Journal of Medicinal Chemistry

Article

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O^2-Sulfonylethyl Protected Isopropylamine Diazen-1-ium-1,2-diolates as Nitroxyl (HNO) Donors: Synthesis, β-Elimination Fragmentation, HNO Release, Positive Inotropic Properties, and Blood Pressure Lowering Studies

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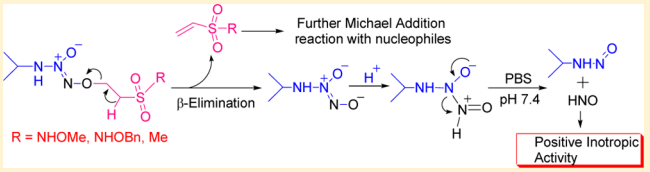
Supporting Information

ABSTRACT: New types of nonexplosive O^2-sulfonylethyl protected (-$CH_2CH_2SO_2R$; R = OMe, NHOMe, NHOBn, Me) derivatives of isopropylamine diazen-1-ium-1,2-diolate (IPA/NO) (**2**−**5**) were developed that are designed to act as novel HNO donors. These compounds, with suitable half-lives (6.6−17.1 h) at pH 7.4, undergo a base-induced β-elimination reaction that releases a methyl vinyl sulfone product and the parent IPA/NO anion which subsequently preferentially releases HNO (46−61% range). Importantly, the O^2-methylsulfonylethyl compound **5** exhibited a significant in vitro inotropic effect up to 283% of the baseline value and increased the rates of contraction and relaxation but did not induce a chronotropic effect. Furthermore, compound **5** (22.5 mg/kg po dose) provided a significant reduction in blood pressure up to 6 h after drug administration. All these data suggest that O^2-sulfonylethyl protected derivatives of IPA/NO, which are efficient HNO donors, could have potential applications to treat cardiovascular disease(s) such as congestive heart failure.



INTRODUCTION

Nitroxyl (HNO), the one-electron reduced and protonated congener of nitric oxide (NO), exerts some biological activities similar to that of NO. For example, HNO and NO are effective vasodilators and inhibitors of platelet aggregation and adhesion[1] and show in vitro and in vivo anticancer activity.[2] HNO has attracted recent attention because of some of its unique biological and pharmacological properties that differ from NO. In this regard, HNO exerts a positive inotropic action on heart and it also shows lusitropic properties. Both properties contribute to increased cardiac output.[3−5] HNO also induces beneficial effects in the treatment of ischemia−reperfusion injury.[6] These distinct biological actions indicate that HNO release from HNO donors constitutes a drug design strategy applicable to the treatment of heart failure.

Several HNO donor compounds have been discovered that have been subjected to extensive studies. Angeli's salt (AS, $Na_2N_2O_3$) is a well-known HNO donor that releases HNO in the pH 4−8 range.[7] The structural simplicity of Angeli's salt limits its extensive modification for medicinal chemistry utility.[7] In our ongoing studies to design novel anti-inflammatory drugs that act as HNO donors and are devoid of cardiovascular side effects, we synthesized phenyl-[8] and alkylsulfohydroxamic acid[9] derivatives. Unfortunately, these sulfohydroxamic acids required a nonphysiological alkaline pH to release HNO. As interest in HNO as a therapeutic agent continues to grow, new and mechanistically unique HNO donors are urgently needed in this challenging research area.

Diazeniumdiolates derived from secondary amines usually act as NO donors under physiological conditions because of the ease of protonation at the amine nitrogen (N^3) and then fragmentation to furnish the amine and 2 equiv of NO (Figure 1). In contrast, diazeniumdiolates of primary amines such as isopropylamine diazeniumdiolate (IPA/NO), which is a HNO donor, undergo preferential protonation at N^2 prior to decomposition to HNO and a nitrosamine in the pH range 5−13 (Figure 1).[10,11] Recently a novel O^2-acetyloxymethyl IPA/NO (AcOM-IPA/NO) was reported by the Keefer group that is a "pure" HNO donor with an ability to strengthen contraction of beating cardiac myocytes.[12] In a previous study, we reported new types of O^2-(*N*-methoxy-2-ethanesulfonylamide) protected secondary amine diazeniumdiolates (representative compound **1**, Figure 1).[13] NO release studies showed that these compounds are able to release NO in the presence of bases such as the basic natural amino acids arginine and histidine or the non-nucleophilic organic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in phosphate buffered

Received: September 10, 2012
Published: October 16, 2012

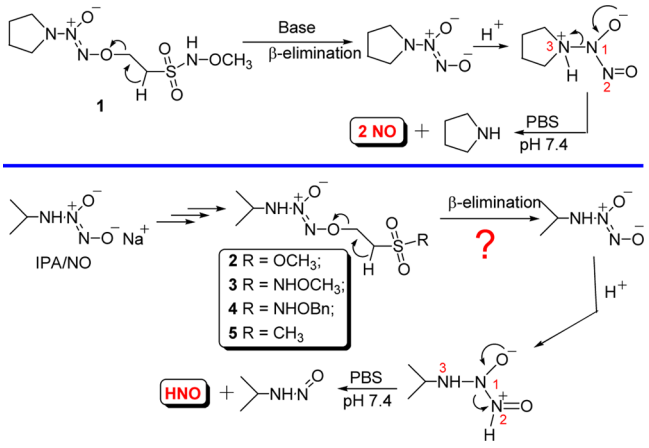


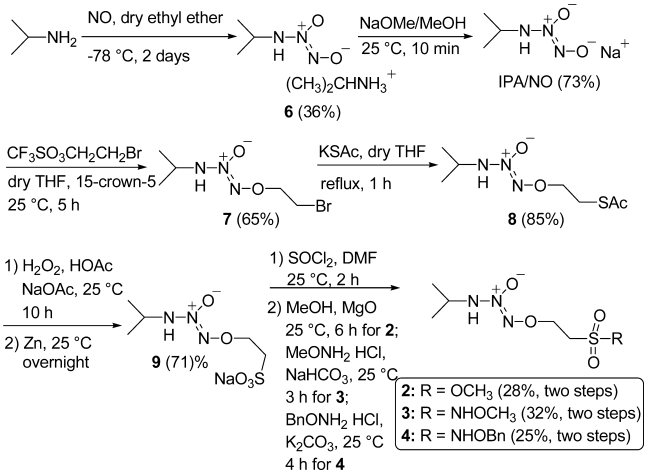
Figure 1. Rationale for the design of novel base-sensitive HNO donors **2**–**5**.

solution (PBS) at pH 7.4 via a β-elimination cleavage reaction. Accordingly, it was of interest to investigate whether protecting the primary amine IPA/NO using an O^2-sufonylethyl group would provide a hitherto-unknown class of stable (nonexplosive) and readily isolated O^2-protected IPA/NO that acts as an HNO donor via a β-elimination reaction at physiological relevant conditions. Herein, we now describe the synthesis of a group of O^2-sulfonylethyl IPA/NO compounds **2**–**5**, half-lives, fragmentation mechanism, HNO/NO release studies, positive inotropic properties, and antihypertensive activities.

