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Ethanesulfohydroxamic Acid Ester Prodrugs of Nonsteroidal Anti-inflammatory Drugs (NSAIDs): Synthesis, Nitric oxide and Nitroxyl Release, Cyclooxygenase Inhibition, Anti-inflammatory, and Ulcerogenicity Index Studies

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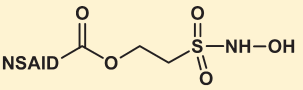
ABSTRACT: The carboxylic acid group of the anti-inflammatory (AI) drugs indomethacin, (*S*)-naproxen and ibuprofen was covalently linked via a two-carbon ethyl spacer to a sulfohydroxamic acid moiety ($CH_2CH_2SO_2NHOH$) to furnish a group of hybrid ester prodrugs that release nitric oxide (NO) and nitroxyl (HNO). Biological data acquired for this hitherto unknown class of ethanesulfohydroxamic acid ester prodrugs showed (i) all compounds exhibited superior NO, but similar HNO, release properties relative to arylsulfohydroxamic acids, (ii) the (*S*)-naproxen and ibuprofen prodrug esters are more potent AI agents than their parent NSAID, (iii) the indomethacin prodrug ester, in contrast to indomethacin which is highly ulcerogenic, showed no visible stomach lesions [ulcer index (UI) = 0 for a 80 μmol/kg oral dose] while retaining potent AI activity, and iv) that the indomethacin prodrug ester, unlike indomethacin which is an ulcerogenic selective COX-1 inhibitor, is a selective COX-2 inhibitor (COX-2 selectivity index = 184) devoid of ulcerogenicity that is attributed to its high COX-2 SI and/or ability to release cytoprotective NO.



NSAID = Indomethacin, (*S*)-Naproxen, Ibuprofen

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) of the aryl-(heteroaryl)acetic acid class (**1**−**3**, see Figure 1) exert their therapeutic effects by inhibiting cyclooxygenase-derived prostaglandin synthesis. However, inhibition of the constitutive cyclooxygenase-1 (COX-1) isozyme is responsible for gastrointestinal (GI),[1,2] renal,[3,4] and hepatic[5] side effects often observed in patients undergoing long-term treatment. Selective cyclooxygenase-2 (COX-2) inhibitors, despite their safe profile in the GI tract, may cause an increased risk of adverse cardiovascular effects such as thrombosis and stroke in some patients undergoing chronic treatment.[6]

Nitric oxide (NO) is an effective vasodilation agent, an inhibitor of platelet aggregation and adhesion,[7] and it provides a promising concept to suppress vascular side effects observed with NSAID use.[8,9] Nitroxyl (HNO) is (i) the reduced form of nitric oxide (NO), (ii) an effective vasodilation agent and inhibitor of platelet aggregation and adhesion like NO,[10] (iii) a positive inotropic cardiac agent,[11−13] (iv) a protector against cardiac ischemia-reperfusion injury,[14] and (v) it is resistant to superoxide radical anion.[11] Benzenesulfohydroxamic acid ($PhSO_2NHOH$), common name of Piloty's acid (PA), can serve as a HNO and/or NO donor.[15] In this regard, we recently reported a novel group of nonselective COX inhibitors (**5**) wherein the $MeSO_2$ COX-2 pharmacophore present in 1,1-diphenyl-2-(4-methanesulfonylphenyl)hex-1-ene (**4**) was replaced by a SO_2NHOH NO/HNO donor prodrug moiety.[16] These $ArylSO_2NHOH$ compounds **5** showed moderate in vitro COX-1 and COX-2 inhibitory, and in vivo anti-inflammatory (AI), activities relative to the reference drugs celecoxib, ibuprofen, and aspirin. The arylsulfohydroxamic acids **5** acted as NO donors at physiogical pH that was increased in the presence of a strong oxidant, but a nonphysiological alkaline pH was required to release HNO.

The simplest alkylsulfohydroxamic acid CH_3SO_2NHOH is reported to release HNO at a rate comparable to the well-known HNO donor Angeli's salt (sodium trioxodinitrate) at physiological pH.[17,18] Accordingly, it was of interest to investigate whether coupling a short-chain alkylsulfohydroxamic acid to the CO_2H group of NSAIDs would provide a hitherto-unknown class of ester compounds that act as dual NO and HNO donors having good NO/HNO release properties at physiological pH. In continuation of our ongoing studies to design novel AI drugs that are devoid of adverse ulcerogenic and/or cardiovascular side effects, we now describe the synthesis of a group of $CH_2CH_2SO_2NHOH$ and $CH_2CH_2SO_2NHOMe$ ester prodrugs (**9a**−**e**) of the NSAIDs indomethacin, ibuprofen, (*S*)-naproxen, and the reference compound phenylacetic acid (Scheme 1), in vitro NO and HNO release, in vitro COX-1/COX-2 inhibition, and in vivo ulcerogenic index studies, and their evaluation as AI agents.

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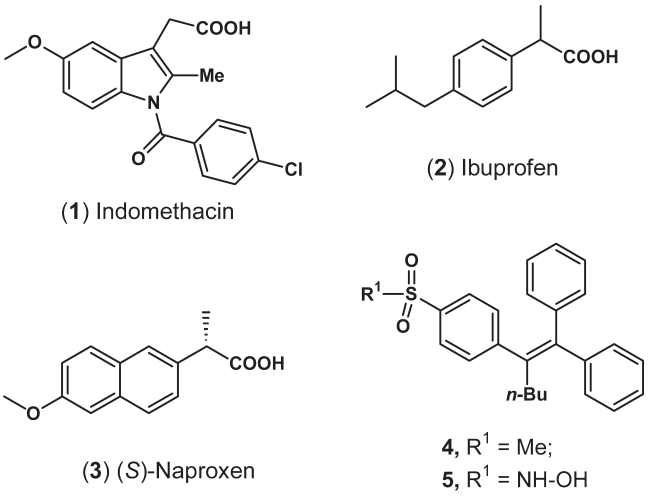


Figure 1. Chemical structures of some representative NSAIDs (**1**−**3**), a representative selective COX-2 inhibitory triaryl olefin (**4**), and an acyclic triaryl olefin possessing a sulfohydroxamic acid dual NO/HNO donor moiety (**5**).

RESULTS AND DISCUSSION

Chemistry. Reaction of indomethacin, (*S*)-naproxen, phenylacetic acid, or ibuprofen with oxalyl chloride in dry CH_2Cl_2 and a catalytic amount of DMF furnished the respective acid chloride (**6a**, **6b**, **6c**, or **6d**) as previously reported.[19] Because indomethacin acid chloride (**6a**) is unstable at high temperature (>130 °C), condensation of **6a** with 2-hydroxyethanesulfonic acid sodium salt was carried out using dry pyridine as solvent at 60 °C to prepare the carbonyloxyethanesulfonic acid sodium salt (**7a**).[20] In contrast, reaction of the (*S*)-naproxen, phenylacetic, and ibuprofen acid chlorides with 2-hydroxyethanesulfonic acid sodium salt at 130 °C in the absence of a solvent afforded the respective carbonyloxyethanesulfonic acid sodium salt (**7b**, **7c**, or **7d**).[21] Subsequent reaction of each sulfonic acid sodium salt **7a**−**d** with thionyl chloride in DMF provided the respective sulfonyl chloride **8a**−**d**. Reaction of each sulfonyl chloride **8a**−**d** with hydroxylamine hydrochloride in the presence of potassium carbonate in dry THF afforded the respective target products **9a**−**d**. A similar reaction of the ethanesulfonyl chloride **8d** with methoxylamine hydrochloride in the presence of sodium bicarbonate in dry THF furnished the target $CH_2CH_2SO_2NHOMe$ product **9e** (Scheme 1).

