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Diazen-1-ium-1,2-diolated nitric oxide donor ester prodrugs of 1-(4-methanesulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylic acids: Synthesis, nitric oxide release studies and anti-inflammatory activities

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ABSTRACT

A new group of hybrid nitric oxide-releasing anti-inflammatory drugs (NONO-coxibs) wherein an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**11a–c**) NO-donor moiety is attached directly to the carboxylic acid group of 1-(4-methanesulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylic acids were synthesized. The diazen-1-ium-1,2-diolate compounds **11a–c** all released a low amount of NO upon incubation with phosphate buffer (PBS) at pH 7.4 (7.7–9.3% range). In comparison, the percentage of NO released was significantly higher (67.5–73.6% of the theoretical maximal release of two molecules of NO/molecule of the parent hybrid ester prodrug) when the diazen-1-ium-1,2-diolate ester prodrugs were incubated in the presence of rat serum. These incubation studies suggest that both NO and the anti-inflammatory 1-(4-methanesulfonylphenyl)-5-(4-H, 4-F or 4-Me-phenyl)-1*H*-pyrazol-3-carboxylic acid (**9a–c**) would be released from the parent NONO-coxib upon in vivo cleavage by non-specific *serum* esterases. The 1-(4-methanesulfonylphenyl)-5-(4-H, 4-F or 4-Me-phenyl)-1*H*-pyrazol-3-carboxylic acids (**9a–c**) exhibited AI activities (ID_{50} = 85.2–104.4 mg/kg po range) between that exhibited by the reference drugs aspirin (ID_{50} = 128.7 mg/kg po) and celecoxib (ID_{50} = 10.8 mg/kg po). Hybrid ester anti-inflammatory/NO-donor prodrugs (NONO-coxibs) offers a potential drug design concept targeted toward the development of anti-inflammatory drugs that are devoid of adverse ulcerogenic and/or cardiovascular effects.

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1. Introduction

The development of celecoxib,[1] rofecoxib,[2] valdecoxib,[3] and etoricoxib[4,5] validated the original concept that selective cyclooxygenase-2 (COX-2) inhibitors would be effective anti-inflammatory agents with a diminished gastrointestinal (GI) and renal toxicity.[6–9] These drugs preferentially inhibit the inducible COX-2 isozyme that causes inflammation relative to the constitutive COX-1 isozyme that provides gastroprotection and maintains vascular homeostasis. This initial apparently safe pharmacological profile of selective COX-2 inhibitors was relatively short-lived. It was not long until evidence surfaced suggesting that highly selective COX-2 inhibitors alter the balance in the COX pathway resulting in a decrease in the level of the desirable vasodilatory and anti-aggregatory prostacyclin (PGI_2) in conjunction with an increase in the level of the undesirable prothrombotic thromboxane A_2 (TxA_2). This alteration resulted in increased incidences of an adverse cardiovascular thrombotic event such as myocardial infarction.[10] Accordingly, the clinical use of rofecoxib and valdecoxib were voluntarily terminated due to adverse cardiovascular effects associated with their use.[11]

Nitric oxide (NO), like PGI_2, plays an important cytoprotective role in GI homeostasis by helping to maintain mucosal blood flow, by optimizing mucus secretion, and by inhibiting platelet and inflammatory-cell activation.[12–15] These actions of NO could enhance the gastro-sparing features of selective COX-2 inhibitors and potentially induce peripheral vasodilation to circumvent the elevation in blood pressure exhibited by selective COX-2 inhibitors that decrease the physiological level of PGI_2. In this regard, hybrid selective COX-2 inhibitors possessing a NO-donor moiety (NO-coxibs) have been investigated as a method to increase the clinical safety of COX-2 inhibitors. Examples of NO-coxibs (see Fig. 1) having a nitrate ester NO-donor moiety include the oxazole (**1**) which exhibits anti-inflammatory activity similar to that of valdecoxib with anti-thrombotic action at higher doses,[16] the pyrazoles which are selective COX-2 inhibitors (**2a–b**) that exhibit anti-inflammatory activity and low gastric toxicity (**2b**),[17] and the imidazoles (**3a–b**) that exhibit a NO-dependent vasodilator activity.[18] In earlier studies, we described novel acrylate ester prodrugs such as **4**,[19] and related analogs,[20] having a NO-donor diazen-1-ium-1,2-diolate moiety that are effectively cleaved by esterases to release the parent COX-2 inhibitory acrylic acid and NO.

