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Novel (*E*)-2-(aryl)-3-(4-methanesulfonylphenyl)acrylic ester prodrugs possessing a diazen-1-ium-1,2-diolate moiety: Design, synthesis, cyclooxygenase inhibition, and nitric oxide release studies

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Abstract—A novel group of hybrid nitric oxide-releasing anti-inflammatory drugs (**11**) possessing a 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate, or 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate, nitric oxide (·NO) donor moiety attached via a one-carbon methylene spacer to the carboxylic acid group of (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids were synthesized. These ester prodrugs (**11**) all exhibited in vitro inhibitory activity against the cyclooxygenase-2 (COX-2) isozyme (IC_{50} = 0.94–31.6 μM range). All compounds released ·NO upon incubation with phosphate buffer (PBS) at pH 7.4 (3.2–11.3% range). In comparison, the percentage of ·NO released was significantly higher (48.6–75.3% range) when these hybrid ester prodrugs were incubated in the presence of rat serum. 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. O^2-[(*E*)-2-(4-Acetylaminophenyl)-3-(4-methanesulfonylphenyl)acryloyloxymethyl]-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**11f**) is a moderately potent (IC_{50} = 0.94 μM) and selective (SI > 104) COX-2 inhibitor that released 73% of the theoretical maximal release of two molecules of ·NO/molecule of the parent hybrid ester prodrug upon incubation with rat serum. Hybrid ester ·NO-donor prodrugs offer a potential drug design concept for the development of anti-inflammatory drugs that are devoid of adverse ulcerogenic and/or cardiovascular side effects.

1. Introduction

The decreased incidence of gastrointestinal toxicity associated with the use of selective cyclooxygenase-2 (COX-2) inhibitors for the treatment of rheumatoid diseases has been clinically validated.[1,2] Despite the relatively safe pharmacological profile of selective COX-2 inhibitors, precaution is required regarding their use in patients at risk for an adverse cardiovascular event such as myocardial infarction. In this regard, the clinical use of rofecoxib and valdecoxib was terminated due to adverse cardiovascular effects.[3] This increased risk is thought to be triggered by a reduction in the level of the desirable platelet aggregation inhibitor and vasodilatory prostacyclin (PGI_2) in conjunction with an increased level of the undesirable potent platelet activator and aggregator thromboxane A_2 (TxA_2).[4]

Nitric oxide (·NO) is an important mediator of gastrointestinal mucosal defense, exerting many of the same actions as prostaglandins in the gastrointestinal tract,[5] and ·NO has been shown to reduce the severity of gastric injury in experimental models.[6,7] The concept[8] that linking an ·NO-releasing moiety to a non-steroidal anti-inflammatory drug (NSAID) may reduce the toxicity of the latter was confirmed by an animal study which showed that ·NO-diclofenac (**1**, see Fig. 1) spared the gastrointestinal tract even though it suppressed prostaglandin synthesis as effectively as the parent drug.[9] One limitation of ·NO-releasing NSAIDs having a nitrooxyalkyl group is the fact that production of ·NO from organic nitrate esters requires a three-electron reduction, and

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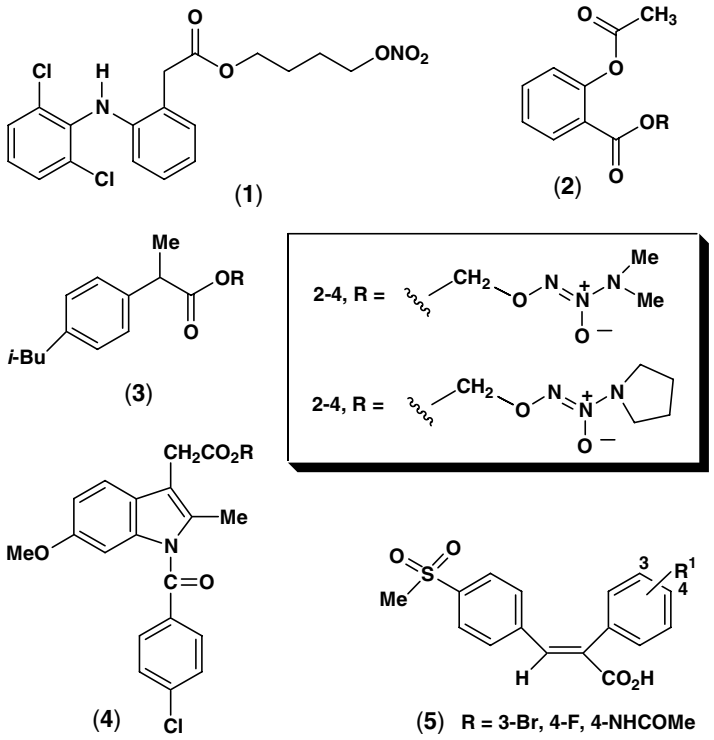
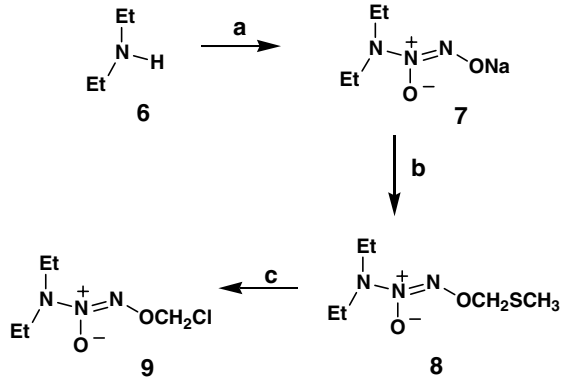