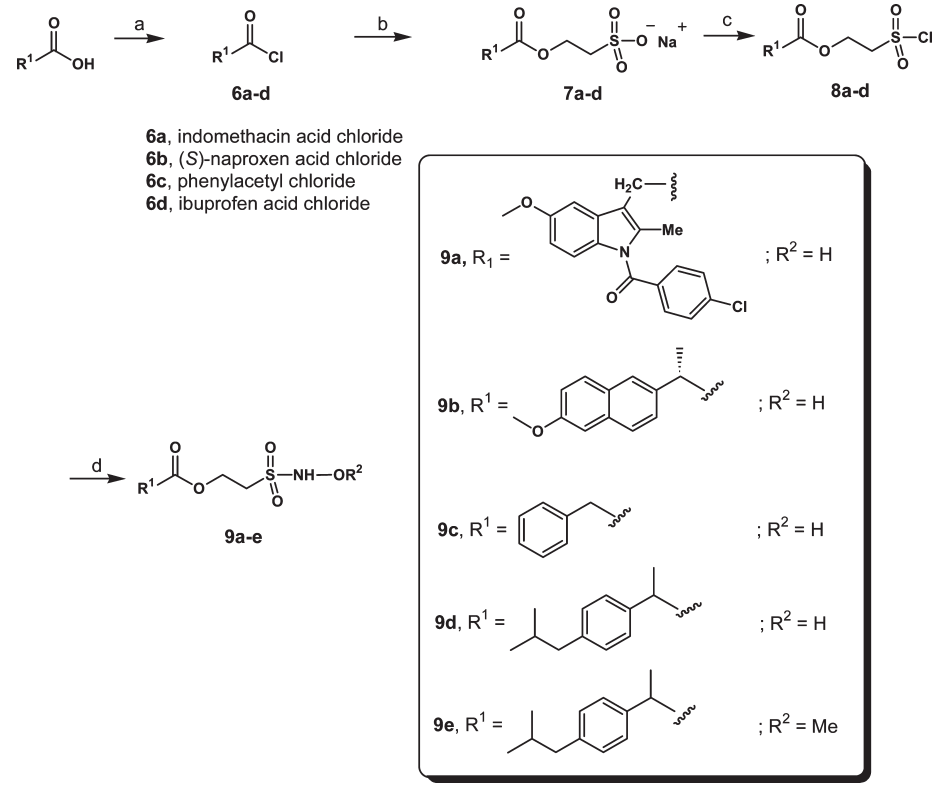
Drug Design Rationale. In a recent study, we reported a novel group of acyclic triaryl olefins possessing an arylsulfohydroxamic acid moiety (**5**) which act as dual NO/HNO donors.[16] The ethanesulfohydroxamic acid ester prodrugs **9a**−**d** described in the current investigation were designed based on the expectation that (i) an ethanesulfohydroxamic acid prodrug moiety would possess similar HNO and/or NO release properties to that of methanesulfohydroxamic acid ($MeSO_2NHOH$), thereby providing release of HNO at physiological pH, attempts to synthesize the $-CH_2SO_2NHOH$-(OMe) analogues of the $-CH_2CH_2SO_2NHOH$(OMe) compounds **9a**−**e** have to date not been successful, and (ii) esterase induced-cleavage of the ester group (see Figure 2) would furnish the parent NSAID indomethacin, (*S*)-naproxen, or ibuprofen along with the putative hydroxyethanesulfohydroxamic acid ($HOCH_2CH_2SO_2NHOH$).

Nitric Oxide Release. The % NO released from the *N*-hydroxy (methoxy) ethanesulfonamides **9a**−**e** and ethanesulfohydroxamic acid (**EA**) upon incubation in phosphate-buffered saline (PBS at pH 7.4) or PBS containing rat serum were measured by quantitation of nitrite using the Griess reaction (see data in Table 1). The % NO released from the ethanesulfohydroxamic acids esters **9a**−**d** in PBS at pH 7.4 varied over a 44.5−54.3% range, which is indicative of much higher NO release relative to that previously reported for a group of arylsulfohydroxamic acids **5** (4.3−11.4% range).[16] The % NO release from **9a**−**d** and **EA** was suppressed (1.3−4.8% range) in PBS containing rat serum. One plausible explanation for this reduction in NO release because NO is not expected to react with serum thiols,[11] could be due to the likely probability that the highly lipophilic (log *P* = 2.1−3.4 range; see data in Table 2) sulfohydroxamic acids **9a**−**b**, **d**−**e** undergo strong protein binding to rat serum which results in suppressed NO release. This observation is consistent with a similar serum effect for arylsulfohydroxamic acids **5**.[16] These data suggest that the sulfohydroxamic acid moiety present in the prodrugs **9a**−**d** should possess some degree of stability in the circulatory system. In contrast, the *N*-methoxyl ethanesulfonamide **9e** showed a much lower % NO release in PBS and PBS containing rat serum (4.3% and 0.9%, respectively) relative to the *N*-hydroxyl ethanesulfonamides (**9a**−**d**).

Indirect Assay of Nitroxyl (HNO) Release As Nitrous Oxide (N_2O). Quantitative gas chromatographic analysis of N_2O, which arises from HNO dimerization and dehydration under anaerobic conditions (HNO + HNO → [HONNOH] → N_2O + H_2O), was employed because direct HNO detection continues to be difficult.[22] HNO release from **9a**−**e** and ethanesulfohydroxamic acid (**EA**) was measured using three MeOH-based solvent mixtures (see data in Table 1) based on the fact that methanesulfohydroxamic acid releases HNO at a rate comparable to Angeli's salt at physiological pH,[17,18] an alkaline pH can enhance the amounts of HNO released from the sulfohydroxamic acid moiety,[23] and HNO reacts rapidly with thiols to form disulfides and hydroxylamine or sulfinamides.[24] The percentage of N_2O arising from **9a**−**d** and ethanesulfohydroxamic acid in MeOH/TBS was very low (<1%). In contrast, in the presence of the base NaOH that produces a nonphysiological alkaline pH, the % N_2O produced was substantially larger (20−25% range). In comparison, the % N_2O produced in the MeOH/NaOH/GSH solvent system was much smaller (≤2% and ≤7% for 2 and 24 h incubations, respectively) because addition of the thiol glutathione (GSH) reacts with HNO, thereby resulting in the expected decrease in N_2O production observed. This latter group of experiments in which the incubation solvent system contains GSH provides strong evidence for the release of HNO from **9a**−**d** and the subsequent dimerization of the released HNO to N_2O.

