{-1} cm^{-1}); $[\alpha]^{18.8}_D = -23.8$ (0.0068, $CHCl_3$); IR (neat) 2978 (C-H aliphatic), 1749 (*C*═O); ^{1}H NMR ($CDCl_3$) δ 1.70−1.78 (m, 1H, pyrrolidin-1-yl H-3), 1.90−1.97 (m, 2H, pyrrolidin-1-yl H-4), 2.10 (s, 3H, $COCH_3$), 2.12−2.22 (m, 1H, pyrrolidin-1-yl H′-3), 2.36 (s, 3H, C*H*$_3$), 3.61−3.67 (m, 1H, pyrrolidin-1-yl H-5), 3.72 (s, 2H, C*H*$_2$CO$_2$), 3.76−3.82 (m, 1H, pyrrolidin-1-yl H′-5), 3.84 (s, 3H, OC*H*$_3$), 4.51 (dd, $J = 9.2$, 4.4 Hz, 1H, pyrrolidin-1-yl H-2), 5.69 (d, $J = 7.2$ Hz, 1H, -CO$_2$CH*H*′O$_2$C-), 5.72 (d, $J = 7.2$ Hz, 1H, -CO$_2$C*H*H′O$_2$C-), 5.75 (d, $J = 5.6$ Hz, 1H, -OC*H*H′OAc), 5.85 (d, $J = 5.6$ Hz, 1H, -OCH*H*′OAc), 6.67 (dd, $J = 8.8$, 2.4 Hz, 1H, indolyl H-6), 6.90 (d, $J = 9.2$ Hz, 1H, indolyl H-7), 6.95 (d, $J = 2.4$ Hz, indolyl H-4), 7.47 (ddd, $J = 8.8$, 2.4, 2.0 Hz, 2H, benzoyl H-2, H-6), 7.66 (ddd, $J = 8.4$, 2.4, 1.6 Hz, 2H, benzoyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 13.3, 20.8, 22.2, 27.4, 30.0, 50.7, 55.7, 61.3, 79.6, 87.1, 101.1, 111.4, 111.8, 114.9, 129.1, 130.3, 130.7, 131.1, 131.4, 133.7, 136.2, 139.3, 156.1, 168.2, 169.3, 169.4, 169.7. Anal. ($C_{28}H_{29}ClN_4O_{10}$) C, H, N.

Anti-inflammatory Assay. The test compounds **23** and **26**, as well as the reference drugs aspirin and indomethacin were evaluated by using the in vivo rat carrageenan-induced foot paw edema model reported previously.[43]

Analgesic Activity. Analgesic activity was determined by using a 4% sodium chloride-induced writhing (abdominal constriction) assay previously reported.[44]

Nitric Oxide Release Assay. NO gas measurements were performed by using a Sievers NOA, model 280i, by using a method described earlier.[41] The instruments were calibrated before each experiment with nitrogen as the zero gas. Mixtures of NO/He (certified standards, MG Industries, Morrisville, PA) at different concentrations were injected into the reaction chamber, recording the area under the curve (AUC) for each peak and plotting μmol of NO vs peak area. Linear regression analysis of resultant graphs showed correlation coefficients of 0.999 or better. Measurement of NO released from the test compounds was performed by injecting the prodrug dissolved in DMSO into a clean, dry, NOA measurement cell (sealed with a septum) containing deoxygenated 0.1 M phosphate-buffered solution (3 mL, pH 7.4) and 50 μM diethylenetriaminepentaacetic acid. The NO generated from the samples was carried from the phosphate-buffered solution to the NOA via a constant nitrogen purge. Integration of the resulting AUC was used to calculate the amount of NO released from each test compound on the basis of the calibration curve. The experiments with PLE (10 μL of a suspension in 3.2 M ammonium sulfate) were carried out in phosphate-buffered solution (3 mL) at pH 8.0.

Acute Ulcerogenesis Assay. The ability to produce gastric damage was evaluated according to reported procedures.[45−48] Ulcerogenic activity was evaluated after oral administration of aspirin (1.38 mmol/kg), indomethacin (0.08 mmol/kg), or an equimolar amount of the corresponding test compound (**23** or **26**). All drugs were suspended and administered in 1.7 mL of a 1% methylcellulose solution. Control rats received oral administration of vehicle (1.7 mL of 1% methylcellulose solution). Food, but not water, was removed 24 h before administration of test compounds. Six hours after oral administration of the drug, rats were euthanized in a CO_2 chamber, and their stomachs were removed, cut out along the greater curvature of the stomach, gently rinsed with water, and placed on ice. The number and the length of ulcers observed in each stomach were determined by using magnifier lenses. The severity of each gastric lesion was measured along its greatest length (1 mm, rating of 1; 1−2 mm, rating of 2; and >2 mm, rating according to their length in millimeter). The UI for each test compound was calculated by adding the total length (L, in mm) of individual ulcers in each stomach and averaging over the number of animals in each group ($n = 4$): $UI = (L_1 + L_2 + L_3 + L_4)/4$.

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Supporting Information Available: A table of combustion data is presented for compounds **23**, **26**, **22**, and **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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