Figure 1. Chemical structures of ·NO-diclofenac (**1**), the ·NO-donor diazen-1-ium-1,2-diolate ester prodrugs of aspirin (**2**), ibuprofen (**3**), indomethacin (**4**), and (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids (**5**).

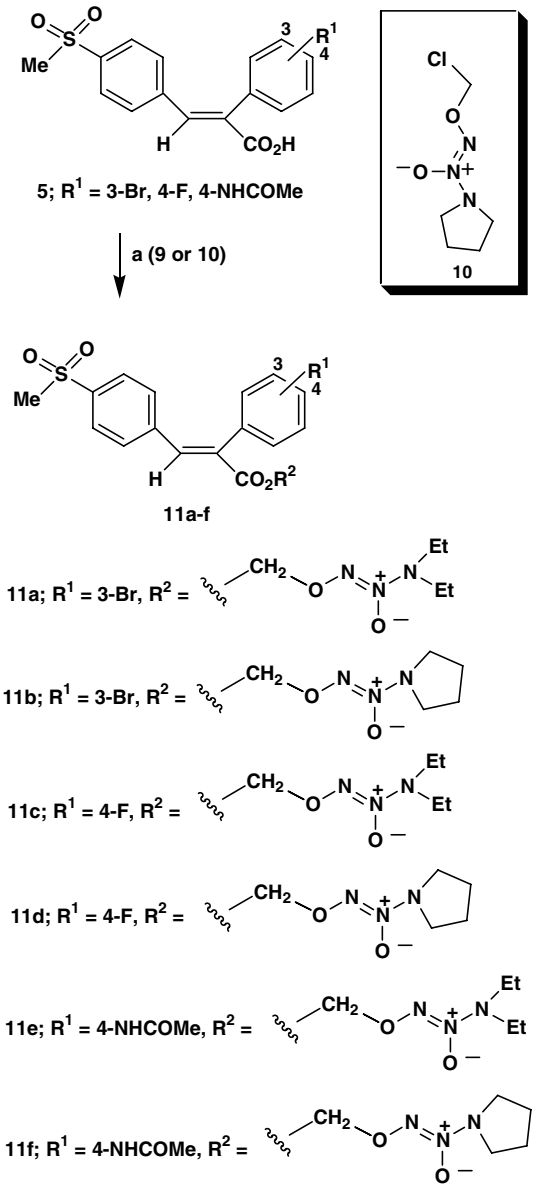
this metabolic activation decreases in efficiency on continued use of the drugs, contributing to 'nitrate tolerance'.[10] In contrast, O^2-unsubstituted *N*-diazen-1-ium-1,2-diolates have the potential to release up to 2 equivalents of ·NO with half-lives that correlate well with their pharmacological durations of action. In an earlier study, we showed that a novel group of hybrid ·NO-releasing 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 methylene spacer to the carboxylic acid group of aspirin (**2**), ibuprofen (**3**), and indomethacin (**4**) 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.[11] Recently, we described a group of acyclic (*E*)-2-(phenyl)-3-(4-methanesulfonylphenyl)acrylic acids that exhibit COX isozyme and/or 5-lipoxygenase (5-LOX) inhibitory activities.[12] We now report a group of hybrid ester prodrugs (**11a**–**f**) in which a diazen-1-ium-1,2-diolate ·NO-donor moiety has been coupled via a one-carbon methyl spacer to a select group of (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids (**11**). It is expected that hybrid ·NO-donor prodrugs of this type will be devoid of adverse ulcerogenic and cardiovascular effects.