RESULTS AND DISCUSSION

Chemistry. The target O^2-sulfonylethyl IPA/NO compounds **2**–**4** were synthesized as depicted in Scheme 1,

Scheme 1. Synthesis of O^2-Protected IPA/NO Analogues 2–4



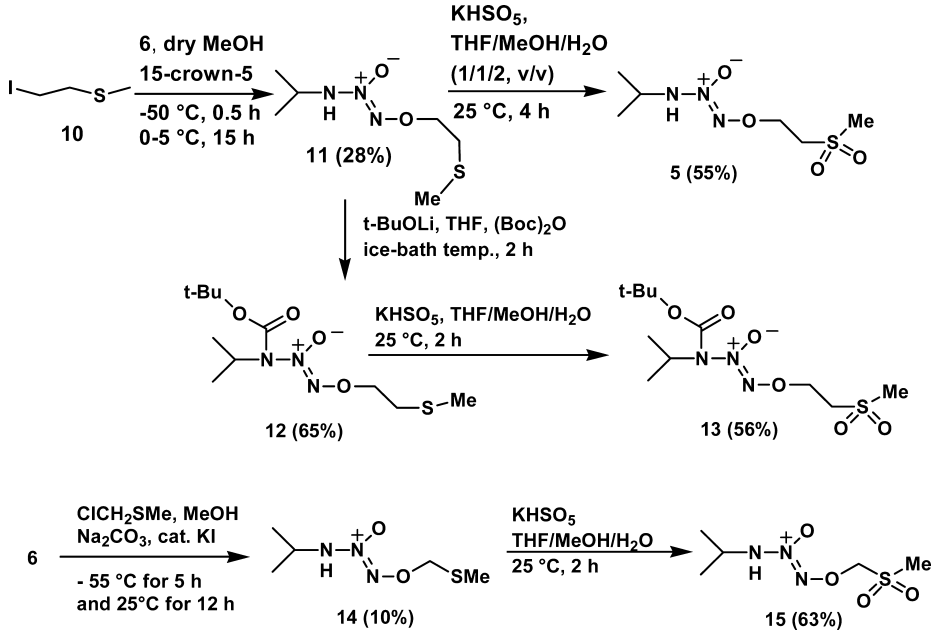
employing a more efficient O^2-alkylation reaction of IPA/NO. The yield for the reaction of IPA/NO with 2-iodoethyl thioacetate, which was initially used to prepare O^2-(2-acetylthioethyl) IPA/NO **8** according to a previous report,[13] is very low (<2%). Fortunately, the reaction of 2-bromo-1-(trifluoromethanesulfonyloxy)ethane with IPA/NO furnished O^2-(2-bromoethyl) IPA/NO **7** in a 65% yield, since the trifluoromethanesulfonate moiety is a good leaving group for the O^2-alkylation of diazeniumdiolates.[14] Subsequent transformation of the bromo compound **7**, upon reaction with potassium thioacetate in dry THF at reflux, afforded the desired acetylthioethyl intermediate **8** in 85% yield. The thioacetate moiety in **8** was converted to the sodium sulfonate analogue **9** in the presence of H_2O_2 in a solution of acetic acid. Reaction of the sodium sulfonate **9** with thionyl chloride in DMF afforded the corresponding relatively unstable sulfonyl chloride product which was used immediately in the subsequent reaction without further purification. Reaction of this sulfonyl chloride with magnesium oxide in dry MeOH afforded the target methyl sulfonate **2**. Similar reactions of this sulfonyl chloride with methoxylamine hydrochloride, or *O*-benzylhydroxylamine hydrochloride, in the presence of sodium bicarbonate or potassium carbonate in dry THF furnished the respective *N*-methoxysulfonylamide **3** or *N*-benzyloxysulfonylamide **4** target product.

The synthetic reaction sequence used to prepare O^2-(2-methylsulfonylethyl) IPA/NO **5** is illustrated in Scheme 2. Since the previously unknown 2-methylthio-1-(trifluoromethanesulfonyloxy)ethane (structure not shown) is a rather unstable compound, 2-(methylthio)ethyl iodide **10**[15] was found to be a useful alternative alkylating agent. Accordingly, this key O^2-alkylation reaction was performed using the iodo compound **10** with isopropylamine diazeniumdiolate ammonium salt **6** rather than the corresponding IPA/NO sodium salt in dry MeOH to yield the O^2-alkylated product **11**. The improved yield in this O^2-alkylation reaction (28% yield using the ammonium salt **6** relative to 8% for the sodium salt) is likely due to the superior solubility in organic solvent and higher reactivity of the ammonium salt **6**.[16] Oxidation of the methylthio compound **7** using the mild oxidant potassium peroxymonosulfate ($KHSO_5$) afforded the target methyl sulfone compound **5**. The *N*-Boc compound **13** and the one-carbon homologue of compound **5** (compound **15**), required for mechanistic NO and HNO release studies, were prepared using the procedures illustrated in Scheme 2. In this regard, reaction of the amine **11** with $(Boc)_2O$ and *t*-BuOLi afforded the *tert*-butyl carbamate **12** (65%) that was oxidized using $KHSO_5$ to give the target compound **13** (56%). Condensation of the IPA/NO salt **6** with chloromethyl methylsulfide in the presence of Na_2CO_3 gave the O^2-methylthiomethyl compound **14** (10%) which was subsequently oxidized using $KHSO_5$ to furnish the target product **15**.

Mechanistic Decomposition of 5 Using ^{1}H NMR Spectrometry. ^{1}H NMR has been used for the first time to provide confirmation for the proposed base-induced β-elimination for O^2-sulfonylethyl NONOates. In this regard, compound **5** (20 mM) was mixed with 1 equiv of DBU in CD_3OD in a NMR tube. This mixture was shaken at 25 °C for exposure times of 0, 0.5, 1, and 4 h, respectively. The ^{1}H NMR spectrum was recorded at these 4 times, and the spectra are presented in parts b, c, d, e of Figure 2, respectively. A comparison of the spectra in Figure 2a and Figure 2b indicates that addition of DBU to the solution of **5** in CD_3OD results in a rapid base-induced β-elimination reaction to furnish $H_2C{=}CHSO_2Me$ (=C*H*, δ 6.93; H_{trans}, δ 6.34; H_{cis}, δ 6.16, and J_{trans} = 17 Hz, J_{cis} = 10 Hz). The $H_2C{=}CHSO_2Me$ intermediate product is gradually consumed, as illustrated by the NMR spectral changes that occur proceeding from (b) to (c) and then to (d). These spectral changes indicate that a Michael reaction of methyl vinyl sulfone with deuterium methoxide anion produces compound **16** (see structure in Figure 2). The two geminal methylene protons on carbon adjacent to oxygen

dx.doi.org/10.1021/jm301303p | J. Med. Chem. 2012, 55, 10262–10271

Scheme 2. Synthesis of the O^2-Protected IPA/NO Analogue 5, the N^3-Carbamoyl Compound 13 and the One-Carbon Homologue 15