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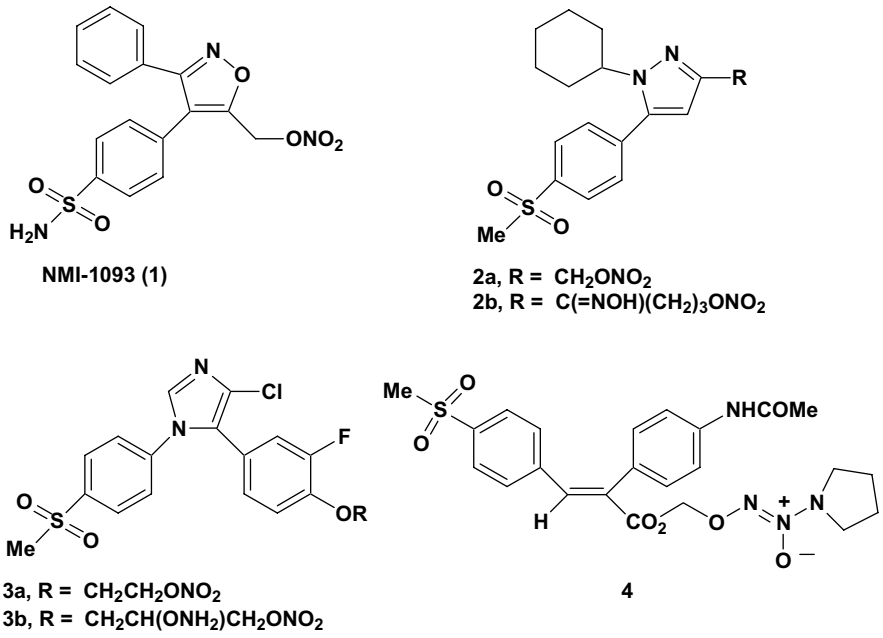


Figure 1. Some selective cyclooxygenase-2 (COX-2) inhibitors that possess a nitric oxide donor nitrate (**1–3**), or diazen-1-ium-1,2-diolate (**4**), moiety.

