Acyclic triaryl olefins possessing a sulfohydroxamic acid pharmacophore: synthesis, nitric oxide/nitroxyl release, cyclooxygenase inhibition, and anti-inflammatory studies†

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Nitric oxide (NO) and its reduced form nitroxyl (HNO), effective vasodilation agents that can inhibit platelet aggregation and adhesion, could suppress adverse cardiovascular effects associated with the use of selective COX-2 inhibitors. In this regard, a sulfohydroxamic acid (SO_2NHOH) substituent, that can act as a dual NO/HNO donor moiety, was inserted at the *para*-position of the C2 phenyl ring of acyclic 2-alkyl-1,1,2-triaryl olefins previously shown to be potent and highly selective COX-2 inhibitors. Although this new group of 1,1-diaryl-2-(4-hydroxyaminosulfonylphenyl)alk-1-enes exhibited weak inhibition of the constitutive cyclooxygenase-1 (COX-1) and inducible COX-2 isozymes, *in vivo* studies showed anti-inflammatory potencies that were generally intermediate between that of the reference drugs aspirin and ibuprofen. All compounds released NO (5.6–13.5% range) upon incubation with phosphate buffer which was increased further (8.3–25.6% range) in the presence of the oxidant $K_3(FeCN_6)$.The low release of HNO in MeOH-buffer (< 2% at 24 h incubation) was much higher at alkaline pH (11–37% range). The concept of designing better anti-inflammatory drugs possessing either an effective HNO, or dual NO/HNO, donor moiety that are devoid of adverse ulcerogenic and/or cardiovascular side effects warrants further investigation.

Introduction

Selective cyclooxygenase-2 (COX-2) inhibitors, such as rofecoxib (**1**)[1] celecoxib (**2a**),[2] valdecoxib (**3a**)[3] and the acyclic 2-butyl-1,1,2-triaryl (*Z*)-olefin (**4**),[4] reduce inflammation and pain by inhibiting the peripheral production of prostaglandins (see structures in Fig. 1). Although the risk of gastric irritation and peptic ulceration associated with the use of selective COX-2 inhibitors is extremely low, there is an increased incidence of adverse cardiovascular events such as thrombosis and stroke.[5] Highly selective COX-2 inhibitors may decrease the level of the beneficial vasodilator and platelet aggregation inhibitor prostacyclin (PGI_2) concurrent with a contraindicated increase in the level of the potent platelet activator and aggregator thromboxane A_2 (TxA_2). This biochemical alteration in the arachidonic acid COX biosynthetic pathway has been attributed to the increased incidence of cardiovascular thrombotic events observed.[6]

The biological activity and biological chemistry of nitrogen oxide species in mammalian systems continues to attract considerable attention.[7] Nitric oxide (NO) is an effective vasodilation agent, an inhibitor of platelet aggregation and adhesion,[8] and it provides an attractive method to suppress vascular side effects associated with NSAID use.[9] In recent studies, we showed that a variety of NO donor ester prodrugs of non-steroidal anti-inflammatory drugs (NSAIDs) are effectively cleaved by esterases to release the parent COX-2 inhibitory compound and NO.[10] Nitroxyl (HNO), the reduced form of NO, exhibits unique biological and pharmacological properties compared to other nitrogen oxides.[11] In the cardiovascular system, HNO exerts a positive inotropic cardiac effect that is independent from β-adrenergic signaling by directly enhancing cardiac sarcoplasmic reticulum Ca^{2+}

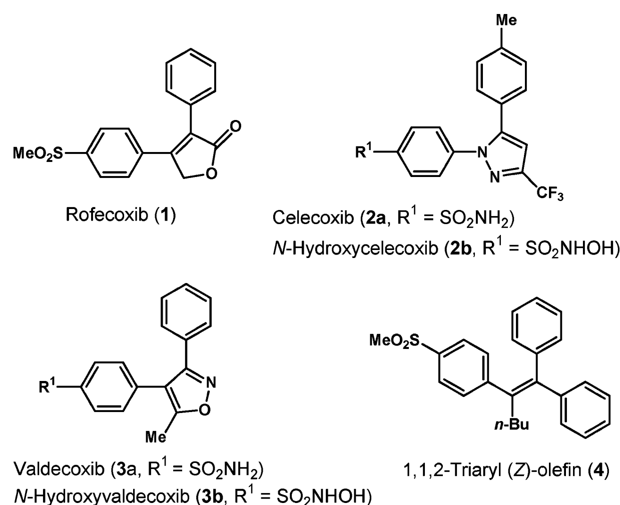

Fig. 1 Some representative selective cyclooxygenase-2 (COX-2) inhibitors (**1**, **2a**, **3a**), the sulfohydroxamic acid analog of celecoxib (**2b**), the sulfohydroxamic acid metabolite of valdecoxib (**3b**), and an acyclic selective COX-2 inhibitor (**4**) with potent anti-inflammatory activity.

