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Diazen-1-ium-1,2-diolated and nitrooxyethyl nitric oxide donor ester prodrugs of anti-inflammatory (*E*)-2-(aryl)-3-(4-methanesulfonylphenyl)acrylic acids: Synthesis, cyclooxygenase inhibition, and nitric oxide release studies

Khaled R. A. Abdellatif, Morshed Alam Chowdhury, Ying Dong, Qiao-Hong Chen and Edward E. Knaus*

Dentistry Pharmacy Centre, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, 3-118 Edmonton, Alta., Canada T6G 2N8

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Abstract—A new group of hybrid nitric oxide-releasing anti-inflammatory drugs wherein an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**11a–d**), or 2-nitrooxyethyl (**12a–d**), ·NO-donor moiety is attached directly to the carboxylic acid group of (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids were synthesized. The 2-nitrooxyethyl ester prodrugs (**12a–d**) all exhibited in vitro inhibitory activity against the cyclooxygenase-2 (COX-2) isozyme (IC_{50} = 0.07–2.8 μM range). All compounds released a low amount of ·NO upon incubation with phosphate buffer (PBS) at pH 7.4 (1.0–4.8% range). In comparison, the percentage ·NO released was significantly higher (76.2–83.0% range) when the diazen-1-ium-1,2-diolate ester prodrugs were incubated in the presence of rat serum, or moderately higher (7.6–10.1% range) when the nitrooxyethyl ester prodrugs were incubated in the presence of L-cysteine. These incubation studies suggest that both ·NO and the parent anti-inflammatory (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acid would be released upon in vivo cleavage by non-specific *serum* esterases in the case of the diazen-1-ium-1,2-diolate esters (**11a–d**), or interaction with systemic thiols in the case of the nitrate esters (**12a–d**). O^2-Acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (*E*)-3-(4-methanesulfonylphenyl)-2-phenylacrylate (**11a**) released 83% of the theoretical maximal release of 2 molecules of ·NO/molecule of the parent hybrid ester prodrug upon incubation with rat serum. Hybrid ester anti-inflammatory/·NO donor prodrugs offer a potential drug design concept targeted toward the development of anti-inflammatory drugs that are devoid of adverse ulcerogenic and/or cardiovascular effects.

1. Introduction

Inhibition of cyclooxygenase-derived prostaglandin synthesis, the major mechanism by which non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory effect, is also responsible for adverse gastroduodenal erosions and ulcerations with an incidence that is near 15% in chronic NSAID users.[1] Nitric oxide (·NO) is also a beneficial mediator of gastrointestinal mucosal protection that induces many actions similar to prostaglandins in the gastrointestinal tract.[2] In this respect, ·NO has been shown to reduce the severity of gastric injury in experimental models.[3,4] A biologically relevant class of O^2-unsubstituted *N*-diazen-1-ium-1,2-diolates (NONOates) have the potential to release ·NO (first-order kinetics) without metabolic activation, possess structural diversity that allows controllable rates of ·NO-release, and are amenable to facile derivatization chemistry that facilitates targeting of ·NO to specific organ and/or tissue sites.[5] The concept[6] that linking an ·NO-releasing moiety to a NSAID may reduce the gastrointestinal toxicity of the latter has been validated by in vivo ulcerogenicity studies. Accordingly, we showed that a novel group of hybrid NO-releasing NONO-NSAID ester prodrugs possessing a 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate or 1-(*N*,*N*-dimethylamino)-diazen-1-ium-1,2-diolate moiety attached via a one-carbon spacer, or an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate moiety attached directly to the carboxylic acid group of aspirin

Keywords: Anti-inflammatory acrylic ester prodrugs; Cyclooxygenase-1 and -2 inhibition; Diazen-1-ium-1,2-diolate and nitrooxyethyl nitric oxide donors.
* Corresponding author. Tel.: +1 780 492 5993; fax: +1 780 492 1217; e-mail: eknaus@pharmacy.ualberta.ca