2. Chemistry

O^2-Chloromethyl-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**9**) was prepared according to a modified procedure reported by Tang et al.,[13] as illustrated in Scheme 1. Thus, the reaction of diethylamine (**6**) with nitric oxide gas (40 psi) at 25 °C in the presence of NaOMe afforded O^2-sodium 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**7**) in 26% yield. The sodium salt was alkylated with chloromethyl methyl sulfide to afford O^2-(methylthiomethyl)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**8**), which was subsequently reacted with sulfuryl chloride in dichloromethane for 8 h to afford the O^2-chloromethyl-protected diazeniumdiolate (**9**) in quantitative yield. The target ·NO-acrylic acid ester prodrugs **11a**–**f** were synthesized in moderate-to-good yield (23–69%) by condensation of the sodium salt of the acrylic acid (**5**, R^1 = 3-Br, 4-F or 4-NHCOMe) with the O^2-chloromethyl compound **9**, or **10**, using the polar aprotic solvent hexamethylphosphoramide (HMPA) as illustrated in Scheme 2.



Scheme 1. Reagents and conditions: (a) nitric oxide (40 psi), NaOMe, MeOH, ether, 25 °C, 5 h; (b) $ClCH_2SMe$, HMPA, 25 °C, 72 h; (c) SO_2Cl_2, CH_2Cl_2, 25 °C, 8 h.



Scheme 2. Reagents and conditions: (a) Na_2CO_3, HMPA, 25 °C, 43 h.

3. Results and discussion

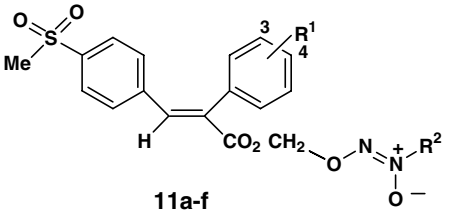
A group of acyclic (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids that exhibited interesting combinations of COX-1/COX-2 and/or 5-LOX/15-LOX isozyme inhibitory activities were recently reported.[12] Three compounds from within this group were selected for elaboration to hybrid ester prodrugs (**11a**–**f**) in which a diazen-1-ium-1,2-diolate ·NO-donor moiety is coupled via a one-carbon methyl spacer to the (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids (**5**, R^1 = 3-Br, 4-F, 4-NHCOMe). In vitro structure–activity relationships acquired for this group of hybrid ester prodrugs (**11a**–**f**) showed that they exhibit a broad range (moderately potent-to-inactive) of COX-1 (IC_{50} = 1.0 to >100 μM), and COX-2 (IC_{50} = 0.94–31.6 μM range, see data in Table 1), inhibitory activities. Compounds having a —NEt_2 moiety were generally more potent inhibitors of COX-1 than the corresponding 1-pyrrolinyl analogs (**11a** > **11b**; **11c** > **11d**; but **11e** and **11f** were both inactive). In contrast, all compounds inhibited COX-2 irrespective of the combination of R^1 (3-Br, 4-F, 4-NHCOMe), and R^2 (—NEt_2, 1-pyrrolinyl), substituents. Compounds having a 4-F or 4-NHCOMe R^1-substituent exhibited a higher COX-2 selectivity index (SI) in the 0.43 to >106 range relative to the non-selective 3-Br compounds (COX-2 SI = 0.03 to 0.22). The most potent (IC_{50} = 0.94 μM), and selective (SI > 106), COX-2 inhibitor having a R^1 = 4-NHCOMe substituent in conjunction with a R^2 1-pyrrolidinyl moiety (**11f**) was less promising than the reference drugs celecoxib (COX-2 IC_{50} = 0.07 μM, SI = 473) and rofecoxib (COX-2 IC_{50} = 0.50 μM, SI > 200). COX-1 and COX-2 inhibitory data for the parent acrylic acids (**5**) are listed in Table 1.[12]