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† Electronic supplementary information (ESI) available: Experimental procedures and spectroscopic data for compounds **6–12**, **16–19** and **21–29** described in the paper are given, and ^1H NMR scans for all compounds **6–12**, **13a–g**, **16–19** and **21–29**. See DOI: 10.1039/c005066k

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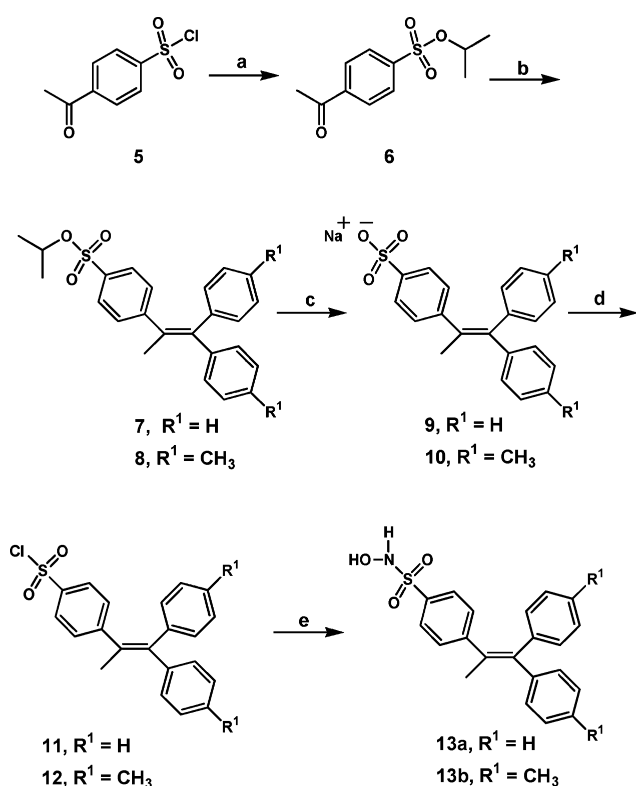
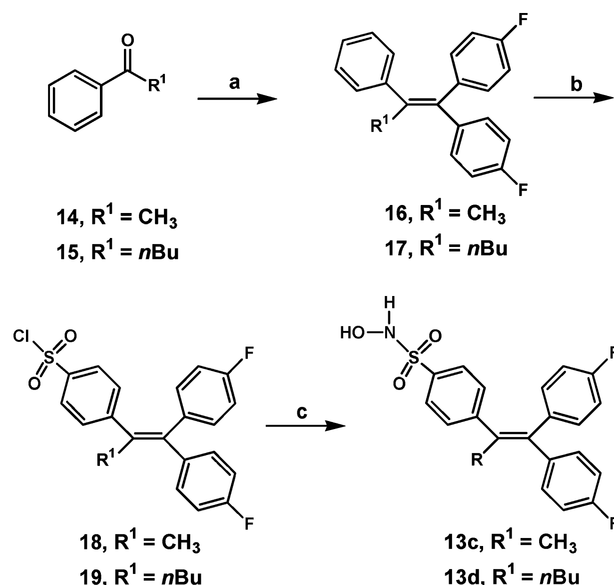
recycling.[11,12] HNO also protects heart tissue against ischemia-reperfusion injury,[13] it effectively inhibits human platelet aggregation in a rapid and concentration-dependent manner,[14] and it is resistant to superoxide radical anion.[11] Benzenesulfohydroxamic acid ($PhSO_2NHOH$), well known as Piloty's acid (PA), can serve as a HNO and/or NO donor.[15] In this regard, PA can decompose to HNO and sodium phenylsulfinate ($C_6H_5SO_2Na$) *via* either a base-catalyzed deprotonation mechanism[16] or by the release of NO and benzenesulfinic acid ($C_6H_5SO_2H$) upon oxidative decomposition by O_2, H_2O_2, $[Fe(CN)_3]^{3-}$ [17a] or by metal complexes.[17b]

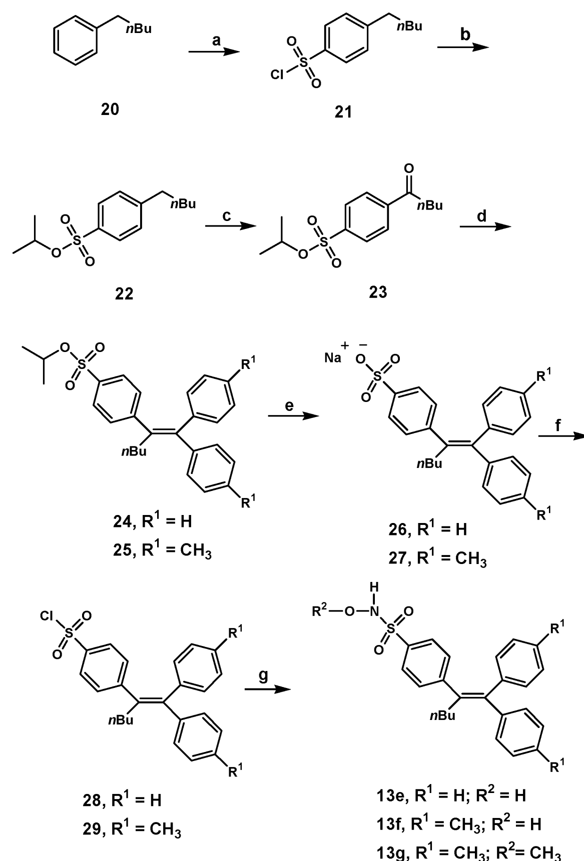
The design of selective COX-2 inhibitors that simultaneously act as NO/HNO donors, to the best of our knowledge, has not been reported. In a previous study we showed that 1,1-diphenyl-2-(4-methanesulfonylphenyl)hex-1-ene (**4**) was a highly potent and selective COX-2 inhibitor that exhibited potent anti-inflammatory activity.[4] It was therefore of interest to determine whether replacement of the $MeSO_2$ COX-2 pharmacophore present in the (*Z*)-olefin **4** by a SO_2NHOH moiety would provide a hitherto unknown class of compounds that act as dual NO and HNO donors. As part of this ongoing program, we now describe the synthesis of a group of acyclic 2-alkyl-1,1,2-triaryl (*Z*)-olefins possessing a *para*-$HONHSO_2$, or *para*-$MeONHSO_2$, substituent on the C2 phenyl ring (**13a–g**) as illustrated in Schemes 1–3, *in vitro* NO and HNO release and COX-1/COX-2 inhibition studies, and evaluation as anti-inflammatory agents.