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(**1a**–**c**), ibuprofen (**2a**–**c**) and indomethacin (**3a**–**c**) showed approximately equipotent anti-inflammatory activity to the parent NSAID, extensive in vitro ester cleavage by nonspecific serum esterase, and no (aspirin and ibuprofen), or minimal (indomethacin), in vivo ulcerogenicity[7,8] (see structures in Fig. 1). Animal studies carried out by others have shown that nitrate-based NO-NSAIDs such as NO-naproxen (**4**),[9] NO-flurbiprofen (**5**),[10,11] NO-diclofenac (**6**),[12] and NO-aspirin (**7**)[13] are gastrointestinal sparing while simultaneously suppressing prostaglandin synthesis similar to the parent drugs.[14–16]

A novel class of acyclic (*E*)-2-(phenyl)-3-(4-methanesulfonylphenyl)acrylic acids (**8**) that exhibit COX isozyme and/or 5-lipoxygenase (5-LOX) inhibitory activities were reported recently.[17] We now present a group of hybrid ester prodrugs in which (i) an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**11a**–**d**), or (ii) 2-nitrooxyethyl (**12a**–**d**), ·NO-donor moiety is attached directly to the carboxylic acid group of a select group of (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids (**8**, R = 4-H, 4-OMe, 4-F, 4-Br). It is expected that these hybrid ·NO-donor prodrugs will be devoid of adverse ulcerogenic and cardiovascular effects based on their ability to release cytoprotective and vasodilatory ·NO.

2. Chemistry

A group of (*E*)-2-(phenyl)-3-(4-methanesulfonylphenyl) acrylate esters possessing an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate ester moiety (**11a**–**d**) were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, nucleophilic displacement of the mesyloxy group present in the mesylate **9** by the respective sodium salt of the acrylic acid (**8a**–**d**) in hexamethylphosphoramide (HMPA) afforded the target product (**11a**–**d**) in moderate yield (32–48%). The corresponding 2-nitrooxyethyl acrylate esters **12a**–**d** were prepared in higher yields (76–94%) by the K_2CO_3 mediated coupling of the acrylic acids (**8a**–**d**) with 2-nitrooxyethyl bromide (**10**) in DMF.

3. Results and discussion

A group of acyclic (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids that exhibited interesting combinations of COX-1/COX-2 and/or 5-LOX/15-LOX isozyme inhibitory activities were recently reported.[17] Four compounds from within this group, having a 4-H, 4-OMe, 4-F or 4-Br substituent on the C-2 phenyl ring, were selected for elaboration to hybrid ester prodrugs wherein an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**11a**–**d**), or