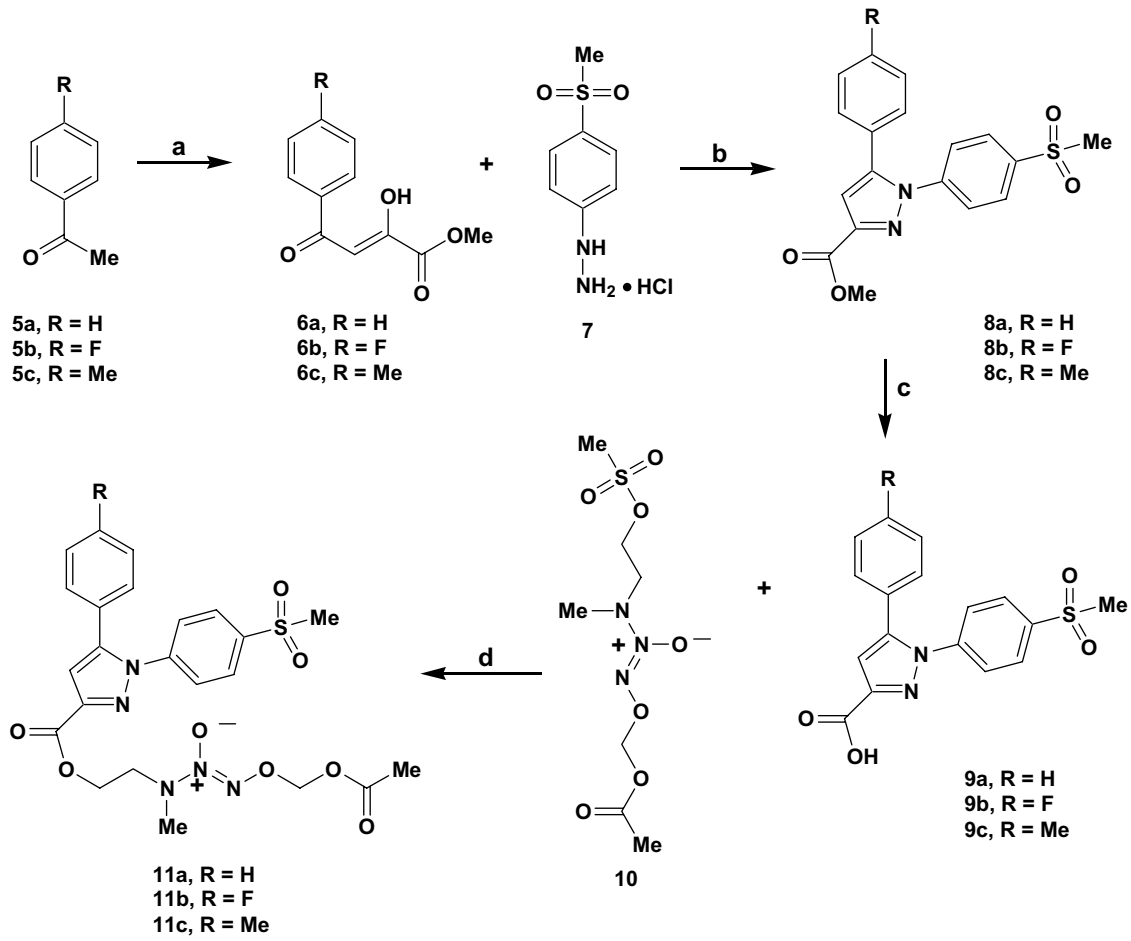
NO-coxibs that release NO from a nitrooxy group (nitrate ester) are disadvantaged by the fact that the production of NO requires a demanding three-electron reduction. The efficacy of this metabolic activation process can decrease on prolonged use of a nitrate ester NO-donor drug culminating in nitrate tolerance.[21–23] Our group has introduced and developed the concept of 'NONO-coxibs', which is based on the linkage of a *N*-diazen-1-ium-1,2-diolate moiety to the structure of a selective COX-2 inhibitor such as the acrylate **4**[19] shown in Figure 1, and other NO-donor analogs thereof.[20] These studies were initiated to address safety and efficacy issues related to classical selective COX-2 inhibitors, and organic nitrate-based NO-coxibs. Diazen-1-ium-1,2-diolate ions, after their cleavage from the parent hybrid NONO-coxib, can release up to two equivalents of NO without further metabolic activation, they are structurally diverse, and they possess a rich derivatization chemistry that facilitates delivery of NO to specific organ and/or tissue sites.[24] These features distinguish the NONO-coxib **4** (Fig. 1) from nitrate-based NO-coxibs (**1**–**3**) which require redox activation before NO is released. Our ongoing research program is targeted toward the design of improved anti-inflammatory agents devoid of adverse GI and cardiovascular effects. We now report the synthesis and nitric oxide release data for a group of hybrid diazen-1-ium-1,2-diolated nitric oxide donor ester prodrugs of 1-(4-methanesulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylic acids (**11a–c**), and the anti-inflammatory activities for the parent 1-(4-methanesulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylic acids (**9a–c**).