Scheme 1 Reagents and conditions: (a) isopropanol, 4-dimethylaminopyridine (DMAP), dry CH_2Cl_2, 25 °C, 4 h; (b) benzophenone for compound **7**, 4,4′-dimethylbenzophenone for compound **8**, Zn, $TiCl_4$, dry THF, reflux, 2.5 h; (c) NaI, acetone, reflux, 16 h; (d) $SOCl_2$, DMF, 25 °C, 1 h; (e) $HONH_2 \cdot HCl$, MgO, THF–H_2O–MeOH = 30 : 2 : 3 (v/v/v), 25 °C, 3 h.



Scheme 2 Reagents and conditions: (a) 4,4′-difluorobenzophenone, Zn, $TiCl_4$, dry THF, reflux, 4.5 h; (b) $ClSO_3H$, $CHCl_3$, 25 °C, 2 h; (c) $HONH_2 \cdot HCl$, MgO, THF–H_2O–MeOH = 30 : 2 : 3 (v/v/v), 25 °C, 3 h.



Scheme 3 Reagents and conditions: (a) $ClSO_3H$, $CHCl_3$, 25 °C, 4 h; (b) isopropanol, DMAP, dry CH_2Cl_2, 25 °C, 4 h; (c) $KMnO_4$, $FeCl_3$, acetone, −78 °C for 2 h and then 25 °C for 2 h; (d) benzophenone for compound **24**, 4,4′-dimethylbenzophenone for compound **25**, Zn, $TiCl_4$, dry THF, reflux, 2.5 h; (e) NaI, acetone, reflux, 16 h; (f) $SOCl_2$, DMF, 25 °C, 1 h; (g) $HONH_2 \cdot HCl$ for **13e** and **13f**; $CH_3ONH_2 \cdot HCl$ aqueous solution for **13g**, MgO for **13e** and **13f**, K_2CO_3 for **13g**, THF–H_2O–MeOH = 30 : 2 : 3 (v/v/v), 25 °C, 3 h.

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Results and discussion

Chemistry

Reactants that possess a benzenesulfonyl chloride, or benzenesulfohydroxamic acid, moiety are not able to withstand reductive McMurry olefination reaction conditions. Therefore, an isopropyl protection strategy[18] involving the reaction of 4-acetylbenzenesulfonyl chloride (**5**) with isopropanol was used for the preparation of isopropyl 4-acetylbenzenesulfonate (**6**) that was required for the synthesis of the target products **13a** and **13b** (Scheme 1). The subsequent McMurry cross-coupling reaction[4] of **6** with either benzophenone, or 4,4′-dimethylbenzophenone, afforded the respective olefins **7** and **8** which upon reaction with sodium iodide in acetone at reflux furnished the corresponding sulfonic acid sodium salts **9** and **10**.[19] Treatment of **9** or **10** with thionyl chloride in DMF, provided the respective sulfonyl chloride **11** or **12**.[20] Subsequent reaction of the sulfonyl chlorides **11** and **12** with hydroxylamine hydrochloride in the presence of the mild base magnesium oxide[21] afforded the respective target products **13a** and **13b**.

A geometry optimized PM3 calculation[22] for 1,1-di-(4-fluorophenyl)-2-phenylhex-1-ene (**17**) showed that the electron density was highest at the *para*-position (–0.100) relative to the *ortho* (–0.091) and *meta* (–0.098) position of the C2 phenyl ring. Accordingly, electrophilic chlorosulfonation of the olefins **16** and **17** provided the expected benzenesulfonyl chlorides **18** and **19** as illustrated in Scheme 2. A similar PM3 calculation for 1,1-di-(4-methylphenyl)-2-phenylhex-1-ene also indicated that electron density was highest at the *para*-position (–0.062) relative to the *ortho* (–0.054) and *meta* (–0.061) position of the C2 phenyl ring. However, the chlorosulfonation reaction in this latter instance occurred on a 4′-methylphenyl ring rather than at the expected *para*-position of the C2 phenyl ring. Reaction of the sulfonyl chlorides **18** and **19** with hydroxylamine hydrochloride afforded the respective target product **13c** or **13d**.

Hex-1-enes **13e–g** were prepared using methodologies similar to those employed for the preparation of **13a** and **13b** (see Scheme 3). Thus, chlorosulfonation of amylbenzene (**20**) furnished the *para*-chlorosulfonation product **21** in good yield. Ferric chloride assisted permanganate benzylic oxidation[23] of the isopropyl sulfonate **22** afforded the ketone **23** in moderate yield. After completion of the McMurry cross-coupling reactions, olefins **24** and **25** were deprotected to give the respective sodium sulfonate salts **26** and **27**. Reaction of the sulfonyl chlorides **28** and **29**, prepared from the sodium sulfonate salts **26** and **27**, with hydroxylamine, or methoxyamine, hydrochloride afforded the respective target products **13e–f** or **13g**.

The target sulfohydroxamic acids (**13a–f**), unlike *N*-hydroxycelecoxib(**2b**, Fig. 1) which is unstable,[24] are stable products that are readily isolated and purified.