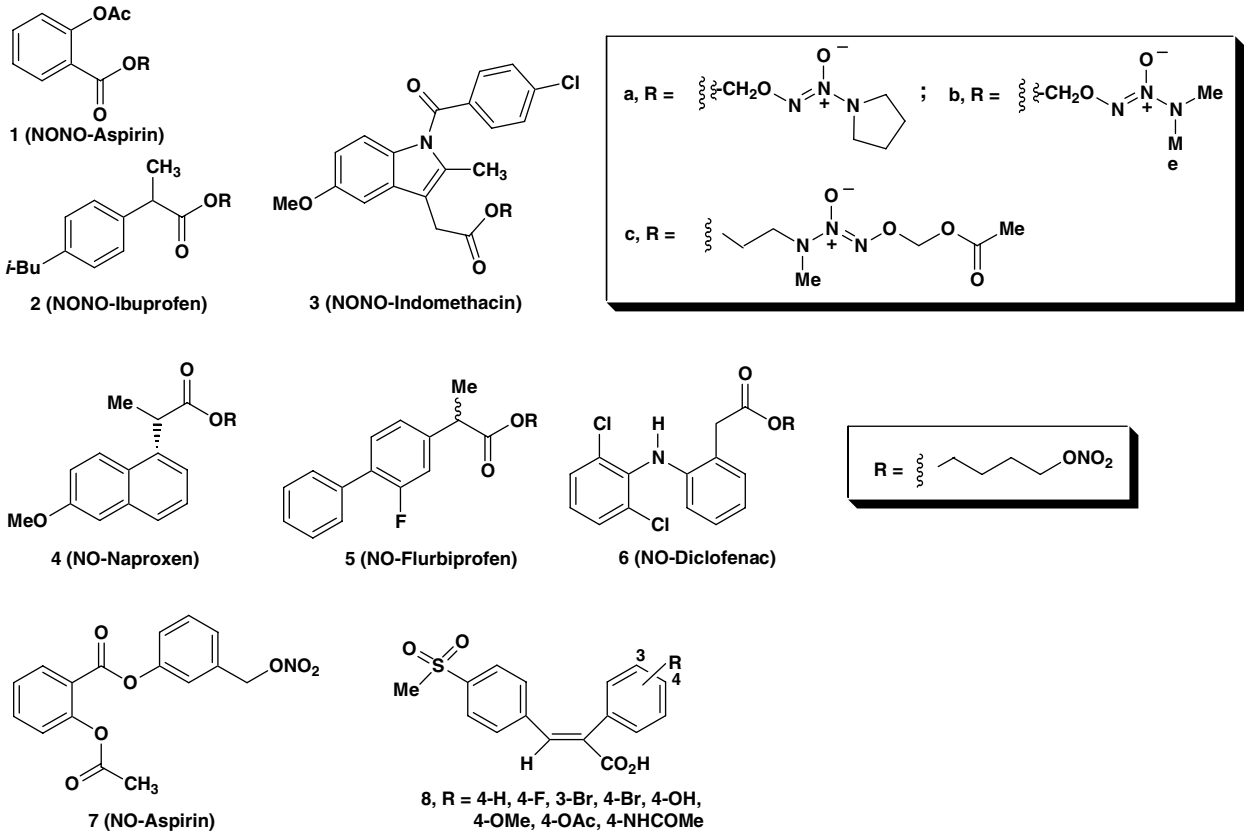
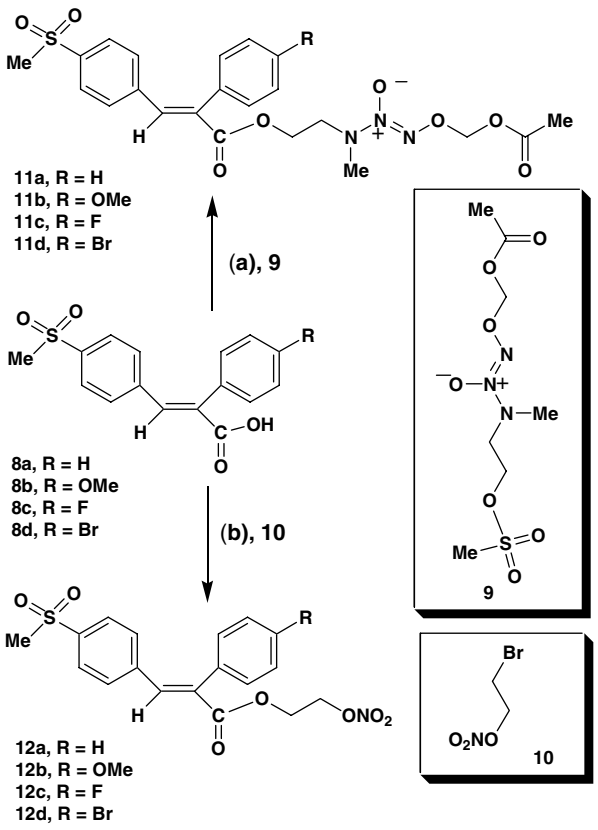


Figure 1. Chemical structures of some diazen-1-ium-1,2-diolate ester prodrugs of aspirin (**1a**–**c**), ibuprofen (**2a**–**c**) and indomethacin (**3a**–**c**), some nitrooxybutyl ester prodrugs of naproxen (**4**), flurbiprofen (**5**) and diclofenac (**6**), the 3-(nitrooxymethylphenyl) ester of aspirin (**7**), and (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids (**8**).



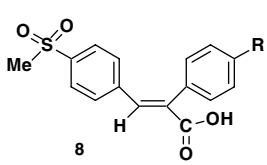
Scheme 1. Reagents and conditions: (a) Na_2CO_3, hexamethylphosphoramide (HMPA), 25 °C, 72 h; (b) K_2CO_3, dimethylformamide (DMF), 25 °C, 36 h.