2. Chemistry

A group of 1-(4-methanesulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylate esters possessing an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate ester moiety (**11a–c)** were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, Claisen condensation of acetophenone (**5a**), 4-fluoroacetophenone (**5b**), or 4-methylacetophenone (**5c**) with diethyl oxalate gave the respective 2,4-diketo ester (**6a–c**). Subsequent reaction of the ester **6a**, **6b**, or **6c** with (4-methylsulfonylphenyl)hydrazine hydrochloride (**7**) in ethanol at reflux furnished the corresponding pyrazole ester (**8a**, **8b** or **8c**) in high yield (81–91%). It is well documented that this latter reaction occurs in a regiospecific manner to yield the 1,5-diarylpyrazole product when a phenylhydrazine hydrochloride is employed and the reaction is carried out in ethanol at reflux temperature.[1] Alkaline hydrolysis of the esters (**8a–c**) using LiOH gave the respective acid (**9a**, **9b** or **9c**) in good yield (63–91%). Nucleophilic displacement of the mesyloxy group present in the mesylate **10** upon reaction with the respective sodium carboxylate of **9a**, **9b** or **9c** in hexamethylphosphoramide (HMPA) afforded the target O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate 1-(4-methanesulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylates (**11a–c**).

3. Results and discussion

Three plausible positions on a generic 1,5-diaryl-3-substituted-pyrazole structure were considered for attachment of an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate NO-donor moiety via an ester moiety (see Fig. 2). The R^1 position on the N^1-phenyl ring requires a COX-2 pharmacophore such as a $MeSO_2$ or H_2NSO_2 substituent for potent and selective COX-2 inhibitory activity.[1] Ahlstrom et al., in an elegant study that investigated CYP2C9 structure—metabolism relationships to optimize the metabolic stability of COX-2 inhibitors, showed that the pyrazole ring C-3 position (R^2 substituent) has very few steric restrictions with respect to COX-2 suggesting that COX-2 inhibition properties should be retained.[25] It was also suggested that a compound containing a R^2 carboxyl substituent at the C-3 position may undergo an electrostatic interaction with Arg120 in the binding pocket of the COX-2 enzyme. We envisaged that a R^3 carboxyl substituent on the pyrazole C-5 phenyl ring was not tolerable since the R^3 methyl substituent (benzylic carbon) in celecoxib (see structure in Fig. 2) undergoes sequential metabolic biotransformation ($Me \rightarrow CH_2OH \rightarrow CO_2H \rightarrow CO_2$-glucuronide conjugate) to inactive metabolites.[26] Accordingly, it was decided based on this structural information to couple the O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate NO-donor moiety to a pyrazole ring C-3 CO_2H group via an ester moiety to prepare the target NONO-coxib hybrid ester prodrugs (**11a–c)**.



Scheme 1. Reagents and conditions: (a) diethyl oxalate, NaOMe, MeOH, reflux, 2 h; (b) EtOH, reflux, 3 h; (c) THF/MeOH, LiOH (2 M), 25 °C, 15 h; (d) Na_2CO_3, hexamethylphosphoramide (HMPA), 25 °C, 96 h.

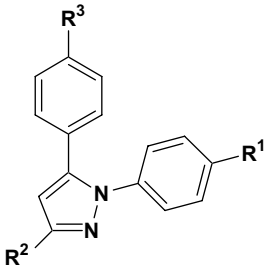
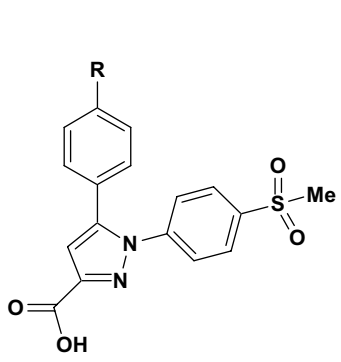


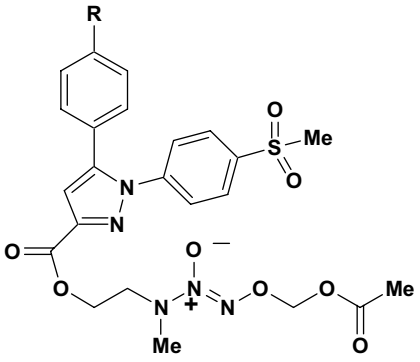
Figure 2. Generic 1,5-diaryl-3-substituted-pyrazole selective COX-2 structure based on the structure of celecoxib ($R^1 = SO_2NH_2$, $R^2 = CF_3$, $R^3 = Me$).