Drug design rationale

A novel group of acyclic triaryl (*Z*)-olefins possessing a small C2 alkyl substituent, such as 1,1-diphenyl-2-(4-methylsulfonylphenyl)hex-1-ene (**4**) that exhibited optimal COX-2 inhibitory potency (IC_{50} = 0.014 μM) and selectivity (SI > 7124), were recently reported by our group.[4] In this study, the *N*-hydroxy (methoxy) benzenesulfonamide analogues **13a–g** were designed based on the expectation that: i) a sulfohydroxamic moiety (SO_2NHOH) would act as a COX-2 pharmacophore similar to a $MeSO_2$ or H_2NSO_2 substituent at the *para*-position of a phenyl ring on a 5-membered heterocyclic ring template that confers potent and selective COX-2 inhibitory activity,[1–3] ii) the sulfohydroxamic acids **13a–f** will exhibit anti-inflammatory activity since *N*-hydroxyvaldecoxib (**3b**) is an active metabolite resulting from *N*-hydroxylation of valdecoxib (**3a**)[25] in mice[26] and humans,[27] and iii) the SO_2NHOH moiety will act as a NO/HNO donor to release NO and/or HNO under different conditions[15–17] which could circumvent the adverse vascular side effects of potent COX-2 inhibitors.[9,14]

Cyclooxygenase-1 (COX-1) and -2 enzyme inhibition

In vitro COX-1/COX-2 enzyme inhibition studies (Table 1) showed that the *N*-hydroxysulfonamides **13a–f** were weak inhibitors of COX-1 (IC_{50} = 6.0 to 53.7 μM range) and COX-2 (IC_{50} = 44.3 to 101 μM range) that resulted in low COX-2 selectivity indexes (SI < 1; COX-2 selectivity index = COX-1 IC_{50}/COX-2 IC_{50}). In contrast, the *N*-methoxybenzenesulfonamide analogue **13g** did not inhibit the COX-1 or COX-2 isozymes at the highest test compound concentration (100 μM). One could interpret this latter structure–activity observation as an indication that elaboration of the $MeSO_2$ moiety present in compound **4**, or the SO_2NHOH moiety present in **13f**, to a SO_2NHOMe moiety may not be adaptive to insertion into the COX-2 secondary pocket binding site.[4] The COX-2 SI for **13a–f** remained low (< 1) irrespective of their molecular volumes which spanned a range of 315–399 Å^3 (see data in Table 1). The observation that *N*-hydroxyvaldecoxib (**3b**, COX-1 IC_{50} = 96.2 μM; COX-2 IC_{50} = 1.1 μM; COX-2 SI = 88) is a much weaker and less selective COX-2 inhibitor than valdecoxib (**3a**, COX-1 IC_{50} = 157.2 μM; COX-2 IC_{50} = 0.04 μM; COX-2 SI = 3930), in conjunction with the fact that **3b** is a much more potent anti-inflammatory agent than **3a**, indicates that caution must be exercised in the interpretation of COX isozyme inhibitory data.[25]

Anti-inflammatory activity

The oral anti-inflammatory (AI) activities (% inhibition of inflammation for a 100 mg kg^{-1} oral dose or ED_{50} value) for the sulfohydroxamic acids **13a–g** were determined using a carrageenan-induced rat foot paw edema model (see data in Table 1). Within this group of compounds, **13f** (ED_{50} = 88 mg kg^{-1}) and **13e** (ED_{50} = 114 mg kg^{-1}), together with **13a**, **13c** and **13d** that showed a 49.0–55.6% inhibition of inflammation for a 100 mg kg^{-1} oral dose, exhibited relatively similar AI activities. Their moderately potent AI activity is greater than that of the reference drug aspirin, but less than that of the non-selective COX-2 inhibitor ibuprofen and the highly selective COX-2 inhibitors celecoxib and the acyclic (*Z*)-olefin **4**. The effect of the R^2-substituent on AI activity was variable. In the prop-1-ene group (**13a–c**; R^1 = Me), the relative AI potency order was F ≥ H > Me whereas, in the hex-1-ene group (**13d–f**, R^1 = *n*-Bu) the relative potency order was Me ≥ F > H. It should be noted that the *N*-methoxybenzenesulfonamide (SO_2NHOMe) compound **13g**, which showed no inhibition of COX-1 and COX-2 (IC_{50} > 100 μM), exhibited weak *in vivo* AI activity. One plausible explanation for this observation is that the SO_2NHOMe group in **13g** undergoes metabolic *O*-demethylation