The percent of ·NO released from the hybrid ester prodrugs (**11a**–**f**) upon incubation in phosphate-buffered saline (PBS at pH 7.4), and in the presence of rat serum, was determined (see data in Table 1). One type of chemical modification used to control the rate of ·NO release from diazen-1-ium-1,2-diolates is the attachment of alkyl substituents to the O^2-position.[14] O^2-substituted-diazen-1-ium-1,2-diolates are stable compounds that hydrolyze slowly even in acidic solution.[15] Consistent with these observations, when compounds **11a**–**f** were incubated in PBS at pH 7.4, the percentage of ·NO released varied from 3.2% to 11.3% which is indicative of slow ·NO release. On the other hand, the effect of non-specific esterases present in rat serum on the ·NO release properties of compounds **11a**–**f** was substantially higher (48.6–75.3% 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. The hybrid ester prodrugs **11a**–**f** cannot release ·NO prior to cleavage of the ester group. This requirement is consistent with the observation that the O^2-sodium diazen-1-ium-1,2-diolates **7** and **12**, which do not possess an ester group that requires prior ester cleavage, release 85% and 94% of the theoretical maximal release of two molecules of ·NO/molecule of the parent ·NO-donor. A plausible mechanism for the ester hydrolysis of hybrid ester prodrugs containing a diazen-1-ium-1,2-diolate moiety, and the subsequent release of two molecules of ·NO, was described in an earlier study.[11] The hybrid ester ·NO-donor prodrugs **11a**–**f** were designed with a one-carbon methylene spacer between the carboxy 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 ester cleavage would spontaneously eliminate formaldehyde to produce the free diazen-1-ium-1,2-diolate compound that can subsequently fragment to release two molecules of ·NO.

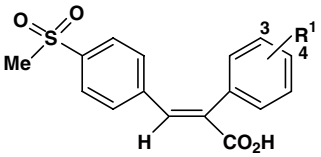
4. Conclusions

A group of hybrid ester prodrugs (**11a**–**f**) were designed in which a diazen-1-ium-1,2-diolate ·NO-donor moiety was coupled via a one-carbon methyl spacer to a select group of (*E*)-3-(4-methanesulfonylphenyl)-2-(phenyl)acrylic acids (**5**). Structure–activity and biological stability studies showed that these ester prodrugs (i) retain in vitro

Table 1. In vitro COX-1 and COX-2 inhibition, and percent (%) nitric oxide release, data for the diazeniumdiolate acrylate esters (**11a–f**), the acrylic acids (**5**), O^2-sodium 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**7**), and O^2-sodium 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**12**)



11a-f



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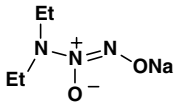

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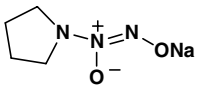

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Compound	R^1	R^2	IC_{50}[a] (μM)		COX-2 SI[b]	% ·NO released[c]	
			COX-1	COX-2		PBS[d]	Serum[e]
11a	3-Br	—NEt_2	1.1	31.6	0.03	3.2	59.2
11b	3-Br	1-pyrrolidinyl	2.5	11.5	0.22	6.9	48.6
11c	4-F	—NEt_2	1.0	2.3	0.43	7.1	75.3
11d	4-F	1-pyrrolidinyl	>100	10.8	>9.2	11.3	67.1
11e	4-NHCOMe	—NEt_2	>100	16.8	>5.9	6.5	70.4
11f	4-NHCOMe	1-pyrrolidinyl	>100	0.94	>106	9.9	73.0
5	3-Br	—	>100[f]	0.31[f]	>322[f]	—	—
5	4-F	—	0.4[f]	36.0[f]	—	—	—
5	4-NHCOMe	—	3.2[f]	2.5[f]	1.3[f]	—	—
Aspirin	—	—	0.35	2.4	0.15	—	—
Celecoxib	—	—	33.1	0.07	473	—	—
Rofecoxib	—	—	>100	0.50	>200	—	—
7	—	—	—	—	—	85.0	85.1
12	—	—	—	—	—	94.0[g]	—

[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 2 mol of ·NO/mol of the test compound. The result is the mean value of three 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] Data taken from literature.[12]

[g] Data taken from the literature.[11]