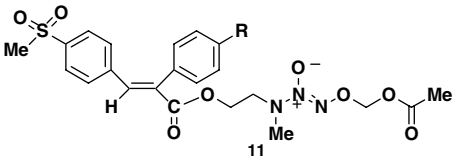
2-nitrooxyethyl (**12a–d**), ·NO-donor moiety is attached directly to the carboxylic acid group of the anti-inflammatory compound (**8a–d**). In vitro structure-activity relationships acquired for the hybrid nitrooxy ester prodrugs (**12a–d**) showed that they exhibited medium-to-high COX-1 (IC_{50} = 0.37–6.2 μM range), and COX-2 (IC_{50} = 0.07–2.8 μM range), inhibitory activities (see data in Table 1). The OMe (**12b**) and F (**12c**) analogs were non-selective COX-2 inhibitors, whereas the H (**12a**) and Br (**12d**) analogs showed COX-2 selectivity indexes of 34.4 and 5.3, respectively. Esterase cleavage of the acrylic acid ester moiety in the ester prodrugs (**11a–d**) would liberate the parent acrylic acids (**8a–d**) for which the COX-1 and COX-2 inhibitory data previously acquired[17] are listed in Table 1.

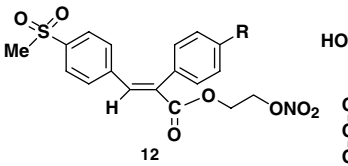
The rate of ·NO release from diazen-1-ium-1,2-diolates can be decreased by chemical modification involving the attachment of alkyl substituents to the O^2-position.[18] Thus, O^2-substituted-diazen-1-ium-1,2-diolates are stable compounds that hydrolyze slowly even in acidic solution.[19] The percent ·NO released from the hybrid O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate ester prodrugs (**11a–d**) upon incubation in phosphate-buffered-saline (PBS at pH 7.4), and in the presence of rat serum, was determined by quantitation of nitrite using the Griess reaction (see data in Table 1). In this regard, the percentage of ·NO released upon incubation of **11a–d** in PBS at pH 7.4 varied over a narrow 3.5–4.8% range which is indicative of slow ·NO release.[20] In contrast, the effect of non-specific esterases present in rat serum on the ·NO release properties of compounds **11a–d** was substantially higher (76.2–83.0% range). These data indicate the non-specific serum esterases present in rat serum cleave these hybrid prodrug esters more effectively than PBS at pH 7.4. From a mechanistic perspective, the hybrid ester prodrugs **11a–d** cannot release ·NO prior to cleavage of the terminal O^2-acetoxymethyl ester group. This requirement is consistent with the observation that O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**13**), which does not possess an ester group that requires prior ester cleavage, releases 84.9% and 85% of the theoretical maximal release of two molecules of ·NO/molecule of the parent NO donor in PBS and serum, respectively. A plausible mechanism for the ester hydrolysis of hybrid ester prodrugs containing an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate ester moiety, and the subsequent release of two molecules of ·NO, was described in an earlier study.[8] The hybrid ester ·NO-donor prodrugs **11a–d** were designed with a one-carbon methylene spacer between the terminal acetoxy group and the diazen-1-ium-1,2-diolate O^2-atom, such that the O^2-(hydroxymethyl)diazen-1-ium-1,2-diolate compound formed after terminal acetoxy cleavage would spontaneously eliminate formaldehyde to produce the free diazen-1-ium-1,2-diolate compound (NONOate) that can subsequently fragment to release two molecules of ·NO (NONO-donor). Comparative studies showed that the extent of ·NO release upon incubation of the hybrid nitrooxyethyl ester prodrugs (**12a–d**) in PBS at a pH of 7 was lower (1.0–3.3% range) than the corresponding O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate ester prodrugs (**11a–d**, 3.5–4.8% range). The percentage of ·NO released from the nitrooxyethyl compounds **12a–d** was greater in the presence of 5 mM L-cysteine (7.6–10.1% range) than in the absence of L-cysteine. This latter observation is consistent with reports that ·NO release from organic nitrates is facilitated by thiols.[21,22]