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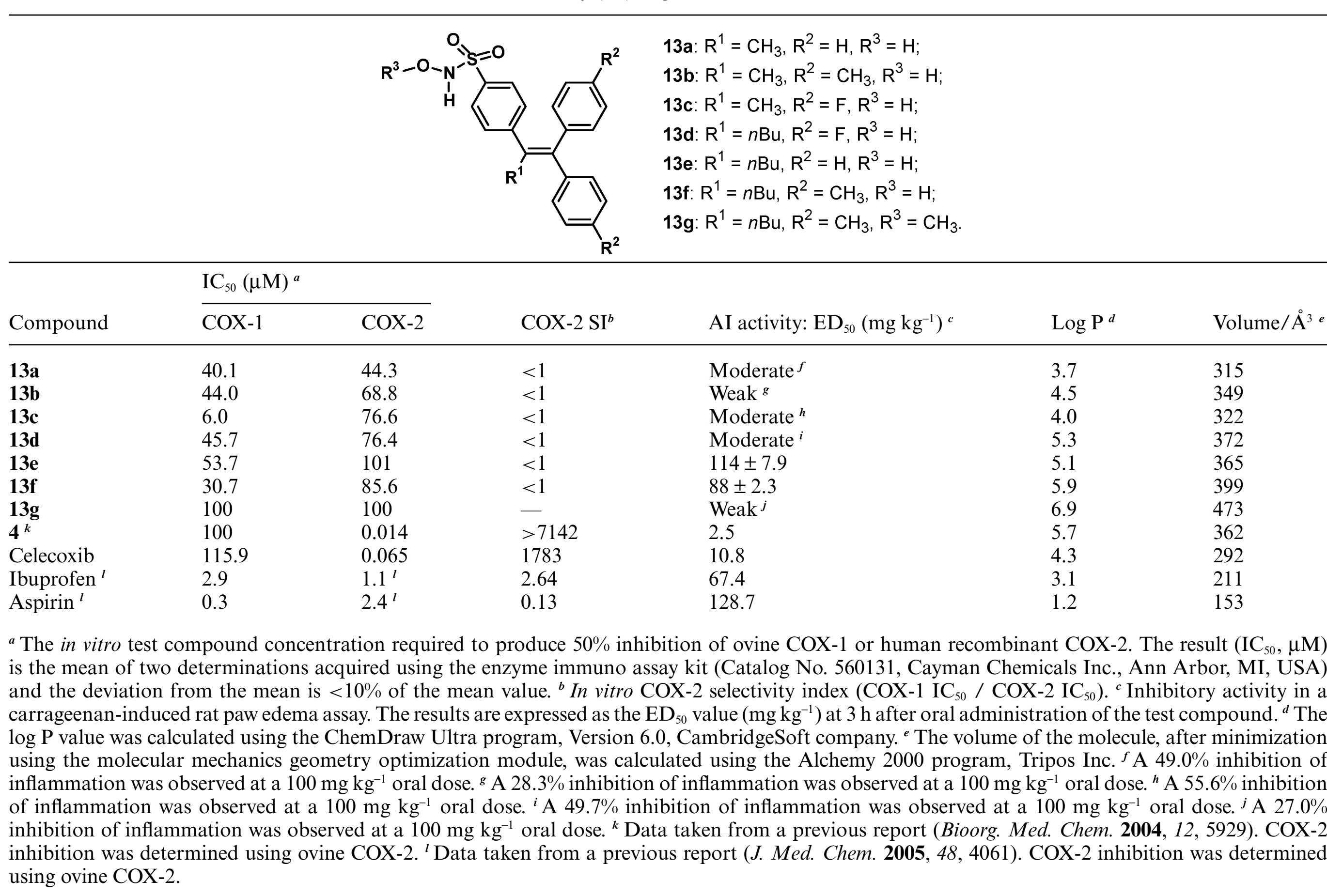
Table 1 *In vitro* COX-1 and COX-2 inhibition, anti-inflammatory (AI), log P and volume data



	IC_{50} (μM) [a]					
Compound	COX-1	COX-2	COX-2 SI[b]	AI activity: ED_{50} (mg kg^{-1}) [c]	Log P [d]	Volume/Å^3 [e]
13a	40.1	44.3	<1	Moderate [f]	3.7	315
13b	44.0	68.8	<1	Weak [g]	4.5	349
13c	6.0	76.6	<1	Moderate [h]	4.0	322
13d	45.7	76.4	<1	Moderate [i]	5.3	372
13e	53.7	101	<1	114 ± 7.9	5.1	365
13f	30.7	85.6	<1	88 ± 2.3	5.9	399
13g	100	100	—	Weak [j]	6.9	473
4 [k]	100	0.014	>7142	2.5	5.7	362
Celecoxib	115.9	0.065	1783	10.8	4.3	292
Ibuprofen [l]	2.9	1.1 [l]	2.64	67.4	3.1	211
Aspirin [l]	0.3	2.4 [l]	0.13	128.7	1.2	153

[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 (Catalog 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 ED_{50} value (mg kg^{-1}) at 3 h after oral administration of the test compound. [d] The log P value was calculated using the ChemDraw Ultra program, Version 6.0, CambridgeSoft company. [e] The volume of the molecule, after minimization using the molecular mechanics geometry optimization module, was calculated using the Alchemy 2000 program, Tripos Inc. [f] A 49.0% inhibition of inflammation was observed at a 100 mg kg^{-1} oral dose. [g] A 28.3% inhibition of inflammation was observed at a 100 mg kg^{-1} oral dose. [h] A 55.6% inhibition of inflammation was observed at a 100 mg kg^{-1} oral dose. [i] A 49.7% inhibition of inflammation was observed at a 100 mg kg^{-1} oral dose. [j] A 27.0% inhibition of inflammation was observed at a 100 mg kg^{-1} oral dose. [k] Data taken from a previous report (*Bioorg. Med. Chem.* **2004**, *12*, 5929). COX-2 inhibition was determined using ovine COX-2. [l] Data taken from a previous report (*J. Med. Chem.* **2005**, *48*, 4061). COX-2 inhibition was determined using ovine COX-2.

to a SO_2NHOH metabolite **13f** that exhibits AI activity (ED_{50} = 88 mg kg^{-1} po).