Cyclooxygenase-1 (COX-1) and -2 Enzyme Inhibition. In vitro COX-1/COX-2 isozyme inhibition studies (Table 2) showed that the ethanesulfohydroxamic acids (**9a**−**b**, **9d**) and the *N*-methoxyl ethanesulfonamide (**9e**) ester prodrugs were more potent inhibitors of COX-2 (IC_{50} = 0.4−15.8 μM range) than COX-1 (IC_{50} = 1.1−77.7 μM range). Within this group of compounds, the *N*-methoxyl ethanesulfonamide ester of ibuprofen **9e** showed a higher COX-2 selectivity index than the parent NSAID ibuprofen where the SI's are 23.3 and 2.9, respectively. The observation that the ethanesulfohydroxamic acid ester of ibuprofen **9d** showed a much lower COX-2 SI (0.07) compared

Scheme 1[a]



[a] Reagents and conditions: (a) oxalyl chloride, dry CH_2Cl_2, DMF, 25 °C, 12 h; (b) 2-hydroxyethanesulfonic acid sodium salt, dry pyridine, 60 °C, 24 h for **7a**, 2-hydroxyethanesulfonic acid sodium salt, 130 °C, 4 h for **7b**−**d**; (c) $SOCl_2$, DMF, 25 °C, 1 h; (d) dry THF, K_2CO_3, HO-NH_2·HCl, 25 °C, 2 h for **9a**−**d**, dry THF, $NaHCO_3$, MeO-NH_2·HCl, 25 °C, 2 h, for compound **9e**.

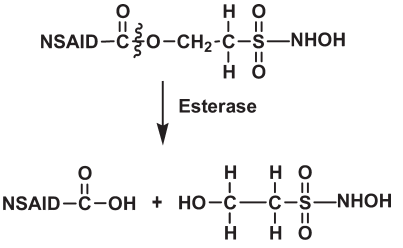
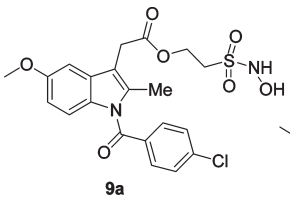


Figure 2. Putative esterase induced cleavage of the ester group to release the parent NSAID and 3-hydroxyethanesulfohydroxamic acid.

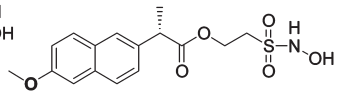
to the *N*-methoxyl analogue **9e** (23.3) indicates that the *N*-methoxyl group must enhance binding to the COX-2 enzyme. It is most noteworthy that the COX-2 SI for the ethanesulfohydroxamic acid ester of indomethacin **9a** (COX-2 SI = 184) is completely reversed relative to the parent NSAID indomethacin, which is a highly ulcerogenic selective COX-1 inhibitor (COX-2 SI = 0.02). The lipophilicity of **9a** (log *P* = 3.4) and indomethacin (log *P* = 3.6) are relatively similar. The molecular volume of the primary COX-2 binding site and its associated secondary pocket is about 25% larger (394 Å^3) than the volume of the smaller (316 Å^3) COX-1 binding site.[25] Accordingly, it is highly probable that the larger molecular volume of **9a** (381 Å^3) compared to that of the much smaller molecular volume of indomethacin (298 Å^3) is an important contributor to the COX-2 selectivity observed for **9a** (see data in Table 2). The phenylacetic acid analogue **9c**, as expected, did not inhibit the COX-1 or COX-2 isozymes at the highest test compound concentration tested (100 μM). Similarly, **EA** did not inhibit COX-1 or COX-2 at the highest concentration (100 μM) tested.

Anti-inflammatory Activity. AI ID_{50} values acquired for the ethanesulfohydroxamic acid esters of indomethacin (**9a**) and (*S*)-naproxen (**9b**), % inhibition of inflammation induced by the phenylacetic acid ester **9c** for a 100 mg/kg oral dose, and % inhibition shown by the ibuprofen prodrug esters **9d** and **9e** for a 327 μmol/kg oral dose were determined using a carrageenan-induced rat foot paw edema model (see data in Table 2). Within this group of compounds, all ester prodrugs of NSAIDs **9a**−**b** and **9d**−**e** showed potent AI activities. In this regard, the (*S*)-naproxen analogue **9b** showed a more potent AI activity (ID_{50} = 11.8 μmol/kg po) than (*S*)-naproxen (ID_{50} = 29.7 μmol/kg po).[26] On the other hand, the COX-2 selective indomethacin analogue **9a** is a less potent AI agent (ID_{50} = 19.1 μmol/kg po) relative to indomethacin (ID_{50} = 11.7 μmol/kg po). Despite the fact that the ethanesulfohydroxamic acid ester of ibuprofen **9d** (COX-2 SI = 0.07) and the *N*-methoxyl ethanesulfonamide ester of ibuprofen **9e** (COX-2 SI = 23.3) showed distinctly different COX-2 selectivity indexes, **9d** and **9e** both exhibited more potent, yet similar, AI activities (79.5 and 78.9% inhibition of inflammation for a 327 μmol/kg oral dose) relative to the parent NSAID ibuprofen (AI ID_{50} = 327 μmol/kg po). One plausible explanation for this AI data, because **9e** released a low 4.3% of NO and a negligible amount of HNO (Table 1), could be due to the likely probability that in vivo cleavage of the ester group present in both **9d** and **9e** by an esterase releases a similar

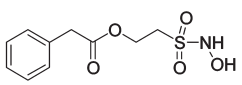
Table 1. Percent (%) Nitric Oxide and Nitrous Oxide Release from 9a−e and the Reference Compound Ethanesulfohydroxamic Acid (EA)



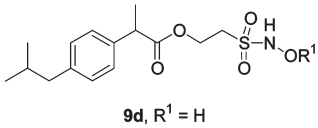
9a



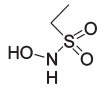
9b



9c



9d, R^1 = H
9e, R^1 = Me



EA

	% NO release[b]		% N_2O release [a]					
			MeOH/TBS [c]		MeOH/base [d]		MeOH/base/GSH [e]	
	PBS [f]	PBS + serum [g]	2 h	24 h	2 h	24 h	2 h	24 h
9a	44.5	3.2	0	0	20	1	1	1
9b	52.9	1.3	0	0	24	23	2	4
9c	54.3	4.8	0	0	23	21	0	7
9d	46.3	2.1	0	0	25	24	0	7
9e	4.3	0.9	0	0	0	0	0	0
EA	54.5	1.4	0	0	24	7	0	0

[a] Percent of N_2O released, based on the condensation of 2 mol of HNO → 1 mol N_2O + H_2O. The result is the mean value of three measurements (n = 3). The HNO donor test compound (**9a−e**, **EA**) concentration is 50 mM in each experiment unless otherwise noted. [b] Percent NO released based on a theoretical maximum release of 1 mol of NO/mol of the sulfohydroxamic acid test compound (**9a−e**) and the reference agent ethanesulfohydroxamic acid (**EA**). The result is the mean value of three measurements (n = 3) where variation from the mean % value was ≤0.2%. [c] MeOH/TBS solvent is comprised of 0.6 mL MeOH and 0.2 mL of 700 mM Tris buffer solution (TBS) at pH 7.00. [d] MeOH/base solvent is comprised of 0.6 mL MeOH and 0.2 mL of 1 M NaOH. [e] MeOH/base/GSH experiments are 100 mM in glutathione (GSH) and 250 mM in NaOH except for compounds **9b** and **EA** which were 25 mM in NaOH. [f] A solution of the test compound (2.4 mL of a 5.0 × 10^{-2} mM) in phosphate buffer at pH 7.4 was incubated at 37 °C for 1.5 h. [g] A solution of the test compound (2.4 mL of a 5.0 × 10^{-2} mM) in phosphate buffer at pH 7.4, to which 90 μL rat serum had been added, was incubated at 37 °C for 1.5 h.