The percent of NO released from the hybrid ester prodrugs (**11a**–**c**) 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.[27] O^2-substituted-diazen-1-ium-1,2-diolates are stable compounds that hydrolyze slowly even in acidic solution.[28] Consistent with these observations, when compounds **11a–c** were incubated for 1.5 h in PBS at pH 7.4, the percentage of NO released varied from 7.7% to 9.3% 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–c** was substantially higher (67.5–73.6% range). These data indicate that 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**–**c** cannot release NO prior to cleavage of the acetoxy moiety present in the terminal O^2-acetoxymethyl-1-(*N*-methylamino)diazen-1-ium-1,2-diolate NO-donor moiety. This requirement is consistent with the observation that the O^2-sodium diazen-1-ium-1,2-diolate **12**, which does not possess an ester group that requires prior ester cleavage, releases 84.5% and 85% of the theoretical maximal release of two molecules of NO/molecule of the parent NO donor. Two plausible pathways for the ester hydrolysis of hybrid ester prodrugs containing an O^2-acetoxymethyl-1-[*N*-(2-ethoxy)-*N*-methylamino]diazen-1-ium-1,2-diolate moiety, and the subsequent release of acetic acid, formaldehyde, two molecules of NO, and *N*-methylethanolamine was described in an earlier study.[29] The hybrid ester NO-donor prodrugs **11a–c** 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 cleavage of the acetoxy group 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. In contrast, cleavage of the second ester group attached directly to the C-3 position of the pyrazole ring, that releases the parent coxib **9a–c**, can occur either prior to, or after, NO release has occured.

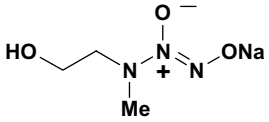
Table 1
Percentage (%) of nitric oxide release data for the diazeniumdiolate pyrazole esters (**11a–c**) and O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**12**), and in vivo anti-inflammatory activities for the 1-(4-methanesulfonylphenyl)-5-(4-substituted-phenyl)-1*H*-pyrazol-3-carboxylic acids (**9a–c**)



9a-c



11a-c



12

Compound	R	% NO released[a]		AI activity[d]: ID_{50} (mg/kg)
		PBS[b]	Serum[c]	
9a	H	—	—	104.4
9b	F	—	—	104.1
9c	Me	—	—	85.2
11a	H	7.7	67.5	—
11b	F	9.3	73.6	—
11c	Me	9.2	70.9	—
12	—	84.5	85.0	—
Celecoxib	—	—	—	10.8
Aspirin	—	—		128.7

[a] Percent of nitric oxide released based on a theoretical maximum release of 2 mol of NO/mol of the diazen-1-ium-1,2-diolate test compounds (**11a–c**, **12**). The result is the mean value of three measurements ($n = 3$) where variation from the mean % value was $\leqslant 0.5\%$.
[b] 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.
[c] 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.
[d] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as the ID_{50} value (mg/kg) at 3 h after oral administration of the test compound.