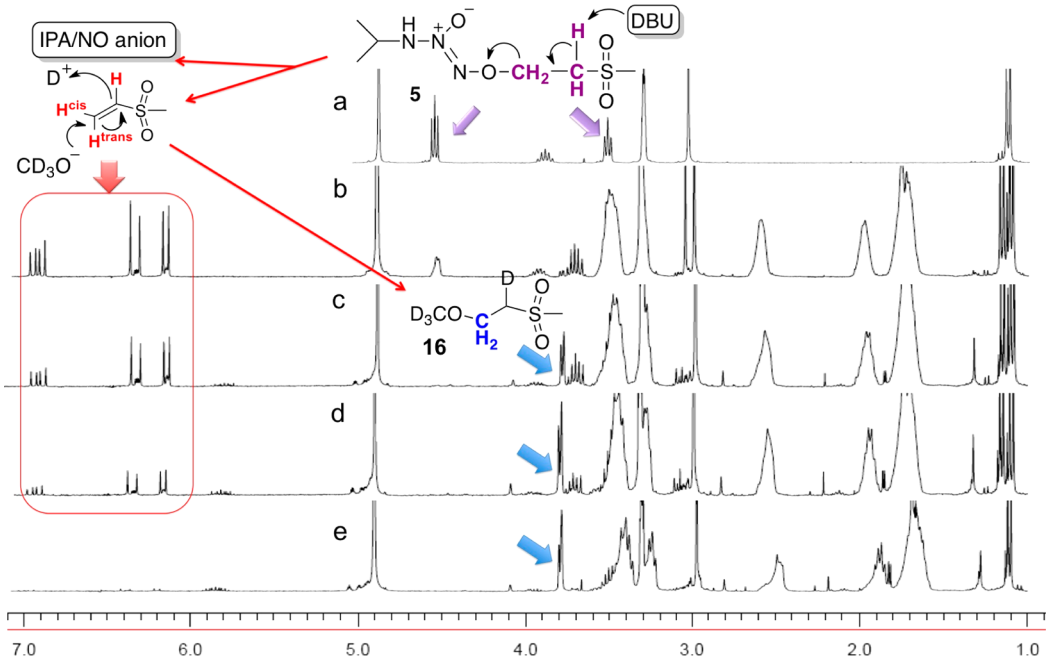


Figure 2. Partial ^{1}H NMR spectrum of compound **5** (20 mM) without/with DBU (20 mM) in CD_3OD. The spectrum of compound **5** (20 mM) only is shown in (a). The spectra of a mixture of **5** (20 mM) with DBU (20 mM) in CD_3OD after shaking at 25 °C for 0, 0.5, 1, and 4 h are shown in (b), (c), (d), and (e), respectively.

in **16** appear as a doublet (J_{vic} = 5.5 Hz) at δ 3.77. The chemical mechanism for the fragmentation of **5**, which releases methyl vinyl sulfone as a short-lived intermediate, is shown in Figure 2. Methyl vinyl sulfone, a potentially toxic product from the β-fragmentation reaction, undergoes a facile Michael reaction with nucleophiles such as methoxide indicated above and with glutathione (GSH) to furnish GS-$CH_2CH_2SO_2CH_3$ (see data in Supporting Information). This provides strong credence that the short-lived methyl vinyl sulfone should not induce adverse in vivo toxicity.

Half-Life Determination of 2–5. The half-lives of target compounds **2–5** were measured using a previously reported spectrophotometric method[17] via monitoring of the decrease in the absorbance of the diazeniumdiolate chromophore at about 245 nm wavelength at pH 7.4 phosphate buffer at 37 °C. Examples of the spectral changes occurring with time are shown in Figure S1 in Supporting Information. A linear regression of the $\ln(A_t - A_\infty)$ versus time plots generally showed good linearity (R^2, 0.97–0.99). The observed half-lives ($t_{1/2}$) for compounds **2**, **3**, **4**, and **5** in pH 7.4 buffer solution containing 5% DMSO at 37 °C were 17.1, 8.9, 6.6, and 15.3 h, respectively,

dx.doi.org/10.1021/jm301303p | J. Med. Chem. 2012, 55, 10262–10271

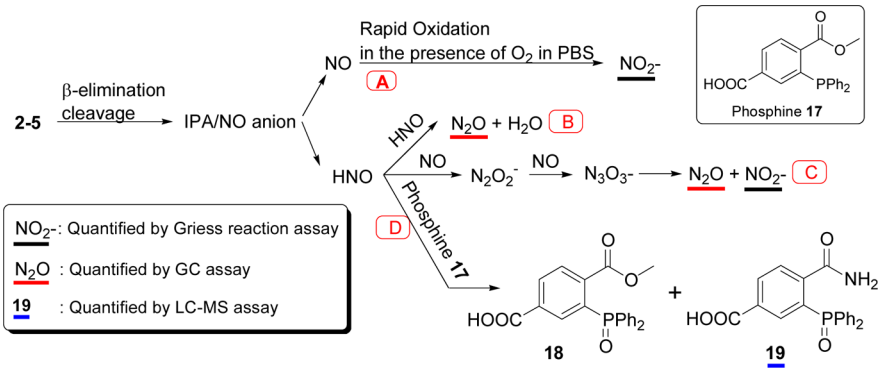


Figure 3. Plausible reactions of HNO and NO resulting in the formation of NO_2^-, N_2O, and/or HNO during in vitro incubation studies.

Table 1. Percent (%) NO_2^-, N_2O, and HNO Release from 2−5, 13, 15, IPA/NO, and AS in Buffer Solution at Different pH Values

conditions		2	3	4	5	13	15	IPA/NO	AS
					NO_2^{-a}				
pH 7.4[b]		10.6	0.8	6.0	1.8	28.6	0.4	62.0	
pH 10.0[c]		42.0	10.4	24.0	26.0	25.4	4.6	36.0	
					N_2O[d]				
MeOH/TBS 7.4[e]	0.5 h	1.4	5.9	3.8	0.5			9.4	
	2 h	1.9	12.9	9.7	1.6			8.3	
	48 h	1.9	33.5	27.5	10.4			7.0	
MeOH/TBS 7.4/GSH[f]	0.5 h	0.2	0.4	0.3	1.3			6.1	
	2 h	0.4	0.5	0.4	0.9			7.4	
	48 h	0.2	1.8	0.8	0.1			6.8	
MeOH/TBS 10.0[g]	0.5 h	16.7	52.6	56.8	52.9			5.9	
	2 h	15.1	62.9	61.3	62.2			5.7	
MeOH/TBS 10.0/GSH[h]	0.5 h	1.2	17.3	24.2	0.4			8.5	
	2 h	1.3	29.0	30.3	2.2			9.5	
					HNO[i]				
2 h			46.2 ± 1.6	47.3 ± 1.2	51.2 ± 2.8	5.1 ± 0.8	0.31 ± 0.1	5.4 ± 0.06	85.9 ± 1.3
48 h			51.3 ± 2.0	55.4 ± 1.8	61.4 ± 1.5	7.2 ± 0.5	4.0 ± 0.1	5.1 ± 1.3	85.7 ± 3.6