Table 2. In Vitro COX-1 and COX-2 Inhibition, Anti-inflammatory (AI), Ulcer Index (UI), log *P*, and Molecular Volume (Å^3) Data

	IC$_{50}$ (μM) [a]						
compd	COX-1	COX-2	COX-2 SI [b]	AI activity: ID$_{50}$ (μmol/kg) [c]	ulcer index [d]	log *P* [e]	volume (Å^3) [f]
9a	77.7	0.42	184	19.1	0	3.4	381
9b	4.5	2.3	1.9	11.8		2.1	297
9c	>100	>100		weak [g]		0.65	212
9d	1.1	15.8	0.07	potent [h]		2.9	295
9e	14.6	0.63	23.3	potent [i]		3.4	311
indomethacin	0.13	6.9	0.02	11.7	64.5 ± 10.5	3.6	298
(*S*)-naproxen	0.18	12.4	0.01	29.7		3.0	214
ibuprofen	4.0	1.4	2.9	327		3.8	211
EA	>100	>100					

[a] The in vitro test compound concentration required to produce 50% inhibition of ovine COX-1 or human recombinant COX-2. The result (IC$_{50}$, μM) is the mean of two determinations acquired using the enzyme immuno assay kit (catalogue no. 560131, Cayman Chemicals Inc., Ann Arbor, MI, USA), and the deviation from the mean is <10% of the mean value. [b] In vitro COX-2 selectivity index (COX-1 IC$_{50}$/COX-2 IC$_{50}$). [c] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as the inhibitory dose (ID$_{50}$ value in μmol/kg, determined from a dose−response curve, required to decrease inflammation by 50%) at 3 h after oral administration of the test compound. [d] Calculated by adding the total length (in mm) of individual ulcers in each stomach and averaging over the number of animals (n = 3) in each group. Data are presented as mean total length ± SEM at 6 h after oral administration of the test compound. [e] The log *P* value was calculated using the ChemDraw Ultra program, version 11.0, CambridgeSoft company. [f] The volume of the molecule, after minimization using the molecular mechanics geometry optimization module, was calculated using the Alchemy 2000 program, Tripos Inc. [g] A 12.4% (n = 3, SEM = 4.6) inhibition of inflammation was observed for a 100 mg/kg oral dose. [h] A 79.5% (n = 3, SEM = 2.4) inhibition of inflammation was observed for a 327 μmol/kg oral dose. [i] A 78.9% (n = 3, SEM = 9.2) inhibition of inflammation was observed for a 327 μmol/kg oral dose.

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quantity of ibuprofen thereby providing similar AI activities. The phenylacetic acid ester **9c**, as expected, exhibited weak AI activity (12.4% inhibition of inflammation for a 100 mg/kg po dose).

Ulcer Index (UI) Assay. The most common side effects associated with the chronic use of NSAIDs such as indomethacin include the development of gastric erosions, ulcer formation, and sometimes severe bleeding that is attributed to inhibition of the constitutive COX-1 isozyme. It was therefore anticipated that the ethanesulfohydroxamic acid ester of indometachin **9a**, in view of its COX-2 selectivity and ability to release NO which is a beneficial mediator of GI mucosal protection that induces many actions similar to prostaglandins in the GI tract,[27] would be free of contraindicated GI effects. Accordingly, the UI for compound **9a** was determined for comparison to the parent NSAID indomethacin (Table 2). Unlike indomethacin (UI = 64 for a 80 μmol/kg po dose), **9a** showed no visible lesions (UI = 0 for a 80 μmol/kg po dose). This important gastric-sparing property observed for **9a** is attributed to its good COX-2 selectivity and nitric oxide-releasing properties.

CONCLUSIONS

Hybrid NSAIDs that possess a nitrate[28,29] or diazeniumdiolate[30] NO-releasing moiety provided an attractive drug design strategy to improve the safety profile of traditional NSAIDs. However, production of NO from nitrate esters requires a demanding three-electron reduction, and this metabolic activation can lead to nitrate tolerance upon continued use.[31,32] Diazen-1-ium-1,2-diolates, after release of two molecules of NO, also give rise to the secondary amine from which they are derived. This secondary amine can subsequently undergo biotransformation to a potentially toxic and carcinogenic nitrosoamine.[33] To-date no NO-NSAID or NONO-NSAID has achieved clinical approval. The sulfohydroxamic acid moiety is a desirable drug design pharmacophore because it can act as a dual NO/HNO donor. The difficult challenge of utilizing the unique biological and pharmacological properties of HNO, particularly upon the cardiovascular system, has not been adequately investigated.[11−14] In this regard, a synthetic methodology to prepare ethanesulfohydroxamic acid prodrug esters of indomethacin, (*S*)-naproxen, and ibuprofen was developed. Biological studies indicated that this hitherto-unknown group of NSAID-$CO_2CH_2CH_2SO_2N$-HOH compounds showed (i) a much higher NO release at physiological pH relative to a group of arylsulfohydroxamic acid compounds (**5**) previously reported,[16] (ii) that the ethanesulfohydroxamic acid ester of indomethacin **9a** is a selective COX-2 inhibitor, (iii) that the (*S*)-naproxen and ibuprofen prodrug esters **9b** and **9d**−**e** showed more potent AI activities than their respective parent NSAID (*S*)-naproxen or ibuprofen, (iv) that the ethane-SO_2NHOMe compound **9e**, which did not act as a NO/HNO donor, exhibited a more potent AI activity than ibuprofen that is attributed to an esterase induced (Figure 2) ester cleavage to release ibuprofen, and (v) that the indomethacin prodrug ester **9a**, which exhibited potent AI activity, unlike indomethacin was completely devoid of adverse ulcerogenic effects.

EXPERIMENTAL SECTION

General. Melting points were determined on a Thomas−Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded as films on NaCl plates using a Nicolet 550 series II Magna FT-IR spectrometer. ^{1}H NMR spectra were measured on a Bruker AM-300 spectrometer with TMS as the internal standard, where *J* (coupling constant) values are estimated in hertz (Hz). Mass spectra (MS) were recorded on a Water's Micromass ZQ 4000 mass spectrometer using the ESI ionization mode. Microanalyses were performed for C, H, N by the Microanalytical Service Laboratory, Department of Chemistry, University of Alberta. Compounds **7a**−**d** and **8a**−**d** showed a single spot on Macherey-Nagel Polygram Sil G/UV_{254} silica gel plates (0.2 mm) using a low, medium, and highly polar solvent system, and no residue remained after combustion, indicating a purity >95%. Column chromatography was performed on a Combiflash Rf system using either a gold silica (**8a**−**d**) or a C18 (**7a**−**d**) column. All other reagents, purchased from the Aldrich Chemical Co. (Milwaukee, WI), were used without further purification. The in vivo anti-inflammatory and ulcer index assays were carried out using protocols approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

Indomethacin Acid Chloride (6a). Oxalyl chloride (0.52 mL, 6 mmol) was added dropwise to a solution of indomethacin (1.07 g, 3 mmol) and two drops of dry DMF in dry CH_2Cl_2 (20 mL) at 0−5 °C. The reaction mixture was stirred at 25 °C for 12 h, and the solvent was removed in vacuo to give **6a** as a pale-gray solid; yield 94%; mp 124−126 °C (lit. mp 125−127 °C).[19] ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.42 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 4.18 (s, 2H, CH_2COCl), 6.70 (dd, *J* = 9.2, 2.4 Hz, 1H, indolyl H-6), 6.85−6.90 (m, 2H, indolyl H-7 and H-4), 7.48 (dd, *J* = 6.7, 1.8 Hz, 2H, benzoyl H-3 and H-5), 7.68 (dd, *J* = 6.7, 1.8 Hz, 2H, benzoyl H-2 and H-6).