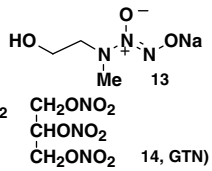
Two disadvantages of ·NO-releasing NSAIDs having a nitrooxyalkyl group are (i) that production of ·NO from organic nitrate esters requires a three-electron reduction, and (ii) this metabolic activation decreases in efficiency on continued use of the drugs contributing to 'nitrate tolerance'.[23] In contrast, O^2-unsubstitued *N*-diazen-1-ium-1,2-diolates have the potential to release up to 2 equivalents of ·NO with half-lives that correlate well with their pharmacological durations of action. In earlier studies, we validated the drug design concept that hybrid NSAID ester prodrugs (NSAID-NONOates) possessing a diazen-1-ium-1,2-diolate ·NO donor moiety constitute a highly effective method to abolish NSAID induced gastrointestinal toxicity (ulcerogencity).[7,8] It is equally probable that incorporation of a vasodilator diazen-1-ium-1,2-diolate NO donor moiety into selective COX-2 inhibitors such as rofecoxib and/or valdecoxib may provide a therapeutic approach to counteract the adverse cardiovascular effects that led to their withdrawal from the clinical market.[24,25]

Table 1. In vitro COX-1 and COX-2 inhibition, and percent (%) nitric oxide release, data for acrylic acids (**8a–d**), diazeniumdiolate acrylate esters (**11a–d**), nitrooxyethyl acrylate esters (**12a–d**), O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**13**), and glycerol trinitrate (**14**)









Compound	R	IC_{50} (μM)[a]		COX-2 S.I.[b]	% ·NO released[c]		
		COX-1	COX-2		PBS[d]	Serum[e]	L-Cysteine[f]
8a	H	1.5[g]	3.0[g]	0.50[g]	—	—	—
8b	OMe	31.6[g]	1.9[g]	16.6[g]	—	—	—
8c	F	0.4[g]	36.0[g]	—	—	—	—
8d	Br	0.88[g]	3.6[g]	0.24[g]		—	—
11a	H	—	—	—	4.8	83.0	—
11b	OMe	—	—	—	3.9	77.6	—
11c	F	—	—	—	3.6	82.1	—
11d	Br	—	—	—	3.5	76.2	—
12a	H	6.2	0.18	34.4	3.0	—	8.5
12b	OMe	1.1	1.4	0.78	1.4	—	9.2
12c	F	1.6	2.8	0.57	3.3	—	7.6
12d	Br	0.37	0.07	5.3	1.0	—	10.1
13	—	—	—	—	84.9	85.0	—
14 (GTN)	—	—	—	—	2.8	—	10.1
Aspirin	—	0.35	2.4	0.15	—	—	—
Celecoxib	—	33.1	0.07	473	—	—	—
Rofecoxib	—	>100	0.50	>200	—	—	—

[a] Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit (Catalog No. 560101, 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] Percent of nitric oxide released based on a theoretical maximum release of (i) 2 mol of NO/mol of the diazen-1-ium-1,2-diolate test compounds (**11a–d**, **13**), (ii) 1 mol of NO/mol of the nitrooxyethyl test compounds (**12a–d**), and (iii) 3 mol of NO/mol of glycerol trinitrate (**14**). The result is the mean value of 3 measurements ($n = 3$) where variation from the mean % value was $\leqslant$0.2%.

[d] A solution of the test compound (2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer at pH 7.4) was incubated at 37 °C for 1.5 h.

[e] A solution of the test compound (2.4 mL of a 1.0×10^{-2} mM solution 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.

[f] A solution of the test compound (2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer at pH 7.4 which contained 5.0 mM L-cysteine) was incubated at 37 °C for 1.5 h.