COX-2 inhibitory activity (COX-2 IC_{50} values in the 0.94–31.6 μM range), (ii) are relatively stable in phosphate-buffered saline at pH 7 where ·NO release is in the 3.2–11.3% range, and (iii) undergo extensive ester cleavage by rat serum esterase(s) that is followed by a significant release of ·NO in the 48.6–75.3% range. O^2-[(*E*)-2-(4-Acetylaminophenyl)-3-(4-methanesulfonylphenyl) acryloyloxymethyl]-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**11f**) is a moderately potent (IC_{50} = 0.94 μM) and selective (SI > 106) COX-2 inhibitor that released 73% of the theoretical maximal release of 2 molecules of ·NO/molecule of the parent hybrid ester prodrug upon incubation with rat serum. Hybrid ester ·NO-donor prodrugs offers 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 D_2O, $CDCl_3$ or DMSO-d_6 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 (Microanalytical Service Laboratory, Department of Chemistry, University of Alberta) and were within ±0.4% of theoretical values. 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. O^2-(Chloromethyl)-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**10**)[13] and the acrylic acids (**5**, R^1 = 3-Br, 4-F, 4-NHCOMe)[12] were prepared according to literature procedures. Nitric oxide gas was purchased from BOC Scientific (Burlington, Ontario).

5.1. O^2-Sodium 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (7)

Diethylamine (**6**, 7.3 g, 0.1 mol) was added to a solution of NaOMe (0.1 mol, 24 mL of a 25% w/v solution in MeOH) and diethyl ether (300 mL) with stirring at 25 °C. This mixture was purged with dry nitrogen for 5 min, and then the reaction was allowed to proceed under an atmosphere of nitric oxide (40 psi internal

pressure) with stirring at 25 °C for 5 h. The product, which precipitated as a fine white powder, was isolated by filtration and then suspended in diethyl ether (100 mL) upon stirring for 15 min. The suspension was filtered, and the solid collected was dried at 25 °C under reduced pressure until a constant weight was achieved after about 1 h to afford **7** as a fine white powder (4.0 g, 26%): mp 200–202 °C; ^{1}H NMR (D_2O) δ 1.12 [t, J = 7.3 Hz, 6H, $N(CH_2CH_3)_2$], 2.93 [q, J = 7.3 Hz, 4H, $N(CH_2CH_3)_2$]. The product **7** was used immediately after drying without further purification for the preparation of compound **8**.

5.2. O^2-(Methylthiomethyl)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (8)

The sodium diazeniumdiolate **7** (2.8 g, 18.2 mmol) was added to a suspension of K_2CO_3 (0.5 g, 3.7 mmol) and HMPA (27 mL) at 4 °C, and this mixture was stirred for 30 min. Chloromethyl methyl sulfide (6.3 g, 21.9 mmol) was added dropwise, and the reaction was allowed to proceed at 25 °C for 72 h with stirring. EtOAc (70 mL) was added to quench the reaction, the solids were filtered off, the organic phase was washed with water (5 × 30 mL), the organic fraction was dried (Na_2SO_4), and the solvent was removed in vacuo to give a liquid residue which was purified by silica gel column chromatography using EtOAc/hexane (1:4, v/v) as eluent. Compound **8** (1.1 g, 32%) was obtained as a pale yellow liquid: IR (film) 1239 (N═N─O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.09 [t, J = 7.0 Hz, 6H, $N(CH_2CH_3)_2$], 2.26 (s, 3H, SCH_3), 2.93 [q, J = 7.0 Hz, 4H, $N(CH_2CH_3)_2$], 5.29 (s, 2H, OCH_2S); MS 215.92 (M + Na). Product **8** was used immediately for the subsequent preparation of the O^2-chloromethyl derivative **9**.

5.3. O^2-(Chloromethyl)-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (9)

A solution of compound **8** (1.1 g, 5.7 mmol) in CH_2Cl_2 (10 mL) was cooled to 4 °C, sulfuryl chloride (1.15 g, 8.55 mmol, 8.5 mL of a 1.0 M solution in CH_2Cl_2) was added dropwise, the ice bath was removed, and the reaction mixture was stirred at 25 °C for 8 h. The solvent was removed in vacuo and the residue was purified by silica gel column chromatography using EtOAc/hexane (1:4, v/v) as the eluent to furnish **9** (1.0 g, 100%) as a yellow oil: IR (film) 1247 (N═N─O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.12 [t, J = 7.0 Hz, 6H, $N(CH_2CH_3)_2$], 3.25 [q, J = 7.0 Hz, 4H, $N(CH_2CH_3)_2$], 5.87 (s, 2H, OCH_2Cl); MS 203.87 (M + Na).