(*S*)-Naproxen Acid Chloride (6b). Compound **6b** was prepared, using the procedure described for the synthesis of **6a**, except that (*S*)-naproxen was used in place of indomethacin, in 97% yield as a pale-gray solid, mp 94−96 °C (lit. mp 90−95 °C).[34] ^{1}H NMR ($CDCl_3$): δ 1.68 (d, *J* = 6.7 Hz, 3H, CH_3), 3.93 (s, 3H, OCH_3), 4.26 (q, *J* = 6.7 Hz, 1H, *CH*Me), 7.15 (dd, *J* = 7.9 Hz, 2.5 Hz, 1H, naphthyl H-3), 7.20 (d, *J* = 2.5 Hz, 1H, naphthyl H-1), 7.36 (dd, *J* = 8.5 Hz, 1.8 Hz, 1H, naphthyl H-7), 7.69−7.78 (m, 3H, naphthyl H-4, H-5 and H-8).

Phenylacetyl Chloride (6c). Compound **6c** was prepared, using the procedure described for the synthesis of **6a**, except that phenylacetic acid was used in place of indomethacin, in 95% yield as **a** yellow oil. ^{1}H NMR ($CDCl_3$): δ 4.15 (s, 2H, ArCH_2), 7.27−7.42 (m, 5H, Ar*H*).

Ibuprofen Acid Chloride (6d). Compound **6d** was prepared using the procedure described for the synthesis of **6a**, except that ibuprofen was used in place of indomethacin, in 94% yield as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 0.91 (d, *J* = 6.7 Hz, 6H, $(CH_3)_2CHCH_2$), 1.59 (d, *J* = 7.4 Hz, 3H, $CHCH_3$), 1.87 (heptet, *J* = 6.7 Hz, 1H, $(CH_3)_2$-*CH*CH_2), 2.48 (d, *J* = 7.4 Hz, 2H, $(CH_3)_2CHCH_2$), 4.10 (q, *J* = 7.4 Hz, 1H, *CH*Me), 7.14−7.22 (m, 4H, phenyl hydrogens).

2-{2-[1-(4-Chlorobenzoyl)-2-methyl-5-methoxyl-1*H*-indoyl-3-yl)]acetoxy}ethanesulfonic Acid Sodium Salt (7a). 2-Hydroxyethanesulfonic acid sodium salt (488 mg, 3.3 mmol), indomethacin acid chloride (1.13 g, 3.0 mmol), and anhydrous pyridine (8 mL) were stirred under argon at 50−60 °C for 24 h. After removal of pyridine, H_2O (10 mL) was added to the residue, and the mixture was washed with EtOAc (2 × 10 mL). The water phase was concentrated under vacuum to give a brown product. Purification using a C18 column with H_2O−acetonitrile (95:5, v/v) as eluent afforded the title compound (313 mg, 22.1%) as a yellow syrup. IR (KBr): 2955, 2894, 1685, 1234, 1159, 1043 cm^{-1}. ESI-MS: 464 [M − Na]$^-$. ^{1}H NMR (DMSO-d_6): δ 2.19 (s, 3H, CH_3), 2.75 (t, *J* = 7.9 Hz, 2H, CH_2SO_3Na), 3.74 (s, 2H, CH_2CO), 3.76 (s, 3H, OCH_3), 4.24 (t, *J* = 7.9 Hz, 2H, $COOCH_2CH_2SO_3Na$), 6.70 (dd, *J* = 9.2, 2.5 Hz, 1H, indolyl H-6), 6.94 (d, *J* = 9.2 Hz, 1H, indolyl H-7), 7.04 (d, *J* = 2.5 Hz, 1H, indolyl H-4), 7.0−7.62 (m, 4H, benzoyl H-2, H-3, H-5 and H-6). ^{13}C NMR (DMSO-d_6): δ 13.1, 50.0, 55.4, 57.6, 61.2, 101.7, 111.5, 112.7, 114.5, 127.2, 129.0, 130.1, 130.5, 134.1, 135.3, 137.5, 155.5, 167.8, 170.3.

(*S*)-2-[2-(6-Methoxynaphthyl-2-yl)propionoyloxy]ethanesulfonic Acid Sodium Salt (7b). An intimate mixture of anhydrous 2-hydroxyethanesulfonic acid sodium salt (400 mg, 2.7 mmol) and (*S*)-naproxen acid chloride (**6b**, 740 mg, 3.0 mmol) was heated at 130 °C until no further hydrogen chloride was evolved (about 4 h). The dark

solid was dissolved in boiling water (20 mL), filtered through a charcoal pad, and extracted with ethyl ether (30 mL) to remove excess (*S*)-naproxen. The aqueous liquor was adjusted to pH 7 with sodium hydroxide and evaporated under reduced pressure. The residue obtained was purified on a C18 column using H_2O−acetonitrile (95:5, v/v) as eluent to furnish **7b** (700 mg, 72.2%) as a white solid; mp 232−235 °C. IR (KBr): 2977, 2941, 1732, 1185 cm^{-1}. ESI-MS: 337 $[M - Na]^-$. ^1H NMR (DMSO-d_6): δ 1.45 (d, J = 7.3 Hz, 3H, CHCH_3), 2.68 (t, J = 7.3 Hz, 2H, CH_2SO_3Na), 3.85 (s, 3H, OCH_3), 3.91 (q, J = 7.3 Hz, 1H, *CH*Me), 4.16−4.26 (m, 2H, COOCH_2CH_2), 7.14 (dd, J = 9.2 Hz, 2.5 Hz, 1H, naphthyl H-3), 7.28 (d, J = 2.5 Hz, 1H, naphthyl H-1), 7.38 (dd, J = 8.0 Hz, 1.3 Hz, 1H, naphthyl H-7), 7.71−7.81 (m, 3H, naphthyl H-4, H-5 and H-8). ^{13}NMR (DMSO-d_6): δ 18.4, 44.4, 49.9, 55.1, 61.2, 105.7, 118.6, 125.5, 126.1, 126.9, 128.3, 129.1, 133.2, 135.6, 157.1, 173.4.