[a]Percent NO_2^- is equal to (the concentration of NO_2^- detected)/(the initial concentration of test compound). The result is the mean value of three measurements (n = 3) where variation from the mean % value was ≤0.2%. [b]A solution of the test compound (2.4 mL of 50 μM) in phosphate buffer at pH 7.4 containing 5% DMSO was incubated at 37 °C for 1.5 h. [c]A solution of the test compound (2.4 mL of 50 μM) in Tris buffer containing 5% DMSO at pH 10.0 was incubated at 37 °C for 1.5 h. [d]Percent of nitrous oxide (N_2O) released is based on the condensation of 2 mol of HNO → 1 mol of N_2O + H_2O. The result is the mean value of three measurements (n = 3). The HNO donor test compound (**2−5** and IPA/NO) concentration is 25 mM in each experiment and all samples were incubated at 37 °C. [e]MeOH/TBS 7.4 solvent comprises 0.4 mL of MeOH and 0.4 mL of 500 mM Tris buffer solution (TBS) that contained 0.2 mM EDTA at pH 7.4. [f]MeOH/TBS 7.4/GSH experiments are 50 mM in glutathione (GSH). [g]MeOH/TBS 10.0 solvent comprises 0.4 mL of MeOH and 0.4 mL of 500 mM TBS that also contained 0.2 mM EDTA at pH 10.0. The solution pH was 9 at the conclusion of the experiment. [h]MeOH/TBS 10.0/GSH experiments are 50 mM in glutathione (GSH). The solution pH was 9 at the conclusion of the experiment. [i]Percent of HNO released, based on a theoretical maximum release of 1 mol of HNO/mol of test compounds **3−5**, **13**, **15**, IPA/NO, and AS, and HNO was selectively trapped by phosphine **17** to produce an equivalent amount of HNO-derived amide **19**. The HNO donor test compound (**3−5**, **13**, **15**, IPA/NO, and AS) concentration is 200 μM. HNO trapping phosphine **17** concentration is 1 mM. The internal standard 4-hydroxybenzophenone concentration is 5.05 μM in each experiment, and all the samples were incubated at 37 °C. The buffer solution contains 7.5% DMSO and 92.5% phosphate buffer (50 μM) at pH 7.4. The incubation mixture was quenched by addition of H_2O_2 after 2 or 48 h. Quantification of the HNO-derived amide **19** was performed using a LC−MS assay.

which are much longer than that of IPA/NO ($t_{1/2}$ = 2.3 min)[18] and that of AcOM-IPA/NO (41 min).[12] These data indicate that **2−5** possess improved chemical stability at physiological conditions relative to IPA/NO.

Nitrite (NO_2^-), Nitrous Oxide (N_2O), and HNO Release Studies. A number of quantitative assays were performed to determine the amount of HNO and NO released from the O^2-sulfonylethyl IPA/NO compounds **2−5** that takes place following β-elimination cleavage. Prior to initiating these assays, compounds **13** and **15** were prepared for use as controls (see structures in Scheme 2). It has been reported that N^3-carbamoyl isopropylaminodiazeniumdiolate acts like secondary amine diazeniumdiolates to release NO rather than HNO, since it does not undergo protonation at the N^3-isopropylamino nitrogen atom.[19] Accordingly, compound **13**, which is a *N*-Boc derivative of compound **5**, is expected to release NO rather than HNO. Compound **15**, a one-carbon homologue of the two-carbon homologue **5**, is not able to undergo a β-elimination cleavage to release the IPA/NO anion. In this regard, compound **15**, unlike IPA/NO, is expected to release a very low, or negligible, amount of HNO.

Quantification of the amount of HNO and NO released from donor compounds requires the use of several assays. Four reactions that NO and/or HNO are expected to undergo

dx.doi.org/10.1021/jm301303p | J. Med. Chem. 2012, 55, 10262−10271

during in vitro incubation studies in buffer at physiological pH are illustrated in Figure 3. IPA/NO functions as a dual donor of HNO and NO at pH 5−8.[10,11] Consequently, compounds **2**−**5** are expected to release both NO and HNO following the β-elimination and O^2-cleavage reaction. Once produced, NO could be rapidly oxidized to nitrite in the presence of oxygen in aqueous PBS solution (reaction A).[20] In contrast, HNO can either dimerize to form N_2O (8×10^6 M^{-1} s^{-1}, reaction B)[21,22] or react with NO to form a hyponitrite radical (with a rate constant of 5.6×10^6 M^{-1} s^{-1}) which subsequently reacts with one more NO to ultimately furnish N_2O and nitrite (reaction C).[23,24] Given this complexity, simply using the amount of NO_2^- to quantify the percentage of NO and using N_2O to quantify the percentage of HNO may lack accuracy. It has been reported that the reaction of the water-soluble phosphine **17** with HNO occurs at a rate comparable to the reaction of HNO with glutathione (2×10^6 M^{-1} s^{-1}), suggesting the utility of **17** as a selective and efficient agent for the detection (trapping) and quantization of HNO.[25,26] On the basis of these data, we proceeded to use three independent assays to measure the release of HNO and/or NO from the test and reference compounds: (a) the colorimetric Griess reaction assay was used to determine the amount of NO_2^-; (b) a gas chromatographic (GC) assay was used to determine the amount of N_2O that was derived from dimerization of HNO or the reaction of HNO and NO; (c) a selective LC−MS assay was developed to determine the percentage of HNO released by quantification of HNO-derived amide **19** produced during a Staudinger reaction. The data from these studies are presented in Table 1.

The % NO_2^- (% NO_2^- is equal to the concentration of NO_2^- measured in the Griess assay/the initial concentration of test compound) released from **2**−**5** in PBS at pH 7.4 and 37 °C varied within a 0.8−10.6% range, which is indicative of slow NO release (Table 1). In contrast, the % NO_2^- released in Tris buffer solution at pH 10 and 37 °C was increased (10.4−42.0% range), which is attributed to a more extensive β-elimination cleavage at alkaline pH that furnishes IPA/NO. The % NO_2^- arising from compound **13** was expectedly higher than that from parent compound **5** in PBS at pH 7.4 (28.6% for **13**; 1.8% for **5**), since the carbamated primary amine diazeniumdiolate acts like a secondary amine to predominately release NO.[19] The one carbon linkage analogue **15** released a very low amount of NO_2^- at both pH 7.4 and 10.0, revealing that the β-elimination-induced O^2-cleavage reaction is not favorable. The observation that IPA/NO releases more NO_2^- at pH 7.4 (62%) than at pH 10 (36%) is consistent with reports that IPA/NO preferentially decomposes to release HNO rather than NO at highly alkaline pH values.[10,11] N_2O release from **2**−**5** and IPA/NO was quantified by gas chromatography (GC) using four MeOH-based solvent mixtures at 37 °C (Table 1). Incubations were also carried out at pH 10, since a highly alkaline pH will accelerate the β-elimination reaction to furnish the IPA/NO anion. Competition incubations experiments containing glutathione (GSH) were also performed, since HNO reacts rapidly with thiols to form disulfides and hydroxylamine or sulfinamides.[27,28] In this regard, the reaction of HNO with GSH competes with the spontaneous dimerization of HNO to N_2O, thereby reducing the amount of N_2O produced. The percentages of N_2O (based on the condensation of 2 mol of HNO → 1 mol of N_2O + H_2O) arising from **3**−**5** in MeOH/TBS at pH 7.4 increased with time. For compound **3**, the percentages of N_2O after incubation for 0.5, 2, and 48 h were 5.9%, 12.9%, and 33.5%, respectively. These data indicate that under physiological relevant conditions, **3**−**5** undergo a β-elimination reaction with expulsion of the free isopropylamine diazeniumdiolate anion that subsequently releases HNO. Interestingly, compound **2** showed low percentages of N_2O production in MeOH/TBS at pH 7.4 even upon incubation for 48 h (1.9%), demonstrating that the O^2-$CH_2CH_2SO_2OMe$ moiety must be relatively stable under physiological conditions. In contrast, in MeOH/TBS at pH 10, the % N_2O produced from compounds **2**−**5** was much higher as expected. For instance, compound **5** liberated 1.6% of N_2O in MeOH/TBS at pH 7.4 relative to 62.2% N_2O in MeOH/TBS at pH 10 for a 2 h incubation. The amount of N_2O released from the SO_2NHOMe **3** (29.0%) and SO_2NHOBn **4** (30.3%) compounds in the MeOH/TBS 10/GSH system was much greater than expected for a 2 h incubation. One plausible explanation for this increased amount of N_2O is attributed to a competition between the reaction of GSH with the vinyl sulfone arising from the β-elimination reaction and the reaction (trapping) of HNO with GSH. In this regard, the vinyl sulfone acts as a Michael acceptor to react with GSH, thereby decreasing the amount of GSH available to react with HNO. Credence for this explanation is based on the observation that GSH reacts readily with methyl vinyl sulfone to furnish GS-$CH_2CH_2SO_2CH_3$ (see data provided as Supporting Information). A second plausible explanation for this increased amount of N_2O is attributed to the reaction C pathway shown in Figure 3. The reaction of HNO with NO to form a hyponitrite radical (5.6×10^6 M^{-1} s^{-1}) is faster than the trapping reaction of HNO by GSH (2×10^6 M^{-1} s^{-1}) such that GSH is not able to stop the generation of N_2O from the hyponitrite radical.