Nitric oxide release

The % NO released from the *N*-hydroxy (methoxy) benzenesulfonamide **13a–g** and **PA** upon incubation in i) phosphate-buffered-saline (PBS at pH 7.4), ii) PBS in the presence of an oxidizing agent, and iii) PBS containing rat serum were measured by quantitation of nitrite using the Griess reaction (see data in Table 2). The % NO released from the sulfohydroxamic acids **13a–f** in PBS at pH 7.4 varied over a 4.3–13.5% range which is indicative of slow NO release. In contrast, the oxidant effect of potassium hexacyanoferrate ($K_3[FeCN_6]$) on the NO release properties of **13a–g** was higher (4.4–25.6% range). This latter observation is consistent with reports that NO release from benzenesulfohydroxamic acid is facilitated by oxidants.[17a] The % NO released from the reference compound PA in PBS (59.7%), or in PBS containing the oxidant (58.4%), was much greater than that observed with **13a–g** in similar NO release experiments. Interestingly, the % NO release from **13a–g** and PA was suppressed (0.38–5.72%) in PBS containing rat serum. One plausible explanation for this observation, since NO is not expected to react with serum thiols,[11] could be due to the likely probability that the highly lipophilic (log P = 3.7–5.9 range; see data in Table 1) sulfohydroxamic acids **13a–f** undergo strong protein binding to rat serum which results in suppressed NO release.

Indirect assay of nitroxyl release (HNO) as nitrous oxide (N_2O)

Direct HNO detection continues to be a difficult analytical challenge. In this regard, gas chromatographic analysis can be used to detect, and determine the percentage of, HNO release indirectly by quantitation of nitrous oxide (N_2O) which arises from HNO dimerization and dehydration under anaerobic conditions (HNO + HNO → [HONNOH] → N_2O + H_2O).[28] Since PA decomposes to HNO in aqueous alkaline solution, and HNO reacts rapidly with thiols to form disulfides and hydroxylamine or sulfinamides,[29] HNO release from **13a–g** was measured using three MeOH-based solvent mixtures (see data in Table 2). In these solvent systems, the percentage of N_2O arising from **13a–f** and PA in MeOH/TBS was low (0–2% range). In contrast, in the presence of the base NaOH, the % N_2O produced was substantially larger (2–71% range). In comparison, the % N_2O produced in the MeOH/NaOH/GSH solvent system was much smaller (0–10% range) since addition of the thiol glutathione (GSH) reacts with nitroxyl (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 **13a–f** and the subsequent dimerization of the released HNO to N_2O.

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Table 2 Percent (%) nitric oxide and nitrous oxide release from **13a–g** and the reference compound Piloty's acid (PA)

	% NO release [a]			% N_2O release [e]					
				MeOH/TBS [f]		MeOH/Base [g]		MeOH/Base/GSH [h]	
	PBS [b]	PBS+Oxidant[c]	PBS+Serum [d]	2h	24h	2h	24h	2h	24h
13a	9.8	25.3	4.45	0	2	36	25	1 [i]	1 [i]
13b	11.4	17.6	3.0	0	0	29	17	1	10
13c	9.4	25.6	3.2	0	0	32	11	1 [j]	2 [j]
13d	5.6	10.7	3.0	0	0	40	34	5	4
13e	7.5	8.3	5.7	0	0	2	16	0	3
13f	13.5	13.5	2.2	0	0	36	37	5	5
13g	4.3	4.4	0.4	0	0	0	0	0	0
PA	59.7	58.4	3.2	0	0	71	31	0	7

[a] Percent of nitric oxide released based on a theoretical maximum release of 1 mol of NO/mol of the sulfohydroxamic test compound (**13a–g**) and the reference agent PA ($PhSO_2NHOH$). The result is the mean value of 3 measurements ($n = 3$) where variation from the mean % value was ≤ 0.2%. [b] A solution of the test compound (2.4 mL of a 5.0 x 10^{-2} mM) in phosphate buffer at pH 7.4 was incubated at 37 °C for 1.5 h. [c] A solution of the test compound (2.4 mL of a 5.0 x 10^{-2} mM) in phosphate buffer at pH 7.4 which contained 5.0 x 10^{-2} mM $K_3(FeCN_6)$, was incubated at 37 °C for 1.5 h. [d] A solution of the test compound (2.4 mL of a 5.0 x 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. [e] Percent of nitrous oxide (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 3 measurements ($n = 3$). The HNO donor test compound (**13a–g**, **PA**) concentration is 50 mM in each experiment unless otherwise noted. [f] 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. [g] MeOH/Base solvent is comprised of 0.6 mL MeOH and 0.2 mL of 1M NaOH. [h] MeOH/Base/GSH experiments are 100 mM in glutathione (GSH) unless otherwise noted. [i] Test compound concentration is 25 mM and GSH concentration is 50 mM. [j] Test compound concentration is 47 mM and GSH concentration is 94 mM.

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Conclusions

A group of 2-alkyl-1,1,2-triaryl olefins were synthesized in which a SO_2NHOR (R = H, Me) NO/HNO donor moiety was placed at the *para*-position on the C2 phenyl ring (**13a–g**). Biological studies showed that the SO_2NHOH group of compounds **13a–f** exhibit i) weak *in vitro* COX-1 and COX-2 inhibitory activity and show low COX-2 SI's, ii) moderate *in vivo* anti-inflammatory activity that is intermediate between that of the reference drugs aspirin and ibuprofen, (iii) a pharmacological acceptable stability in phosphate-buffered saline at pH 7 where NO release is in the 5.6–13.5% range, that NO release is increased in the presence of the oxidant potassium hexacyanoferrate (8.3–25.6% range), but NO release is decreased in the presence of rat serum (2.2–5.7% range), and iv) a low *in vitro* release of HNO in MeOH-buffer (< 2% at 24 h incubation), and a much higher HNO release at alkaline pH (11–37% range at 24 h) that is significantly reduced in the presence of GSH. The design of selective COX-2 inhibitors that contain either an effective HNO, or dual NO/HNO, donor moiety remains nowadays an important Medicinal Chemistry challenge that offers a novel and potential drug design concept for the development of anti-inflammatory drugs that are devoid of adverse ulcerogenic and/or cardiovascular side 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. ^1H 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. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70–230 mesh). All reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification. The *in vivo* anti-inflammatory assay was carried out using a protocol approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

Experimental chemical procedures and spectroscopic data for, and biological assays to evaluate, the target compounds **13a–g** are given below—full experimental procedures and spectroscopic data for the synthesis of compounds **6–12**, **16–19** and **21–29** described in the paper are given in the supporting information.