2-(Phenylacetyloxy)ethanesulfonic Acid Sodium Salt (7c). The title compound was prepared, using a similar procedure to that described for the synthesis of **7b** starting with phenylacetyl chloride, in 54.6% yield as a white solid; mp 252−254 °C. IR (KBr): 2958, 1725, 1199 cm^{-1}. ESI-MS: 243 $[M - Na]^-$. ^1H NMR (DMSO-d_6): δ 2.75 (t, J = 7.3 Hz, 2H, CH_2SO_3Na), 3.64 (s, 2H, ArCH_2), 4.22 (t, J = 7.3 Hz, 2H, COOCH_2), 7.24−7.31 (m, 5H, Ar*H*). ^{13}NMR (DMSO-d_6): δ 40.3, 49.9, 61.2, 126.7, 128.2, 129.2, 134.3, 171.0.

2-[2-(4-Isobutylphenyl)propionoyloxy]ethanesulfonic Acid Sodium Salt (7d). The title compound was prepared, using a similar procedure to that described for the synthesis of **7b** starting with ibuprofen acid chloride in 86.7% yield as a white solid; mp 179−181 °C. IR (KBr): 2962, 2835, 1737, 1656, 1198, 1036 cm^{-1}. ESI-MS: 313 $[M - Na]^-$. ^1H NMR (DMSO-d_6): δ 0.84 (d, J = 6.8 Hz, 6H, $(CH_3)_2$CHCH$_2$), 1.34 (d, J = 7.3 Hz, 3H, CHCH_3), 1.79 (heptet, J = 6.8 Hz, 1H, $(CH_3)_2CH$CH$_2$), 2.40 (d, J = 6.7 Hz, 2H, $(CH_3)_2$CHCH_2), 2.66 (t, J = 7.9 Hz, CH_2SO_3Na), 3.70 (q, J = 7.3 Hz, 1H, *CH*Me), 4.13−4.23 (m, 2H, COOCH_2CH_2), 7.09 (d, J = 8.0 Hz, 2H, phenyl H-3 and H-5), 7.16 (d, J = 8.0 Hz, phenyl H-2 and H-6). ^{13}NMR (DMSO-d_6): δ 18.5, 22.1, 29.5, 44.0, 44.1, 49.8, 61.1, 126.9, 128.9, 137.8, 139.6, 173.7.

2-{2-[1-(4-Chlorobenzoyl)-2-methyl-5-methoxyl-1*H*-indoyl-3-yl)]acetoxy}ethanesulfonyl Chloride (8a). The sulfonic acid sodium salt **7a** (300 mg, 0.615 mmol) was dissolved in DMF (3 mL), and $SOCl_2$ (0.22 mL, 3.08 mmol) was added dropwise. The reaction mixture was allowed to stir at 25 °C for 1 h, poured into cold water (30 mL), and extracted with EtOAc (3 × 30 mL). The combined organic fractions were washed with 2N HCl aqueous solution and brine and dried ($MgSO_4$). After concentration, the resulting brown syrup was purified using ethyl acetate−hexane (1:2, v/v) as eluent to give the title compound (130 mg, 43.3%) as a yellow syrup. IR (film): 2966, 2930, 1749, 1693, 1480, 1378, 1322 cm^{-1}. ESI-MS: 484 $[M + H]^+$. ^1H NMR ($CDCl_3$): δ 2.40 (s, 3H, CH_3), 3.74 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 4.00 (t, J = 5.4 Hz, 2H, CH_2SO_2Cl), 4.68 (t, J = 5.4 Hz, 2H, COOCH_2CH_2), 6.69 (dd, J = 9.2 Hz, 2.5 Hz, 1H, indolyl H-6), 6.89 (d, J = 9.2 Hz, 1H, indolyl H-7), 6.94 (d, J = 2.5 Hz, 1H, indolyl H-4), 7.48 (dd, J = 6.7, 1.8 Hz, 2H, benzoyl H-3, H-5), 7.67 (dd, J = 6.7 Hz, 1.8 Hz, 2H, benzoyl H-2, H-6). ^{13}C NMR (DMSO-d_6): δ 13.3, 55.7, 57.9, 63.6, 68.2, 101.1, 111.5, 111.8, 115.0, 129.1, 130.4, 130.8, 131.2, 133.7, 136.2, 129.4, 156.1, 168.2, 170.1.

(*S*)-2-[2-(6-Methoxynaphthyl-2-yl)propionoyloxy]ethanesulfonyl Chloride (8b). The title compound was synthesized, using a method similar to that used for the preparation of **8a** starting from **7b** in 45.3% yield as a white solid; mp 76−78 °C. IR (film): 2999, 2939, 1743, 1607, 1376, 1160 cm^{-1}. ESI-MS: 357 $[M + H]^+$. ^1H NMR ($CDCl_3$): δ 1.62 (d, J = 7.3 Hz, 3H, CH_3), 3.92 (s, 3H, OCH_3), 3.88−3.96 (m, 3H, *CH*Me and CH_2SO_2Cl), 4.56−4.68 (m, 2H, COOCH_2CH_2), 7.14 (dd, J = 6.7 Hz, 2.4 Hz, 1H, naphthyl H-3), 7.28 (d, J = 2.4 Hz, 1H, naphthyl H-1), 7.39 (dd, J = 8.6 Hz, 1.9 Hz, 1H, naphthyl H-7), 7.67−7.73 (m, 3H, naphthyl H-4, H-5 and H-8). ^{13}C NMR (DMSO-d_6): δ 18.2, 45.2, 55.3, 57.7, 63.4, 105.6, 119.1, 126.0, 126.1, 127.3, 128.9, 129.2, 133.8, 134.6, 157.8, 174.0.

2-(Phenylacetyloxy)ethanesulfonyl Chloride (8c). The sulfonic acid sodium salt **7c** (1.6 g, 6.0 mmol) was dissolved in $SOCl_2$ (10 mL), and a catalytic amount of DMF was added. The mixture was allowed to stir at 25 °C for 3 h. The $SOCl_2$ was removed under reduced pressure at about 40 °C, ethyl acetate (30 mL) was added to the residue that was removed by filtration, and the filtrate was concentrated under vacuum to give a yellow residue which was purified using ethyl acetate−hexane (1/3, v/v) as eluent to give the title compound (1.1 g, 66.6%) as a yellow oil. IR (film): 2963, 1703, 1202 cm^{-1}. ESI-MS: 263 $[M + H]^+$. ^1H NMR ($CDCl_3$): δ 3.69 (s, 2H, ArCH_2), 3.98 (t, J = 6.1 Hz, 2H, CH_2SO_2Cl), 4.66 (t, J = 6.1 Hz, 2H, COOCH_2CH_2), 7.27−7.38 (m, 5H, phenyl hydrogens). ^{13}C NMR (DMSO-d_6): δ 40.9, 57.8, 63.4, 127.4, 128.7, 129.3, 132.9, 170.9.