The percentages of HNO released from compounds **3**−**5**, **13**, **15**, IPA/NO, and AS were determined by quantification of HNO-derived amide **19** produced in the reaction of phosphine **17** in the presence of the test compound using a modified LC−MS assay (Table 1).[26] Compounds **3**−**5** showed high HNO release (46.2−61.4%) in PBS at pH 7.4 for 2 or 48 h incubations. The amount of N_2O released (8.3% at 2 h) from IPA/NO in MeOH/TBS 7.4 is in good agreement with the 5.4% release of HNO determined in the LC−MS assay. Two analogues of compound **5** (the carbamoyl compound **13** and compound **15** having a one-carbon spacer) release a much lower percentage of HNO (0.31−7.17%), indicating that a primary amine moiety and a two-carbon spacer are two important structural requirements that are highly relevant to the concept of designing HNO donor compounds where HNO release is triggered following a β-elimination reaction. Collectively, the three independent assays employed to measure the percentages of NO_2^-, N_2O, and HNO release provide a relatively complete profile of HNO and/or NO release properties of the test compounds. These data acquired revealed that O^2-sulfonylethyl compounds **3**−**5** are good HNO donors compared to the parent IPA/NO at physiological conditions.

Positive Inotropic, Rates of Contraction and Relaxation, and Heart Rate (Cardiac Hemodynamic) Studies. The positive inotropic effect induced by HNO is considered to be a unique biological and pharmacological action that differs from that of NO. It was therefore of interest to carry out the title studies for compound **5** which is an effective HNO donor. To determine whether compound **5** possessed positive inotropic properties, hearts from C57BL/6 mice were perfused with Krebs−Henseleit buffer for 20 min. All hearts had normal baseline contractile function, measured as LVDP (inotropic

Table 2. Cardiac Hemodynamic Parameters Were Measured Using Isolated Perfused Hearts

	baseline	5[a]	isoproterenol[a]
LVDP (cmH_2O)	66 ± 6	187 ± 34*	157
rate of contraction, dP/dt_{max} (cmH_2O/ms)	2619 ± 470	5698 ± 679*	5330
rate of relaxation, $-dP/dt_{min}$ (cmH_2O/ms)	−2389 ± 417	−5330 ± 711*	−5882
HR, perfused (beats/min)	311 ± 48	269 ± 22	334

[a]Values are mean ± SEM: *$p < 0.05$ vs baseline, $n = 4$ (compound **5**), $n = 1$ (isoproterenol).

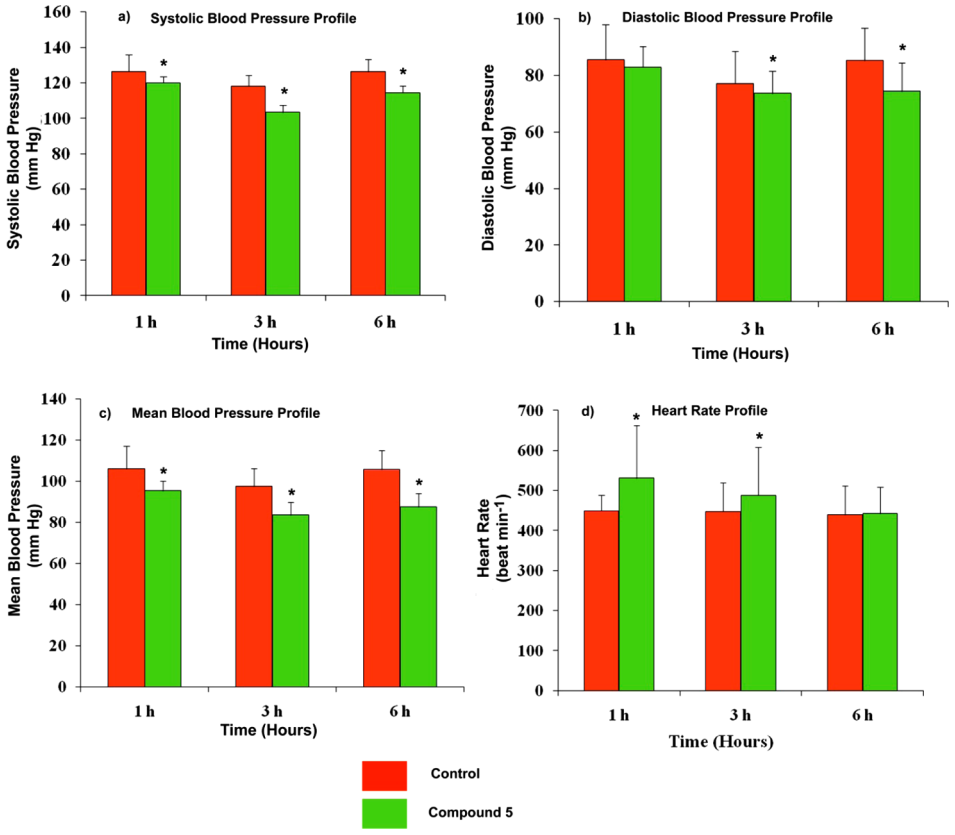


Figure 4. Systolic blood pressure (BP_{sys}), diastolic blood pressure (BP_{dia}), mean blood pressure (BP_{mean}), and heart rate (HR) in conscious mice following oral administration of either vehicle alone (control group, mean ± SD, $n = 6$) or test compound **5** (60.345 μmol/kg or 22.5 mg/kg po; $n = 4$ at 1 h, $n = 5$ at 3 h, and $n = 6$ at 6 h). Data are presented as group mean ± SD. Statistical differences between groups are denoted as ∗, $p < 0.05$.

effect), HR, and diastolic (rate of relaxation, $-dP/dt_{min}$) and systolic (rate of contraction, dP/dt_{max}) contractions (Table 2). Hearts perfused with compound **5** (100 μM) demonstrated a significant increase in LVDP (positive inotropic effect) and relaxation and contraction rates (Table 2) when compared to baseline levels (290% above baseline). Similar inotropic effects were observed with hearts perfused with isoproterenol (Table 2). However, no significant changes to heart rate were observed with compound **5** at the concentration examined. Thus, compound **5** possessed significant inotropic effects where the force of contraction and degree of relaxation were increased without inducing a chronotropic effect.