1,1-Diphenyl-2-(4-hydroxyaminosulfonylphenyl)prop-1-ene (13a). Magnesium oxide (0.263 g, 6.58 mmol) was added to a solution of hydroxylamine hydrochloride (0.200 g, 2.87 mmol) in THF–H_2O–MeOH (30 mL:2 mL:3 mL, v/v/v) prior to the addition of 1,1-diphenyl-2-(4-chlorosulfonylphenyl)prop-1-ene (**11**, 0.300 g, 0.823 mmol). This 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 dried ($MgSO_4$). Removal of the solvent *in vacuo* gave a residue that was purification by flash silica gel column chromatography using n-hexane–EtOAc (2 : 1, v/v) as eluent to afford the title compound **13a** (203 mg, 67.7%) as a white solid, mp 151–153 °C; IR (film): 3411, 3230, 1329, 1168 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.07 (s, 3H, CH_3C=C), 6.87–6.90 (m, 2H, phenyl hydrogens), 7.04–7.11 (m, 3H, phenyl hydrogens), 7.22–7.42 (m, 7H, phenyl hydrogens and sulfonylphenyl H-2, H-6), 7.61 (d, *J* = 7.9 Hz, 2H, sulfonylphenyl H-3, H-5), 9.53 and 9.56 (two d, *J* = 3.7 and 3.1 Hz, respectively, 1H each, *HO*–N*H*). Anal. Calcd for $C_{21}H_{19}NO_3S$: C, 69.02; H, 5.24; N, 3.83. Found: C, 69.01; H, 5.34; N, 3.80.

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The physical and spectral data for **13b–g**, which were prepared using similar methodologies, are listed below.

1,1-Di-(4-methylphenyl)-2-(4-hydroxyaminosulfonylphenyl)prop-1-ene (13b). Yield, 71.2%; white solid; mp 153–154 °C; IR (film): 3411, 3233, 1329, 1172 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.08 (s, 3H, CH_3C═C), 2.16 (s, 3H, ArCH_3), 2.32 (s, 3H, ArCH_3), 6.75 and 6.90 (two d, J = 7.9 Hz, 2H each, tolyl H-3, H-5), 7.10 and 7.20 (two d, J = 7.9 Hz, 2H each, tolyl H-2, H-6), 7.38 (d, J = 7.9 Hz, 2H, sulfonylphenyl H-2, H-6), 7.63 (d, J = 7.9 Hz, 2H, sulfonylphenyl H-3, H-5), 9.54 and 9.58 (two d, J = 3.1 and 3.7 Hz, respectively, 1H each, HO–NH); Anal. Calcd for $C_{23}H_{23}NO_3S$: C, 69.84; H, 5.89; N, 3.54. Found: C, 70.00; H, 6.28; N, 3.57.

1,1-Di(4-fluorophenyl)-2-(4-hydroxyaminosulfonylphenyl)prop-1-ene (13c). Yield, 56.6%; white solid; mp 170–171 °C; IR (film): 3410, 3237, 1229, 1173 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.07 (s, 3H, CH_3C═C), 6.86–6.97 (m, 4H, two 4-fluorophenyl H-3, H-5), 7.19–7.32 (m, 4H, two 4-fluorophenyl H-2, H-6), 7.37 (d, J = 7.9 Hz, 2H, sulfonylphenyl H-2, H-6), 7.63 (d, J = 7.9 Hz, 2H, sulfonylphenyl H-3, H-5), 9.53 and 9.57 (two d, J = 3.1 Hz, 1H each, HO–NH). Anal. Calcd for $C_{21}H_{17}F_2NO_3S$: C, 62.83; H, 4.27; N, 3.49. Found: C, 62.74; H, 4.34; N, 3.50.

1,1-Di-(4-fluorophenyl)-2-(4-hydroxyaminosulfonylphenyl)hex-1-ene (13d). Yield, 62.3%; white solid; mp 143–145 °C; IR (film): 3418, 3240, 2965, 2929, 2858, 1507, 1226, 1170 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.71 (t, J = 7.3 Hz, 3H, $CH_2CH_2CH_2$$CH_3$), 1.13–1.22 (m, 4H, $CH_2$$CH_2CH_2$$CH_3$), 2.35 (t, J = 6.7 Hz, 2H, CH_2C═C), 6.80–7.00 (m, 4H, two 4-fluorophenyl H-3, H-5), 7.20–7.32 (m, 4H, two 4-fluorophenyl H-2, H-6), 7.35 (d, J = 8.5 Hz, 2H, sulfonylphenyl H-2, H-6), 7.64 (d, J = 7.9 Hz, 2H, sulfonylphenyl H-3, H-5), 9.55 and 9.59 (two d, J = 3.7 and 3.1 Hz, respectively, 1H each, HO–NH); Anal. Calcd for $C_{24}H_{23}F_2NO_3S$: C, 64.99; H, 5.23; N, 3.16. Found: C, 65.25; H, 5.33; N, 3.12.