2-[2-(4-Isobutylphenyl)propionoyloxy]ethanesulfonyl Chloride (8d). The title compound was synthesized using a method similar to that used to prepare **8c** starting from **7d**, in 51.7% yield as a yellowish solid; mp 39−40 °C. IR (film): 2966, 1752, 1379, 1167 cm^{-1}. ESI-MS: 355 $[M + Na]^+$. ^1H NMR ($CDCl_3$): δ 0.90 (d, J = 6.7 Hz, 6H, $(CH_3)_2$CHCH$_2$), 1.52 (d, J = 7.3 Hz, 3H, CHCH_3), 1.85 (heptet, J = 7.3 Hz, 1H, $(CH_3)_2CH$CH$_2$), 2.45 (d, J = 7.3 Hz, 2H, $(CH_3)_2$CHCH_2), 3.74 (t, J = 7.3 Hz, CH_2SO_2Cl), 3.94 (q, J = 7.3 Hz, 2H, *CH*Me), 4.59−4.65 (m, 2H, COOCH_2CH_2), 7.11 (d, J = 7.9 Hz, 2H, phenyl H-3 and H-5), 7.20 (d, J = 7.9 Hz, phenyl H-2 and H-6). ^{13}C NMR (DMSO-d_6): δ 18.2, 22.4, 30.1, 44.8, 45.0, 57.7, 63.4, 127.1, 129.4, 136.7, 140.9, 174.0.

2-{2-[1-(4-Chlorobenzoyl)-2-methyl-5-methoxyl-1*H*-indoyl-3-yl)]acetoxy}ethanesulfonyl Hydroxamic Acid (9a). The ethanesulfonyl chloride **8a** (110 mg, 0.23 mmol) was dissolved in dry THF (5 mL), and then hydroxylamine hydrochloride (32 mg, 0.46 mmol) and potassium carbonate (127 mg, 0.92 mmol) were added. The reaction mixture was vigorously stirred at 25 °C until the sulfonyl chloride had completely disappeared (TLC; EtOAc-hexane, 1:1, v/v) in about 2 h. The reaction mixture was filtered through a pad of Celite that provided a clear filtrate which was added to ethyl acetate (20 mL), this mixture was washed with water (20 mL) and brine (20 mL), and the organic fraction was dried ($MgSO_4$). Removal of the solvent from the organic fraction in vacuo gave a residue that was purified by flash silica gel column chromatography using *n*-hexane−EtOAc (2:1, v/v) as eluent to afford the title compound **9a** (75 mg, 68.2%) as a yellow solid; mp 143−144 °C. IR (film): 3385, 3247, 2963, 1685, 1485, 1327 cm^{-1}. ESI-MS: 481 $[M + H]^+$. ^1H NMR (DMSO-d_6): δ 2.20 (s, 3H, CH_3), 3.49 (t, J = 6.1 Hz, 2H, CH_2SO_2NHOH), 3.76 (s, 3H, OCH_3), 3.79 (s, 2H, CH_2), 4.40 (t, J = 6.1 Hz, 2H, COOCH_2CH_2), 6.71 (dd, J = 9.2 Hz, 2.4 Hz, 1H, indolyl H-6), 6.94 (d, J = 9.2 Hz, 1H, indolyl H-7), 7.03 (d, J = 2.4 Hz, 1H, indolyl H-4), 7.62−7.69 (m, 4H, benzoyl H-2, H-3, H-5 and H-6), 9.25 and 9.69 (two d, J = 3.1 Hz, 1H each, *HO*−N*H*). ^{13}C NMR ($CDCl_3$): δ 12.8, 45.8, 55.2, 58.0, 59.5, 100.9, 111.5, 111.8, 114.3, 128.5, 130.0, 130.2, 130.6, 133.3, 135.3, 138.5, 155.5, 167.6, 169.8. Anal. Calcd for $C_{21}H_{21}ClN_2O_7S$: C, 52.45; H, 4.40; N, 5.82. Found: C, 52.38; H, 4.52; N, 5.57.

(*S*)-(+)-2-[2-(6-Methoxynaphthyl-2-yl)propionoyloxy]ethanesulfonylhydroxamic Acid (9b). The title compound was synthesized, using a procedure similar to that used to prepare **9a** starting from **8b**, in 75.7% yield as a white solid; mp 107−109 °C. IR (film): 3386, 3259, 2940, 1739, 1156 cm^{-1}; $[\alpha]^{21.0}_D$ = +22.7 (1.000, $CHCl_3$). ESI-MS: 354 $[M + H]^+$, 371 $[M + NH_4]^+$, 376 $[M + Na]^+$. ^1H NMR (DMSO-d_6): δ 1.47 (d, J = 7.3 Hz, 3H, CH_3), 3.44 (t, J = 6.7 Hz, 2H, CH_2SO_2NHOH), 3.85 (s, 3H, OCH_3), 3.93 (q, J = 7.3 Hz, *CH*Me), 4.28−4.44 (m, 2H, COOCH_2CH_2), 7.14 (dd, J = 9.1 Hz, 2.5 Hz, 1H, naphthyl H-3), 7.28 (d, J = 2.5 Hz, 1H, naphthyl H-1), 7.40 (dd, J = 8.5 Hz, 1.2 Hz, 1H, naphthyl H-7), 7.72−7.80 (m, 3H, naphthyl H-4, H-5 and H-8), 9.23 and 9.66 (two d, J = 3.1 Hz, 1H each, *HO*−N*H*). ^{13}C NMR (DMSO-d_6): δ 18.1, 45.4, 46.0, 58.5, 105.7, 119.4, 125.8, 126.1, 127.6, 128.9, 129.2, 133.8, 134.7, 157.8, 174.1. Anal. Calcd for $C_{16}H_{19}NO_6S$: C, 54.38; H, 5.42; N, 3.96. Found: C, 54.18; H, 5.23; N, 3.93.

2-(Phenylacetyloxy)ethanesulfonylhydroxamic Acid (9c). The title compound was synthesized using a procedure similar to that used to prepare **9a** starting from **8c**, in 61.9% yield as a white solid; mp 96−97 °C. IR (film): 3393, 3255, 1736, 1338, 1150 cm^{-1}. ESI-MS: 282 $[M + Na]^+$. ^1H NMR (DMSO-d_6): δ 3.48 (t, J = 6.1 Hz, 2H, CH_2SO_2NHOH), 3.68 (s, 2H, ArCH_2), 4.37 (t, J = 6.1 Hz, 2H, COOCH_2), 7.25−7.34 (m, 5H, phenyl hydrogens), 9.25 and 9.67 (two d, J = 3.1 Hz, 1H each, $HO-NH$). ^{13}C NMR (DMSO-d_6): δ 40.7, 45.9, 58.1, 126.9, 128.7, 129.0, 133.2, 170.8. Anal. Calcd for $C_{10}H_{13}NO_5S$: C, 46.32; H, 5.05; N, 5.40. Found: C, 46.45; H, 5.05; N, 5.32.