Blood Pressure Studies. Systolic blood pressure (BP_{sys}, mmHg), diastolic blood pressure (BP_{dia}, mmHg), and heart rate (HR, beats min^{-1}) were measured at 1, 3, and 6 h time intervals following oral administration of either the vehicle alone (control group, $n = 6$) or the HNO donor compound **5** (60.345 μmol/kg or 22.5 mg/kg po, $n = 4$ at 1 h; $n = 5$ at 3 h; $n = 6$ at 6 h) to C57 black mice. A minimum of three consecutive measurements were made for each mouse at each time interval, and the mean value is the average of these three measurements. The data obtained for BP_{sys}, BP_{dias}, BP_{mean} (average of BP_{sys} and BP_{dia}), and HR are illustrated in panels a–d of Figure 4 (Table S1 listing numerical data ± standard deviation is provided as Supporting Information).

The O^2-(2-methylsulfonylethyl) compound **5** showed a significant reduction (∗, $p < 0.05$) in BP_{sys} (mmHg) at 1 (120.2), 3 (103.4), and 6 (114.4) hours after drug administration relative to the control group (1 h, 126.5; 3 h, 118.3; 6 h, 126.2; see data in Figure 4a). Significant reductions in BP_{dia} were also observed at 3 and 6 h, but not at 1 h, after drug administration (see data in Figure 4b). The BP_{mean} profile (Figure 4c) shows that there was a significant reduction in BP_{mean} at all three times (1 h, 95.4; 3 h, 83.6; 6 h, 87.5) relative to control values (1 h, 106.0; 3 h, 97.5; 6 h, 105.7).

The HR of mice was determined, and the data are shown in Figure 4d (complete data are provided in Table S1 as Supporting Information). The HR at 1 h (531) and 3 h (488) was higher than, but nearly identical at 6 h (442), that for control mice (449, 448, and 439 beats min^{-1} at 1, 3, and 6 h after drug administration, respectivcly). This in vivo increase in HR is attributed to neuroeffects that increase HR to

dx.doi.org/10.1021/jm301303p | J. Med. Chem. 2012, 55, 10262−10271

compensate for the significant reduction in BP observed. In comparison, the in vitro perfused heart study described previously showed that compound **5** did not induce a chronotropic effect, since there are no neurocompensatory effects to change HR in the in vitro (ex vivo) isolated heart.

CONCLUSIONS

The primary amine O^2-sulfonylethyl IPA/NO compounds **2**−**5** described in this study possess the following distinctive chemical and/or biological advantages compared to the previously reported O^2-sulfonylethyl secondary amine diazeniumdiolates[10] and the parent IPA/NO. (i) O^2-protected IPA/NO analogues **2**−**5**, unlike the unstable and highly explosive parent IPA/NO,[10,11] are stable, readily isolated compounds. (ii) O^2-protected IPA/NO analogues **2**−**5** have longer improved half-lives (6.6−17.1 h) under physiological conditions relative to IPA/NO which has a very short half-life (2.3 min).[18] (iii) ^{1}H NMR spectroscopy, used to investigate the mechanistic fragmentation of **5** in the presence of DBU in CD_3OD solution, provided validation for a previously proposed base-induced β-elimination mechanism.[13] Following the β-elimination reaction to furnish the IPA/NO anion and methyl vinyl sulfone, the latter short-lived vinyl product undergoes subsequent reaction with methoxide to form a Michael addition product. (iv) It is highly probable that compounds **3**−**5** undergo preferential protonation at the primary amino N^3-position prior to decomposition, since a high amount of HNO (46.2−61.4%), as quantified by selective formation of the HNO-derived amide **19** using an efficient LC−MS assay, was released. (v) Compound **5** showed a significant in vitro inotropic effect, increasing the force of contraction and the rate of relaxation without any chronotropic effect; and (vi) compound **5** exhibited an antihypertensive effect, since BP_{mean} was lowered significantly up to 6 h after drug administration. These results reveal that O^2-sulfonylethyl IPA/NO compounds **3**−**5** represent a novel class of chemically stable HNO donor compounds with excellent HNO release properties at physiological pH. The IPA/NO donor compounds described constitute useful lead compounds with relevant pharmaceutical properties for the design of nitroxyl donors with potential applications to treat cardiovascular disease(s) including congestive heart failure.

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 (300 MHz) and ^{13}C NMR (75 MHz) 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). Liquid chromatography−mass spectrometry (LC−MS) data were recorded on a Water's Micromass ZQ 4000 mass spectrometer using the ESI negative ionization mode and a reverse phase C18 column. Microanalyses were performed for C, H, N by the Microanalytical Service Laboratory, Department of Chemistry, University of Alberta, Canada. Unknown compounds **2**−**5**, **7**−**9**, and **11**−**15** showed a single spot on Macherey-NagelPolygram 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 a gold silica (**2**−**5**, **7**−**9**, and **11**−**15**) column. Although a method has been reported[26,29] for the preparation of compound **19**, the amide compound **19** required as a reference sample in the LC−MS assay was more conveniently synthesized via a reaction of phosphine **17** with HNO released from AS and subsequently isolated (purified) on a Combiflash Rf system using a C18 column (complete details are provided as Supporting Information). All reagents, purchased from the Sigma-Aldrich, were used without further purification. In vitro cardiac hemodynamic studies and in vivo blood pressure and heart rate measurements were carried out using protocols approved by the Health Sciences Animal Welfare Committee at the University of Alberta, Canada.

***O^2*-(2-Methoxysulfonylethyl) 1-(Isopropylamino)diazen-1-ium-1,2-diolate (2).** O^2-(2-Oxysulfonylethylsodium salt) 1-(isopropylamino)diazen-1-ium-1,2-diolate (**9**, 250 mg, 1.0 mmol) was dissolved in DMF (2.5 mL), and $SOCl_2$ (0.22 mL, 3.0 mmol) was added dropwise. The reaction mixture was allowed to stir at 25 °C for 1.5 h, poured into cold water (30 mL), and extracted with ethyl ether (3 × 30 mL). The combined organic fractions were washed with 2 N HCl solution and brine, and the organic fraction was dried ($MgSO_4$). After concentration in vacuo at 25 °C, the resulting sulfonyl chloride product obtained as a brown syrup was dissolved in dry MeOH (5 mL), and magnesium oxide (200 mg, 5.0 mmol) was added. The reaction mixture was vigorously stirred at 25 °C until the sulfonyl chloride had completely disappeared (TLC; EtOAc−hexane, 1:2, v/v) in about 6 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 **2** (67 mg, 28%, two steps) as a colorless oil. IR (film): 3275, 2986, 2934, 1742, 1362, 1165 cm^{-1}. ESI-MS: 242 $[M + H]^+$, 264 $[M + Na]^+$. ^{1}H NMR (CD_3OD): δ 1.22 (d, J = 6.7 Hz, 6H, $(CH_3)_2CH$), 3.66 (t, J = 6.1 Hz, 2H, OCH_2CH_2S), 3.83−3.95 (m, 4H, $(CH_3)_2CH$ and SO_3CH_3), 4.53 (t, J = 6.1 Hz, 2H, OCH_2CH_2S), 8.09 (s, 1H, *NH*). ^{13}C NMR (CD_3OD): δ 19.5, 50.2, 57.1, 62.0, 67.9. Anal. Calcd for $C_6H_{15}N_3O_5S$: C, 29.87; H, 6.27. Found: C, 29.90; H, 6.09.