[g] Data taken from the literature.[17]

4. Conclusions

A group of hybrid ester prodrugs in which an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**11a–d**), or 2-nitrooxyethyl (**12a–d**), NO-donor moiety is attached directly to the carboxylic acid group of (*E*)-3-(4-methanesulfonylphenyl)-2-(4-H, 4-OMe, 4-F or 4-Br substituted-phenyl)acrylic acids were synthesized for comparative biological evaluation. Structure-activity and biological stability studies showed that (i) the nitrooxyethyl ester prodrugs (**12a–d**) retain in vitro COX-1 and COX-2 inhibitory activity, (ii) both classes of prodrugs (**11a–d**, **12a–d**) are relatively stable in phosphate-buffered saline at pH 7 where ·NO release is in the 1.0–4.8 % range, and specifically (iii) the O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolates (**11a–d**) undergo extensive ester cleavage by rat serum esterase(s) that is followed by a significant release of ·NO in the 76.2–83.0 % range, (iv) L-cysteine moderately enhances the release of NO (7.6–10.1% range) from the nitrooxyethyl esters (**12a–d**) that requires a metabolically demanding three-electron reduction for the release of NO, and (v) hybrid ester ·NO donor prodrugs offer a potential drug design concept for the development of NSAIDs that are devoid of adverse ulcerogenic and/or cardiovascular side effects.

5. Experimental

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 in $CDCl_3$ 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 other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification. The (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids (**8a**–**d**, R = 4-H, 4-OMe, 4-F, 4-Br),[12] O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino]-diazen-1-ium-1,2-diolate (**9**),[26] 2-bromoethylnitrate (**10**)[22], O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**13**),[26] and glycerol trinitrate (**14**)[27] were prepared according to literature procedures. Nitric oxide gas was purchased from BOC Scientific (Burlington, Ontario).

5.1. General method for preparation of the O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate acrylate esters (11a–d)

Sodium carboxylates of the respective acrylic acids **8a**–**d** (R = H, OMe, F, Br) were prepared in situ by stirring each acid (2.5 mmol) in a suspension of sodium carbonate (0.27 g, 2.5 mmol) and HMPA (3.5 mL) for 19 h at 25 °C. A solution of O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**9**, 2.5 mmol) in HMPA (1.5 mL) was then added, and the reaction was allowed to proceed for 72 h at 25 °C. EtOAc (30 mL) was added, the mixture was washed with water (5 × 15 mL), the organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography using EtOAc/hexane (2:1, v/v) as the eluent. Physical and spectral data for **11a**–**d** are listed below.

5.1.1. O^2-Acetoxymethyl-1-(*N*-ethyl-*N*- methylamino)diazen-1-ium-1,2-diolate (*E*)-3-(4-methanesulfonylphenyl)-2-phenylacrylate (11a). Yield, 48%; white powder; mp 94–96 °C; IR (film) 2972 (C–H aromatic), 2930 (C–H aliphatic), 1753, 1709 (CO_2), 1369, 1151 (SO_2), 1237, 1065 (N═N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H, $COCH_3$), 2.94 (s, 3H, NCH_3), 3.01 (s, 3H, CH_3SO_2), 3.72 (t, J = 5.2 Hz, 2H, CH_2N), 4.40 (t, J = 5.2 Hz, 2H, CO_2CH_2), 5.76 (s, 2H, OCH_2O), 7.17–7.27 (m, 3H, phenyl, H-3, H-4, H-5), 7.30 (d, J = 8.3 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.39–7.41 (m, 2H, phenyl H-2, H-6), 7.75 (d, J = 8.3 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.85 (s, 1H, H-3); MS 513.90 (M+Na). Anal. Calcd for $C_{22}H_{25}N_3O_8S$: C, 53.76; H, 5.13; N, 8.55. Found: C, 53.67; H, 5.23; N, 8.43.

5.1.2. O^2-Acetoxymethyl-1-(*N*-ethyl-*N*- methylamino)diazen-1-ium-1,2-diolate (*E*)-3-(4-methanesulfonylphenyl)-2-(4-methoxyphenyl)acrylate (11b). Yield, 32%; yellow gum; IR (film) 2970 (C–H aromatic), 2927 (C–H aliphatic), 1733, 1712 (CO_2), 1372, 1154 (SO_2), 1254, 1069 (N═N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H, $COCH_3$), 3.01 (s, 3H, NCH_3), 3.03 (s, 3H, CH_3SO_2), 3.74 (t, J = 5.2 Hz, 2H, CH_2N), 3.85 (s, 3H, OCH_3), 4.42 (t, J = 5.2 Hz, 2H, CO_2CH_2), 5.76 (s, 2H, OCH_2O), 6.91 (dd, J = 7.1, 2.0 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.11 (dd, J = 7.1, 2.0 Hz, 2H, 4-methoxyphenyl H-2, H-6), 7.27 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.80 (s, 1H, H-3); MS 543.93 (M+Na). Anal. Calcd for $C_{23}H_{27}N_3O_9S$: C, 51.58; H, 5.38; N, 7.85. Found: C, 51.84; H, 5.82; N, 7.84.