The anti-inflammatory (AI) activities exhibited by the parent 1-(4-methanesulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylic acids (**9a**, R = H; **9b**, R = F; **9c**, R = Me), that would be released upon cleavage of the ester group attached directly to the C-3 position of the pyrazole ring, were determined using a carrageenan-induced rat foot paw edema model (see data in Table 1). The relative potency profile with respect to the R-substituent was Me > F ≈ H. The AI activities exhibited by the hybrid ester prodrugs **11a–c** were not determined in this study since it was previously reported that the same hybrid ester prodrug analogs of aspirin, ibuprofen and indomethacin exhibited similar AI activities to aspirin, ibuprofen and indomethacin for comparable ID_{50} μmol/kg oral dosage regimens.[29] Compounds **9a–c** exhibited AI activities (ID_{50} = 85.2–104.4 mg/kg po range) between that exhibited by the reference drugs aspirin (ID_{50} = 128.7 mg/kg po) and celecoxib (ID_{50} = 10.8 mg/kg po). These AI data are consistent with a qualitative study which showed that ethyl 5-(4-methylphenyl)-1-(4-sulfamoylphenyl)-1*H*-pyrazole-3-carboxylate (66% and 43% inhibition of COX-1 and COX-2) is a less potent inhibitor of the COX isozymes than celecoxib (96% inhibition of both COX-1 and COX-2) at a 100 μM compound concentration.[25]

4. Conclusions

A group of hybrid ester prodrugs (NONO-coxibs) in which an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**11a–c**) NO-donor moiety is attached directly to the carboxylic acid group of 1-(4-methanesulfonylphenyl)-5-(4-H, 4-F or 4-Me-phenyl)-1*H*-pyrazol-3-carboxylic acids (**9a–c)** were synthesized for comparative biological evaluation. Biological stability, NO release, and AI studies showed that (i) the NONO-coxib prodrugs (**11a–c**) are relatively stable in phosphate-buffered saline at pH 7 where NO release is in the 7.7–9.3% range, (ii) the O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolates (**11a–c**) undergo extensive cleavage of the terminal acetoxy group by rat serum esterase(s) that is followed by a significant release of NO in the 67.5–73.6% range, and (iii) the moderate AI activity exhibited by the parent compounds **9a–c** (ID_{50} = 85.2–104.4 mg/kg po range) suggest that an alternative linker group to the ester moiety attached directly to the C-3 position of the pyrazole ring is required to provide more potent AI activity.

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 internal standard, where *J* (coupling constant) values are estimated in Hertz (Hz). Microanalyses were performed for C, H, N (Micro Analytical 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. (4-Methylsulfonylphenyl)hydrazine hydrochloride (**7**),[30] and O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**10**)[31] were prepared according to literature procedures.

5.1. General method for preparation of methyl 2-hydroxy-4-oxo-4-aryl-2-butenoates (6a–c)

A solution of diethyl oxalate (1.63 mL, 12.0 mmol) and either acetophenone **5a**, 4-fluoroacetophenone **5b**, or 4-methylacetophenone **5c** (6.0 mmol) in methanol (10 mL) was added dropwise to a solution of NaOMe in MeOH (2.6 mL of 25% w/v, 12.0 mmol), and the reaction was allowed to proceed at reflux for 2 h. After cooling to 25 °C, the reaction mixture was poured into water (40 mL), acidified with HCl (1 mL of 37% w/v), and extracted with diethyl ether (3× 100 mL). The combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$, filtered, and the solvent was removed in vacuo to afford the respective product **6a–c**. Physical and spectral data for **6a–c** are listed below.

5.1.1. Methyl 2-hydroxy-4-oxo-4-phenyl-2-butenoate (6a)

Yield, 98%; brown solid; IR (film) 3001 (C–H aromatic), 2954 (C–H aliphatic), 1735 (CO_2), 1685 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.98 (s, 3H, OCH_3), 7.10 (s, 1H, butenoate H-3), 7.52 (dd, J = 8.0, 8.0 Hz, 2H, phenyl H-3, H-5), 7.63 (dd, J = 8.0, 8.0 Hz, 1H, phenyl H-4), 8.02 (d, J = 8.0 Hz, 2H, phenyl H-2, H-6), 15.3 (br s, 1H, OH, D_2O exchangeable).