***O^2*-(2-Methoxyaminosulfonylethyl) 1-(Isopropylamino)-diazen-1-ium-1,2-diolate (3).** The same method was used to prepare the intermediate sulfonyl chloride compound as described for the preparation of compound **2**. The resulting brown syrup of the sulfonyl chloride product was dissolved in dry THF (5 mL), and then methoxylamine hydrochloride (167 mg, 2.0 mmol) and $NaHCO_3$ (250 mg, 3.0 mmol) were added. The reaction mixture was vigorously stirred at 25 °C until the sulfonyl chloride had completely disappeared (TLC; EtOAc−hexane, 1:2, v/v) in about 3 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 **3** (82 mg, 32%, two steps) as a colorless oil. IR (film): 3223, 2994, 2945, 1704, 1348, 1163 cm^{-1}. ESI-MS: 257 $[M + H]^+$, 279 $[M + Na]^+$. ^{1}H NMR (DMSO-d_6): δ 1.04 (d, J = 6.8 Hz, 6H, $(CH_3)_2CH$), 3.56 (t, J = 6.1 Hz, 2H, OCH_2CH_2S), 3.67 (s, 3H, $NHOCH_3$), 3.72−3.77 (m, 1H, $(CH_3)_2CH$), 4.54 (t, J = 6.1 Hz, 2H, OCH_2CH_2S), 8.70 (d, J = 9.2 Hz, 1H, $(CH_3)_2CHNH$), 10.2 (s, 1H, $NHOCH_3$). ^{13}C NMR (DMSO-d_6): δ 19.6, 47.3, 47.4, 64.4, 65.6. Anal. Calcd for $C_6H_{16}N_4O_5S$: C, 28.12; H, 6.29. Found: C, 27.82; H, 6.07.

***O^2*-(2-Benzyloxyaminosulfonylethyl) 1-(Isopropylamino)-diazen-1-ium-1,2-diolate (4).** The same method was used to prepare intermediate sulfonyl chloride compound as described for the preparation of compound **2**. The resulting brown syrup of the sulfonyl chloride product was dissolved in dry THF (5 mL), and then *O*-benzyhydroxylamine hydrochloride (319 mg, 2.0 mmol) and K_2CO_3 (276 mg, 2.0 mmol) were added. The reaction mixture was vigorously stirred at 25 °C until the sulfonyl chloride had completely disappeared (TLC; EtOAc−hexane, 1:2, v/v) in about 4 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 **4** (83 mg, 25%, two steps) as a colorless oil. IR (film): 3229, 2981, 2939, 1346, 1160 cm^{-1}. ESI-MS: 355 $[M + Na]^+$. ^1H NMR (DMSO-d_6): δ 1.02 (d, J = 6.7 Hz, 6H, $(CH_3)_2CH$), 3.59 (t, J = 6.1 Hz, 2H, OCH_2CH_2S), 3.70−3.77 (m, 1H, $(CH_3)_2CH$), 4.42 (t, J = 6.1 Hz, 2H, OCH_2CH_2S), 4.89 (s, 2H, CH_2Ar), 7.31−7.38 (m, 5H, *ArH*), 8.70 (d, J = 8.5 Hz, 1H, $(CH_3)_2CHNH$), 10.2 (s, 1H, $NHOCH_2Ar$). ^{13}C NMR (DMSO-d_6): δ 19.6, 47.4, 47.5, 65.6, 78.2, 128.3, 128.8, 135.7. Anal. Calcd for $C_{12}H_{20}N_4O_5S$: C, 43.36; H, 6.07. Found: C, 43.43; H, 5.73.

O^2-(2-Methylsulfonylethyl) 1-(Isopropylamino)diazen-1-ium-1,2-diolate (5). O^2-(2-Methylthioethyl) 1-(isopropylamino)-diazen-1-ium-1,2-diolate (**11**, 260 mg, 1.35 mmol) was dissolved in THF−MeOH (1:1, v/v; 8 mL). A solution of potassium peroxymonosulfate (1.66 g, 2.7 mmol) in water (8 mL) was added dropwise at 0 °C, and the reaction was allowed to proceed for 4 h at 25 °C with stirring. Water (20 mL) was added to the reaction mixture. This solution was extracted with EtOAc (3 × 30 mL). The combined organic fractions were washed with brine (20 mL), and the organic fraction was dried ($MgSO_4$). Removal of the solvent in vacuo afforded a residue which was purified by flash column chromatography using EtOAc−hexane (1:1, v/v) as eluent to furnish the title compound **5** (167 mg, 55%) as a colorless oil, which solidified upon storage in a refrigerator to give **5** as a yellow powder; mp 50−52 °C. IR (film): 3244, 2975, 2934, 1294, 1134 cm^{-1}. ESI-MS: 226 $[M + H]^+$, 248 $[M + Na]^+$, 473 $[2M + Na]^+$. ^1H NMR (CD_3OD): δ 1.12 (d, J = 6.1 Hz, 6H, $(CH_3)_2CH$), 3.03 (s, 3H, SO_2CH_3), 3.51 (t, J = 5.5 Hz, 2H, OCH_2CH_2S), 3.85−3.91 (m, 1H, $(CH_3)_2CH$), 4.55 (t, J = 5.5 Hz, 2H, OCH_2CH_2S). ^{13}C NMR (DMSO-d_6): δ 20.4, 42.9, 49.2, 53.8, 66.8. Anal. Calcd for $C_6H_{15}N_3O_4S$: C, 31.99; H, 6.71; N, 18.65. Found: C, 32.08; H, 6.45; N, 18.32.

O^2-(2-Methylsulfonylethyl) 1-(*N*-Boc-isopropylamino)-diazen-1-ium-1,2-diolate (13). O^2-(2-Methylthioethyl) 1-(*N*-Boc isopropylamino)diazen-1-ium-1,2-diolate (**12**, 100 mg, 0.341 mmol) was dissolved in THF−MeOH (1:1, v/v; 3 mL). A solution of potassium peroxymonosulfate (420 mg, 0.682 mmol) in water (3 mL) was added dropwise at 0 °C, and the reaction was allowed to proceed for 2 h at 25 °C with stirring. Water (10 mL) was added to the reaction mixture. This solution was extracted with EtOAc (3 × 10 mL). The combined organic fractions were washed with brine (10 mL), and the organic fraction was dried ($MgSO_4$). Removal of the solvent in vacuo afforded a residue which was purified by flash column chromatography using EtOAc−hexane (1:1, v/v) as eluent to furnish the title compound **13** (62 mg, 56%) as a yellowish oil, which solidified upon storage in a refrigerator to give **13** as a yellow powder; mp 61−62 °C. IR (film): 2983, 2933, 1747, 1267, 1136 cm^{-1}. ESI-MS: 348 $[M + Na]^+$, 364 $[M + K]^+$. ^1H NMR (CD_3OD): δ 1.25 (d, J = 7.2 Hz, 6H, $(CH_3)_2CH$), 1.48 (s, 9H, $C(CH_3)_3$), 2.99 (s, 3H, SO_2CH_3), 3.35 (t, J = 5.4 Hz, 2H, OCH_2CH_2S), 4.25−4.29 (m, 1H, $(CH_3)_2CH$), 4.68 (t, J = 5.4 Hz, 2H, OCH_2CH_2S). ^{13}C NMR (DMSO-d_6): δ 19.6, 28.0, 43.0, 51.9, 53.9, 67.7, 84.2, 151. Anal. Calcd for $C_{11}H_{23}N_3O_4S$: C, 40.60; H, 7.12; N, 12.91. Found: C, 40.67; H, 7.06; N, 12.66.