5.4. General method for preparation of the diazeniumdiolate acrylate esters (11a–f)

Sodium carboxylates of the respective acrylic acids **5** (R^1 = 3-Br, 4-F, 4-NHCOMe) 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 an O^2-(chloromethyl)diazen-1-ium-1,2-diolate **9** or **10** (2.5 mmol) in HMPA (1.5 mL) was then added, and the reaction was allowed to proceed for 24 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 (1:1, v/v) as the eluent for compounds **11a**–**d**, and acetone/hexane (1:1, v/v) for compounds **11e**–**f**. Physical and spectral data for **11a**–**f** are listed below.

5.5. O^2-[(*E*)-2-(3-Bromophenyl)-3-(4-methanesulfonylphenyl)acryloyloxymethyl]-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (11a)

Yield, 49%; white powder; mp 101–102 °C; IR (film) 2972 (C─H aromatic), 2932 (C─H aliphatic), 1743 (CO_2), 1320, 1149 (SO_2) 1239 (N═N─O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.09 (t, J = 7.0 Hz, 6H, CH_2CH_3), 3.03 (s, 3H, SO_2CH_3), 3.20 (q, J = 7.0 Hz, 4H, CH_2CH_3), 5.98 (s, 2H, OCH_2O), 7.10 (ddd, J = 7.6, 1.5, 1.5 Hz, 1H, bromophenyl H-4), 7.21-7.27 (m, 3H, 4-methanesulfonylphenyl H-2, H-6, bromophenyl H-5), 7.35 (t, J = 1.5 Hz, 1H, bromophenyl H-2), 7.51 (dd, J = 7.6, 1.5 Hz, 1H, bromophenyl H-6), 7.77 (d, J = 8 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.94 (s, 1H, H-3); MS 547.89 (M + Na). Anal. Calcd for $C_{21}H_{24}BrN_3O_6S$: C, 47.91; H, 4.60; N, 7.98. Found: C, 47.97; H, 4.60; N, 7.71.

5.6. O^2-[(*E*)-2-(3-Bromophenyl)-3-(4-methanesulfonylphenyl)acryloyloxymethyl]-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (11b)

Yield, 69%; yellow powder; mp 99–101 °C; IR (film) 2974 (C─H aromatic), 2928 (C─H aliphatic), 1725 (CO_2), 1304, 1151 (SO_2) 1233 (N═N─O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.95 (quintet, J = 6.9 Hz, 4H, pyrrolidinyl H-3, H-4), 3.03 (s, 3H, SO_2CH_3), 3.60 (t, J = 6.9 Hz, 4H, pyrrolidinyl H-2, H-5), 5.91 (s, 2H, OCH_2O), 7.10 (ddd, J = 7.6, 1.5, 1.5 Hz, 1H, bromophenyl H-4), 7.22–7.27 (m, 3H, 4-methanesulfonylphenyl H-2, H-6, bromophenyl H-5), 7.38 (dd, J = 1.5, 1.5 Hz, bromophenyl H-2), 7.51 (ddd, J = 7.6, 1.5, 1.5 Hz, 1H, bromophenyl H-6), 7.77 (dd, J = 6.7, 1.8 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.93 (s,1H, H-3); MS 545.88 (M + Na). Anal. Calcd for $C_{21}H_{22}BrN_3O_6S$: C, 48.10; H, 4.23; N, 8.01. Found: C, 48.44; H, 4.57; N, 7.61.

5.7. O^2-[(*E*)-2-(4-Fluorophenyl)-3-(4-methanesulfonylphenyl)acryloyloxymethyl]-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (11c)

Yield, 23%; yellow oil; IR (film) 2978 (C─H aromatic), 2918 (C─H aliphatic), 1730 (CO_2), 1312, 1150 (SO_2) 1238 (N═N─O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.08 (t, J = 7.0 Hz, 6H, CH_2CH_3), 3.02 (s, 3H, SO_2CH_3), 3.19 (q, J = 7.0 Hz, 4H, CH_2CH_3), 5.98 (s, 2H, OCH_2O), 7.01–7.17 (m, 4H, 4-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 487.98 (M + Na). Anal. Calcd for $C_{21}H_{24}FN_3O_6S$: C, 54.18; H, 5.20; N, 9.03. Found: C, 54.12; H, 4.81; N, 8.88.