2-[2-(4-Isobutylphenyl)propionoyloxy]ethanesulfonylhydroxamic Acid (9d). The title compound was synthesized, using a method similar to that used to prepare **9a** starting from **8d**, in 71.4% yield as a white solid; mp 68−70 °C. IR (film): 3398, 3252, 2959, 1748, 1340, 1158 cm^{-1}. ESI-MS: 347 $[M + NH_4]^+$, 352 $[M + Na]^+$. ^1H NMR (DMSO-d_6): δ 0.84 (d, J = 6.7 Hz, 6H, $(CH_3)_2CHCH_2$), 1.38 (d, J = 6.7 Hz, 3H, $CHCH_3$), 1.77 (heptet, J = 6.7 Hz, 1H, $(CH_3)_2CHCH_2$), 2.40 (d, J = 7.4 Hz, 2H, $(CH_3)_2CHCH_2$), 3.43 (t, J = 6.7 Hz, CH_2SO_2NHOH), 3.76 (q, J = 6.7 Hz, 1H, ArCH), 4.26−4.43 (m, 2H, COOCH_2CH_2), 7.10 (d, J = 7.9 Hz, 2H, phenyl H-3 and H-5), 7.19 (d, J = 7.9 Hz, phenyl H-2 and H-6), 9.22 and 9.66 (two d, J = 2.5 Hz, 1H each, $HO-NH$). ^{13}C NMR (DMSO-d_6): δ 18.1, 22.4, 30.1, 44.9, 45.0, 45.9, 58.5, 127.1, 129.7, 136.9, 141.2, 174.1. Anal. Calcd for $C_{15}H_{23}NO_5S \cdot 0.2H_2O$: C, 54.10; H, 7.08; N, 4.21. Found: C, 54.08; H, 7.00; N, 4.26.

***N*-Methoxyl 2-[2-(4-Isobutylphenyl)propionoyloxy]ethanesulfonylamide (9e).** The sulfonyl chloride **8d** (300 mg, 0.90 mmol) was reacted with methoxylamine hydrochloride (150 mg, 1.8 mmol) that was neutralized with an solution of $NaHCO_3$ (300 mg, 3.57 mmol) in dry THF (14 mL). The reaction was completed using a procedure similar to that described for the isolation of **9a**, to yield the title compound **9e** as a colorless oil (65.1%). IR (film): 3260, 2963, 1745, 1344, 1156 cm^{-1}. ESI-MS: 361 $[M + NH_4]^+$, 366 $[M + Na]^+$. ^1H NMR (DMSO-d_6): δ 0.84 (d, J = 6.7 Hz, 6H, $(CH_3)_2CHCH_2$), 1.37 (d, J = 7.3 Hz, 3H, $CHCH_3$), 1.79 (heptet, J = 6.7 Hz, 1H, $(CH_3)_2CHCH_2$), 2.40 (d, J = 7.3 Hz, 2H, $(CH_3)_2CHCH_2$), 3.46 (t, J = 6.1 Hz, CH_2SO_2NHOMe), 3.64 (s, 3H, SO_2NHOCH_3), 3.75 (q, J = 7.4 Hz, 1H, ArCH), 4.24−4.44 (m, 2H, COOCH_2CH_2), 7.10 (d, J = 7.9 Hz, 2H, phenyl H-3 and H-5), 7.19 (d, J = 7.9 Hz, phenyl H-2 and H-6), 10.2 (s, 1H, SO_2NHOCH_3). ^{13}C NMR (DMSO-d_6): δ 18.1, 22.3, 30.3, 45.0, 45.1, 46.3, 58.5, 64.9, 127.1, 129.7, 138.9, 141.2, 173.8. Anal. Calcd for $C_{16}H_{25}NO_5S \cdot 0.25H_2O$: C, 55.23; H, 7.39; N, 4.03. Found: C, 54.91; H, 7.27; N, 4.17.

Ethanesulfonylhydroxamic Acid (EA). The title compound was prepared by reaction of ethanesulfonyl chloride with hydroxylamine hydrochloride using a procedure similar to that used to prepare **9a**, in 54.0% yield as a colorless oil according to a literature method.[35] IR (film): 3404, 3238, 1333, 1157 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.19 (t, J = 7.3 Hz, 3H, CH_3CH_2), 3.11 (q, J = 7.3 Hz, 2H, CH_3CH_2), 9.16 and 9.50 (two d, J = 3.1 Hz, 1H each, $HO-NH$).

Cyclooxygenase Inhibition Assays. The ability of the test compounds **9a**−**e** listed in Table 2 to inhibit ovine COX-1 and human recombinant COX-2 (IC_{50} value, μM) was determined using an enzyme immuno assay (EIA) kit (catalogue no. 560131, Cayman Chemical, Ann Arbor, MI, USA) according to a previously reported method.[36]

Anti-inflammatory Assay. Anti-inflammatory activity was measured using a carrageenan-induced rat paw edema assay described by Winter et al.[37] Briefly, three male Sprague−Dawley rats weighing 160−180 g were used in each group. Test compounds suspended in water containing 1% methyl cellulose were administered orally for a minimum of three different doses (2.8−112 mg/kg range) 1 h prior to a 0.05 mL subcutaneous injection of 1% carrageenan in 0.9% NaCl solution under the planter skin of the right hind paw. Control experiments were identical, except that the vehicle did not contain a test compound. The volume of the injected paw was measured at 0 and 3 h using a UGO Basile 7141 Plethysmometer (series no. 43201). A dose response curve was constructed which was used to determine the ID_{50} value.

Acute Ulcerogenesis Assay. The ability to produce gastric damage was evaluated according to reported procedures.[38] Ulcerogenic activity was evaluated after oral administration of **9a** (80 μmol/kg po dose) that was suspended 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 12 h before administration of the test compound. 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 millimeters). The UI for **9a** and blank control 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 = 3): UI) $(L1 + L2 + L3)/3$.

Nitric Oxide Release Assay. In vitro nitric oxide release, upon incubation of the test compound (2.4 mL of 5.0×10^{-2} mM) with either (i) phosphate buffer solution (PBS) at pH 7.4 and 37 °C for 1.5 h or (ii) PBS at pH 7.4 and 37 °C to which 90 μL of rat serum had been added, was determined by quantification of nitrite produced by the reaction of nitric oxide with oxygen and water using the Griess reaction. Nitric oxide release data were acquired for test compounds (**9a**−**e**) and the reference compound ethanesulfonylhydroxamic acid (EA) using the reported procedures.[39]

Gas Chromatographic N_2O Analysis. For headspace analysis, substrate (0.04 mmol) was placed in a 10 mL round-bottom flask, which was sealed with a rubber septum and flushed with inert gas. Solvent (0.8 mL) was added, and headspace aliquots (0.25 mL) from three separate experiments were injected at 2 and 24 h onto a 7890A Agilent Technologies Gas Chromatograph equipped with a thermal conductivity detector and a 6 ft × $^1/_8$ in.′ Porapack Q column. The oven operated at 40 °C for 5 min was then ramped to 150 °C over 4.5 min for a total run time of 9.5 min. The purged packed inlet with a total flow (He as carrier gas) of 18 mL/min and a septum purge flow of 3 mL/min was held at 140 °C. The back detector with a reference flow of 9 mL/min and a makeup flow of 6 mL/min was held at 150 °C. The retention time of nitrous oxide was 2.5 min, and yields (% of N_2O produced) were calculated based on a standard curve prepared using known amounts of nitrous oxide gas (Matheson Tri-Gas).

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ABBREVIATIONS USED

AI, anti-inflammatory; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; EA, ethanesulfohydroxamic acid; GI, gastrointestinal; GSH, glutathione; NO, nitric oxide; HNO, nitroxyl; N_2O,

nitrous oxide; NSAIDs, nonsteroidal anti-inflammatory drugs; PBS, phosphate buffer solution; PA, Piloty's acid; SI, selectivity index; TBS, tris buffer solution; UI, ulcer index.

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