O^2-(Methylsulfonylmethyl) 1-(Isopropylamino)diazen-1-ium-1,2-diolate (15). O^2-(Methylthiomethyl) 1-(isopropylamino)-diazen-1-ium-1,2-diolate (**14**, 190 mg, 1.06 mmol) was dissolved in THF−MeOH (1:1, v/v; 5 mL). A solution of potassium peroxymonosulfate (1305 mg, 2.12 mmol) in water (5 mL) was added dropwise at 0 °C, and the reaction was allowed to proceed for 2 h at 25 °C with stirring. Water (15 mL) was added to the reaction mixture. This solution was extracted with EtOAc (3 × 15 mL). The combined organic fractions were washed with brine (15 mL), and the organic fraction was dried ($MgSO_4$). Removal of the solvent in vacuo afforded a residue which was purified by flash column chromatography using EtOAc−hexane (1:1, v/v) as eluent to furnish the title compound **15** (141 mg, 63%) as a colorless oil, which solidified upon storage in a refrigerator to give **15** as a white powder; mp 64−65 °C. IR (film): 3263, 2990, 2938, 1305, 1143, 1084 cm^{-1}. ESI-MS: 212 $[M + H]^+$, 234 $[M + Na]^+$, 250 $[M + K]^+$. ^1H NMR (CD_3OD): δ 1.14 (d, J = 6.6 Hz, 6H, $(CH_3)_2CH$), 2.93 (s, 3H, SO_2CH_3), 3.93−3.97 (m, 1H, $(CH_3)_2CH$), 4.94 (s, 2H, OCH_2S). ^{13}C NMR (DMSO-d_6): δ 20.3, 38.8, 49.4, 84.1. Anal. Calcd for $C_5H_{13}N_3O_4S$: C, 28.43; H, 6.20; N, 19.89. Found: C, 28.68; H, 6.24; N, 19.72.

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 or (ii) TBS at pH 10 at 37 °C for 1.5 h 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 (**2**−**5**) and the reference compound IPA/NO using the reported procedure.[30]

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 the sample incubated at 37 °C, and at desired time-points headspace aliquots (25 μL) were injected via a gastight syringe onto a 7890A Agilent Technologies gas chromatograph equipped with a microelectron capture detector and a 30 m × 0.32 m (25 μm) HP-MOLSIV capillary column. The oven was operated at 200 °C for the duration of the run (4.5 min). The inlet was held at 250 °C and run in split mode (split ratio 1:1) with a total flow (N_2 as carrier gas) of 4 mL/min and a pressure of 37.9 psi. The μECD was held at 325 °C with a makeup flow (N_2) of 5 mL/min. The retention time of nitrous oxide was 3.4 min, and yields were calculated based on a standard curve for nitrous oxide (Matheson Tri-Gas).

LC−MS Quantification of Phosphine-Mediated HNO Trapping. A solution of phosphine **17** in DMSO (25 μL), the test compound (**2**−**5**, **13**, **15**, IPA/NO, or AS) in DMSO (25 μL), and internal standard compound 4-hydroxybenzophenone in DMSO (25 μL) were added to a PBS solution, pH 7.4 (925 μL), providing **17** (1 mM), test compound (200 μM), and 4-hydroxybenzophenone (5.05 μM). The incubation mixture was stirred at 37 °C in a sealed vial for 2 or 48 h. Hydrogen peroxide solution (30% w/v, 5 μL) was added into the incubation mixture to quench the reaction of **17** and HNO. An aliquot (500 μL) was removed and the solution was evaporated to dryness under vacuum (using a Thermo Scientific Savant DNA 120 SpeedVac Concentrator) at about 60 °C. Methanol (500 μL) was added to dissolve the residue. After centrifugation, an aliquot of the clear methanol solution (250 μL) was analyzed by LC−MS (Water's Micromass ZQTM 4000 LC−MS instrument, operating in the ESI negative mode, equipped with a Water's 2795 separation module). Separations were performed in triplicate using a Kromasil 100-5-C18 (100 μm pore size, 5 μm particle size) reverse phase column (2.1 mm diameter × 50 mm length), preceded by a Kromasil 100-5-C18 2.1× guard column. Separations were effected using a gradient going from MeCN/1% aqueous formic acid (40:60, v/v) to MeCN/1% aqueous formic acid (60:40, v/v) over a 12 min period at a flow rate of 0.25 mL/min. Operating parameters were as follows: capillary voltage = 3.5 kV; cone voltage = 20 V; source temperature = 140 °C; sesolvation temperature = 250 °C; cone nitrogen gas flow = 100 L/h; desolvation nitrogen gas flow = 550 L/h. The identities of products **18** (retention time of 6.62 min), **19** (retention time of 3.63 min), and the internal standard 4-hydroxybenzophenone (retention time of 10.89 min) were confirmed by MS (LC−MS chromatograms are provided as Supporting Information). The amount of compound **19** produced upon incubation of the test compound was determined from a standard curve (that was linear over a concentration range of 0.2−10 μg/mL, $R^2 > 0.997$) prepared using an authentic sample of compound **19**. The LC−MS HNO release data presented in Table 1 are the mean of triplicate experiments. A control experiment involving incubation of compound **17** and 4-hydroxybenzophenone under identical conditions, but not containing a test compoud, and subsequent LC−MS analysis showed the absence of **19**.

Determination of Cardiac Hemodynamic Properties for Compound 5. C57BL/6 mouse hearts were perfused in the Langendorff mode as described.[31−33] Briefly, hearts were perfused in a retrograde fashion at constant pressure (90 cmH_2O) with continuously aerated (95%O_2/5%CO_2) Krebs−Henseleit buffer at 37 °C. Hearts were first stabilized for 20 min with buffer and then perfused with compound **5** (100 μM) or isoproterenol (100 μM,

positive ionotropic control) for 20 min. Heart rate (HR), diastolic and systolic rates, and left ventricular developed pressure (LVDP) were obtained.

In Vivo Blood Pressure Measurement. Noninvasive blood pressure and heart rate measurements were performed according to a protocol approved by the Health Sciences Animal Welfare Committee at the University of Alberta (Edmonton, Canada). Following oral administration of test compound **5** (60.345 μmol/kg or 22.5 mg/kg po dose) dissolved in water containing 1% methylcellulose, changes in BP_{sys}, BP_{dia}, BP_{mean}, and HR in C57 black mice were measured in a conscious state at 1, 3 and 6 h time intervals using our previously reported method.[34]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Preparation and characterization data of compounds **6**−**12**, **14**, and **17**−**19**; half-lives determination studies for **2**−**5**; mechanistic decomposition studies using ^{1}H NMR; and numerical blood pressure and heart rate data for control and treated mice. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Vishwanatha Somayaji for spectrometry services. We are also grateful to the Canadian Institutes of Health Research (Grant MOP-14712 to E.E.K and Grant MOP-115037 to J.M.S.) for financial support of this research.

■ ABBREVIATIONS USED

NO, nitric oxide; HNO, nitroxyl; NO_2^-, nitrite; N_2O, nitrous oxide; GSH, glutathione; $KHSO_5$, potassium peroxymonosulfate; PBS, phosphate buffered solution; TBS, Tris buffer solution; AS, Angeli's salt; IPA/NO, isopropylamine diazeniumdiolate; LVDP, left ventricular developing pressure; HR, heart rate

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