5.8. O^2-[(*E*)-2-(4-Fluorophenyl)-3-(4-methanesulfonylphenyl)acryloyloxymethyl]-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (11d)

Yield, 66%; white powder; mp 79–81 °C; IR (film) 2972 (C—H aromatic), 2930 (C—H aliphatic), 1723 (CO_2), 1304, 1150 (SO_2) 1232 (N=N—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.98 (quintet, J = 6.9 Hz, 4H, pyrrolidinyl H-3, H-4), 3.03 (s, 3H, SO_2CH_3), 3.59 (t, J = 6.9 Hz, 4H, pyrrolidinyl H-2, H-5), 5.92 (s, 2H, OCH_2O), 7.02–7.19 (m, 4H, 4-fluorophenyl hydrogens), 7.24 (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 485.95 (M + Na). Anal. Calcd for $C_{21}H_{22}FN_3O_6S$: C, 54.42; H, 4.78; N, 9.07. Found: C, 54.76; H, 4.89; N, 9.07.

5.9. O^2-[(*E*)-2-(4-Acetylaminophenyl)-3-(4-methanesulfonylphenyl)acryloyloxymethyl]-1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (11e)

Yield, 34%; yellow crystals; mp 75–77 °C; IR (film) 3345–3325 (NH), 2979 (C—H aromatic), 2925 (C—H aliphatic), 1726 (CO_2), 1690 (CO), 1313, 1147 (SO_2) 1234 (N=N—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.09 (t, J = 7.0 Hz, 6H, CH_2CH_3), 2.21 (s, 3H, $COCH_3$), 3.03 (s, 3H, SO_2CH_3), 3.20 (q, J = 7.0 Hz, 4H, CH_2CH_3), 5.98 (s, 2H, OCH_2O), 7.12 (d, J = 8.5 Hz, 2H, 4-acetylaminophenyl H-2, H-6), 7.24 (d, J = 7.6 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.30 (s, 1H, NH), 7.52 (d, J = 8.5 Hz, 2H, 4-acetylaminophenyl H-3, H-5), 7.74 (d, J = 7.6 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.88 (s, 1H, H-3); MS 526.87 (M + Na). Anal. Calcd for $C_{23}H_{28}N_4O_7S.2/5H_2O$: C, 54.02; H, 5.63; N, 10.95. Found: C, 54.39; H, 5.62; N, 10.59.

5.10. O^2-[(*E*)-2-(4-Acetylaminophenyl)-3-(4-methanesulfonylphenyl)acryloyloxymethyl]-1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (11f)

Yield, 56%; yellow crystals; mp 130–131 °C; IR (film) 3355–3335 (NH), 2979 (C—H aromatic), 2926 (C—H aliphatic), 1727 (CO_2), 1690 (CO), 1310, 1153 (SO_2) 1235 (N=N—O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.95–1.99 (m, 4H, pyrrolidinyl H-3, H-4), 3.03 (s, 3H, SO_2CH_3), 3.59 (t, J = 6.7 Hz, 4H, pyrrolidinyl H-2, H-5), 5.91 (s, 2H, OCH_2O), 7.13 (d, J = 8.5 Hz, 2H, 4-acetylaminophenyl H-2, H-6), 7.26 (d, J = 8.7 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.40 (s, 1H, NH), 7.52 (d, J = 8.5 Hz, 2H, 4-acetylaminophenyl H-3, H-5), 7.74 (d, J = 8.7 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.87 (s, 1H, H-3); MS 524.99 (M + Na). Anal. Calcd for $C_{23}H_{26}N_4O_7S.1/2H_2O$: C, 54.00; H, 5.32; N, 10.95. Found: C, 54.20; H, 5.59; N, 10.55.

6. 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 immuno assay (EIA) kit (catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA) according to a previously reported method.[16]

7. In vitro nitric oxide release assay

In vitro nitric oxide release, upon incubation of the test compound with either 2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer at pH 7.4, or 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, 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**–**f**), and the reference compounds O^2-sodium 1-(*N*,*N*-diethylamino)diazen-1-ium-1,2-diolate (**7**) and O^2-sodium 1-(pyrrolidin-1-yl)dizen-1-ium-1,2-diolate (**12**), using the reported procedures.[17]

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