1,1-Diphenyl-2-(4-hydroxyaminosulfonylphenyl)hex-1-ene (13e). Yield, 80.3%; white solid; mp 150–152°; IR (film): 3401, 3243, 2964, 2923, 2857, 1325, 1172 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.67 (t, J = 6.7 Hz, 3H, $CH_2CH_2CH_2$$CH_3$), 1.12–1.19 (m, 4H, $CH_2$$CH_2CH_2$$CH_3$), 2.40 (t, J = 7.4 Hz, 2H, CH_2C═C), 6.89 (d, J = 6.7 Hz, 2H, phenyl hydrogens), 7.00–7.10 (m, 3H, phenyl hydrogens), 7.23–7.43 (m, 7H, phenyl hydrogens and sulfonylphenyl H-2, H-6), 7.62 (d, J = 7.9 Hz, 2H, sulfonylphenyl H-3, H-5), 9.55 and 9.59 (two d, J = 3.7 and 3.1 Hz, respectively, 1H each, HO–NH). Anal. Calcd for $C_{24}H_{25}NO_3S$: C, 70.73; H, 6.18; N, 3.44. Found: C, 70.36; H, 6.22; N, 3.32.

1,1-Di-(4-methylphenyl)-2-(4-hydroxyaminosulfonylphenyl)hex-1-ene (13f). Yield, 78.8%; yellowish solid; mp 153–154 °C; IR (film): 3407, 3240, 2957, 2921, 2866, 1327, 1170 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.72 (t, J = 6.7 Hz, 3H, $CH_2CH_2CH_2$$CH_3$), 1.17–1.18 (m, 4H, $CH_2$$CH_2CH_2$$CH_3$), 2.14 (s, 3H, Ar$CH_3$), 2.31 (s, 3H, Ar$CH_3$), 2.40 (t, J = 7.4 Hz, 2H, CH_2C═C), 6.73 and 6.87 (two d, J = 7.9 Hz, 2H each, tolyl H-3, H-5), 7.08 and 7.19 (two d, J = 7.9 Hz, 2H each, tolyl H-2, H-6), 7.35 (d, J = 7.9 Hz, 2H, sulfonylphenyl H-2, H-6), 7.63 (d, J = 7.9 Hz, 2H, sulfonylphenyl H-3, H-5), 9.55 and 9.59 (two d, J = 3.1 Hz, 1H each, HO–NH). Anal. Calcd for $C_{26}H_{29}NO_3S$: C, 71.69; H, 6.71; N, 3.22. Found: C, 71.89; H, 6.78; N, 3.23.

1,1-Di-(4-methylphenyl)-2-(4-methoxyaminosulfonylphenyl)hex-1-ene (13g). Reaction of the sulfonyl chloride **29** with methoxylamine hydrochloride that was neutralized with an aqueous solution of K_2CO_3 yielded the title compound **13g** as a white solid (65.1%); mp 138–140 °C; IR (film): 3235, 2962, 2921, 2868, 1342, 1175 cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.71 (t, J = 6.7 Hz, 3H, $CH_2CH_2CH_2$$CH_3$), 1.11–1.17 (m, 4H, $CH_2$$CH_2CH_2$$CH_3$), 2.13 (s, 3H, Ar$CH_3$), 2.30 (s, 3H, Ar$CH_3$), 2.39 (t, J = 6.7 Hz, 2H, CH_2C═C), 3.60 (s, 3H, OCH_3), 6.71 and 6.85 (two d, J = 8.6 and 7.9 Hz, respectively, 2H each, tolyl H-3, H-5), 7.07 and 7.18 (two d, J = 7.9 Hz, 2H each, tolyl H-2, H-6), 7.36 (d, J = 7.9 Hz, 2H, sulfonylphenyl H-2, H-6), 7.63 (d, J = 7.9 Hz, 2H, sulfonylphenyl H-3, H-5), 10.5 (s, 1H, CH_3O–NH). Anal. Calcd for $C_{27}H_{31}NO_3S$: C, 72.13; H, 6.95; N, 3.12. Found: C, 72.33; H, 7.03; N, 3.16.

In vitro cyclooxygenase inhibition assays

The ability of the test compounds **13a–g** listed in Table 1 to inhibit ovine COX-1 and human recombinant COX-2 (IC_{50} value, μM) was determined using an enzyme immuno assay (EIA) kit (catalog number 560131, Cayman Chemical, Ann Arbor, MI, USA) according to a previously reported method.[30]

In vivo anti-inflammatory assay

Anti-inflammatory activity was measured using a carrageenan-induced rat paw edema assay described by Winter *et al.*[31] Briefly, three male Sprague Dawley rats weighing 160–180g were used in each group. Test compounds suspended in water containing 1% methyl cellulose, were administered orally at different doses (50–150 mg kg^{-1} 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 (Ser. No 43201) for calculation of % inhibition of inflammation.

In vitro 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, ii) PBS containing 50 μM $K_3(FeCN_6)$ at pH 7.4 and 37 °C, or iii) PBS at pH 7.4 and 37 °C to which 90 μL 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 (**13a–g**) and the reference compound Piloty's acid (PA) using the reported procedures.[32]

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) were injected at 2 and 24 h onto a 7890A Agilent Technologies Gas Chromatograph equipped with a thermal conductivity detector and a 6′ x 1/8” Porapack Q column. The oven operated at 40 °C for 5 min was then ramped to 150 °C

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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^{-1} and a septum purge flow of 3 mL min^{-1} was held at 140 °C. The back detector with a reference flow of 9 mL min^{-1} and a make-up flow of 6 mL min^{-1} was held at 150 °C. The retention time of nitrous oxide was 2.5 min, and yields were calculated based on a standard curve for nitrous oxide (Matheson Tri-Gas).

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