5.1.3. O^2-Acetoxymethyl-1-(*N*-ethyl-*N*- methylamino)diazen-1-ium-1,2-diolate (*E*)-2-(4-fluorophenyl)-3-(4-methanesulfonylphenyl)acrylate (11c). Yield, 36%; yellow gum; IR (film) 2965 (C–H aromatic), 2927 (C–H aliphatic), 1735, 1717 (CO_2), 1307, 1148 (SO_2), 1220, 1025 (N═N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H, $COCH_3$), 3.02 (s, 3H, NCH_3), 3.08 (s, 3H, CH_3SO_2), 3.72 (t, J = 5.2 Hz, 2H, CH_2N), 4.45 (t, J = 5.2 Hz, 2H. CO_2CH_2), 5.78 (s, 2H, OCH_2O), 7.01–7.17 (m, 4H, fluorophenyl hydrogens), 7.22 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.76 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.91 (s, 1H, H-3); MS 531.90 (M+Na). Anal. Calcd for $C_{22}H_{24}FN_3O_8S$: C, 51.86; H, 4.75; N, 8.25. Found: C, 51.96; H, 5.01; N, 7.95.

5.1.4. O^2-Acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (*E*)-2-(4-bromophenyl)-3-(4-methanesulfonylphenyl)acrylate (11d). Yield, 39%; yellow gum; IR (film) 2965 (C–H aromatic), 2927 (C–H aliphatic), 1754, 1715 (CO_2), 1304, 1152 (SO_2), 1239, 1073 (N═N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H, $COCH_3$), 3.01 (s, 3H, NCH_3), 3.04 (s, 3H, CH_3SO_2), 3.73 (t, J = 5.2 Hz, 2H, CH_2N), 4.42 (t, J = 5.2 Hz, 2H, CO_2CH_2), 5.76 (s, 2H, OCH_2O), 7.08 (d, J = 8.2 Hz, 2H, 4-bromophenyl H-3, H-5), 7.26 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.52 (d, J = 8.2 Hz, 2H, 4-bromophenyl H-2, H-6), 7.77 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.80 (s, 1H, H-3); MS 591.83 (M+Na). Anal. Calcd for $C_{22}H_{24}BrN_3O_8S$: C, 46.32; H, 4.24; N, 7.37. Found: C, 46.79; H, 4.53; N, 7.23.

5.2. General method for preparation of the 2-nitrooxyethyl acrylate esters (12a–d)

A solution of 2-nitrooxyethyl bromide (**10**, 0.22 mmol), the respective acrylic acid (**8a**–**d**, 0.2 mmol), and K_2CO_3 (0.24 mmol) in dry DMF (10 mL) was stirred at 25 °C for 36 h. Water (40 mL) was added, the mixture was extracted with EtOAc (3 × 300 mL), the extract was washed with water (2 × 40 mL) and then brine (40 mL), the organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography using EtOAc–hexane (1:1, v/v) as eluent. Physical and spectral data for **12a**–**d** are listed below.

5.2.1. 2-Nitrooxyethyl (*E*)-3-(4-methanesulfonylphenyl)-2-phenylacrylate (12a). Yield, 94%; white powder; mp 102–104 °C; IR (film) 2963 (C–H aromatic), 2926 (C–

H aliphatic), 1715 (CO_2), 1632, 1284 (ONO_2), 1306, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.02 (s, 3H, CH_3SO_2), 4.48–4.54 (m, 2H, CO_2CH_2), 4.74 (t, $J = 4.5$ Hz, 2H, CH_2ONO_2), 7.15–7.25 (m, 3H, phenyl, H-3, H-4, H-5), 7.29 (d, $J = 8.3$ Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.34–7.41 (m, 2H, phenyl H-2, H-6), 7.73 (d, $J = 8.2$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.86 (s, 1H, H-3); MS 413.93 (M+Na). Anal. Calcd for $C_{18}H_{17}NO_7S$: C, 54.48; H, 5.13; N, 3.55. Found: C, 54.87; H, 5.19; N, 3.17.

5.2.2. 2-Nitrooxyethyl (*E*)-2-(4-methoxyphenyl)-3-(4-methanesulfonylphenyl)acrylate (12b). Yield, 76%; white powder; mp 58–60 °C; IR (film) 2962 (C–H aromatic), 2927 (C–H aliphatic), 1719 (CO_2), 1633, 1280 (ONO_2), 1308, 1149 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.03 (s, 3H, CH_3SO_2), 3.85 (s, 3H, OCH_3), 4.48–4.54 (m, 2H, CO_2CH_2), 4.73–4.76 (m, 2H, CH_2ONO_2), 6.90 (dd, $J = 7.1$, 2.0 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.10 (dd, $J = 7.1$, 2.0 Hz, 2H, 4-methoxyphenyl H-2, H-6), 7.27 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.80 (s, 1H, H-3); MS 443.89 (M+Na). Anal. Calcd for $C_{19}H_{19}NO_8S$: C, 54.17; H, 4.54; N, 3.32. Found: C, 54.77; H, 4.31; N, 3.24.

5.2.3. 2-Nitrooxyethyl (*E*)-2-(4-fluorophenyl)-3-(4-methanesulfonylphenyl)acrylate (12c). Yield, 94%; white powder; mp 130–132 °C; IR (film) 2962 (C–H aromatic), 2926 (C–H aliphatic), 1717 (CO_2), 1634, 1284 (ONO_2), 1312, 1151 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.03 (s, 3H, CH_3SO_2), 4.50–4.54 (m, 2H, CO_2CH_2), 4.73–4.76 (m, 2H, CH_2ONO_2), 7.05–7.19 (m, 4H, fluorophenyl hydrogens), 7.24 (d, $J = 8.2$ Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.77 (d, $J = 8.2$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.87 (s, 1H, H-3); MS 431.94 (M+Na). Anal. Calcd for $C_{18}H_{16}FNO_7S$: C, 52.81; H, 3.94; N, 3.42. Found: C, 53.31; H, 4.24; N, 3.27.

5.2.4. 2-Nitrooxyethyl (*E*)-2-(4-bromophenyl)-3-(4-methanesulfonylphenyl)acrylate (12d). Yield, 93%; white powder; mp 105–107 °C; IR (film) 2962 (C–H aromatic), 2926 (C–H aliphatic), 1714 (CO_2), 1634, 1282 (ONO_2), 1312, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.04 (s, 3H, CH_3SO_2), 4.47–4.54 (m, 2H, CO_2CH_2), 4.73–4.76 (m, 2H, CH_2ONO_2), 7.07 (d, $J = 8.2$ Hz, 2H, 4-bromophenyl H-3, H-5), 7.25 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.54 (d, $J = 8.2$ Hz, 2H, 4-bromophenyl H-2, H-6), 7.78 (d, $J = 8.5$ Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.88 (s, 1H, H-3); MS 491.80 (M+Na). Anal. Calcd for $C_{18}H_{16}BrNO_7S$: C, 45.97; H, 3.43; N, 2.98. Found: C, 46.08; H, 3.60; N, 3.06.

5.3. In vitro cyclooxygenase (COX) inhibition assays

The ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and COX-2 (IC_{50} value, μM) was determined using an enzyme immunoassay (EIA) kit (catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA) according to a previously reported method.[28]

5.4. In vitro nitric oxide release assay

In vitro nitric oxide release, upon incubation of the test compound at 37 °C for 1.5 h with either 2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer at pH 7.4, with 2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer at pH 7.4 to which 90 μL rat serum had been added, or with 2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer at pH 7.4 which contained 5.0 mM L-cysteine, 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 (**11a–d**; **12a–d**), and the reference compounds O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**13**) and glycerol trinitrate (**14**), using the reported procedures.[29]

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