5.1.2. Methyl 4-(4-fluorophenyl)-2-hydroxy-4-oxo-2-butenoate (6b)

Yield, 89%; brown solid; IR (film) 3060 (C–H aromatic), 2959 (C–H aliphatic), 1733 (CO_2), 1685 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.13 (s, 3H, OCH_3), 7.47 (dd, J = 7.9, 7.9 Hz, 2H, fluorophenyl H-3, H-5), 7.51 (s, 1H, butenoate H-3), 8.10 (dd, J = 7.9, 3.7 Hz, fluorophenyl H-2, H-6).

5.1.3. Methyl 2-hydroxy-4-(4-methylphenyl)-4-oxo-2-butenoate (6c)

Yield, 97%; white solid; IR (film) 3007 (C–H aromatic), 2952 (C–H aliphatic), 1731 (CO_2), 1684 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.63 (s, 3H, phenyl CH_3), 4.13 (s, 3H, OCH_3), 7.45 (s, 1H, butenoate H-3), 7.50 (d, J = 8.5 Hz, 2H, methylphenyl H-3, H-5), 8.10 (d, J = 8.5 Hz, 2H, methylphenyl H-2, H-6).

5.2. General method for preparation of methyl 1-(4-methanesulfonylphenyl)-5-aryl-1*H*-pyrazole-3-carboxylates (8a–c)

(4-Methylsulfonylphenyl)hydrazine hydrochloride (**7**, 0.979 g, 4.4 mmol) was added to a stirred solution of the dione **6a**, **6b**, or **6c** (4.0 mmol) in EtOH (50 mL), and the reaction mixture was heated at reflux with stirring for 3 h. After cooling to 25 °C, the solvent was removed in vacuo. The residue was dissolved in EtOAc (50 mL), washed with water and brine, the organic fraction was dried ($MgSO_4$), filtered, and solvent was removed in vacuo to afford 8a–**c** for which the physical and spectral data are listed below.

5.2.1. Methyl 1-(4-methanesulfonylphenyl)-5-phenyl-1*H*-pyrazole-3-carboxylate (8a)

Yield, 81%; pale brown powder; IR (film) 2960 (C–H aromatic), 2925 (C–H aliphatic), 1730 (CO_2), 1322, 1155 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.09 (s, 3H, SO_2CH_3), 4.00 (s, 3H, OCH_3), 7.09 (s, 1H, pyrazole H-4), 7.23–7.28 (m, 3H, phenyl H-3, H-4, H-5), 7.40 (d, J = 7.3 Hz, 2H, phenyl H-2, H-6), 7.57 (d, J = 8.9 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.94 (d, J = 8.9 Hz, 2H, methanesulfonylphenyl H-3, H-5).

5.2.2. Methyl 5-(4-fluorophenyl)-1-(4-methanesulfonylphenyl) -1H-pyrazole-3-carboxylate (8b)

Yield, 65%; pale brown powder; IR (film) 2955 (C–H aromatic), 2926 (C–H aliphatic), 1735 (CO_2), 1319, 1154 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.10 (s, 3H, SO_2CH_3), 4.00 (s, 3H, OCH_3), 7.06 (s, 1H, pyrazole H-4), 7.11 (dd, J = 8.5, 8.5 Hz, 2H, fluorophenyl H-3, H-5), 7.23 (dd, J = 8.5, 3.7 Hz, 2H, fluorophenyl H-2, H-6), 7.56 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.96 (d, J = 8.9 Hz, 2H, methanesulfonylphenyl H-3, H-5).

5.2.3. Methyl 1-(4-methanesulfonylphenyl)-5-(4-methylphenyl)-1H-pyrazole-3-carboxylate (8c)

Yield, 91%; white powder; IR (film) 2952 (C–H aromatic), 2922 (C–H aliphatic), 1732 (CO_2), 1317, 1153 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.39 (s, 3H, phenyl CH_3), 3.07 (s, 3H, SO_2CH_3), 3.99 (s, 3H, OCH_3), 7.04 (s, 1H, pyrazole H-4), 7.11 (d, J = 8.1 Hz, 2H, methylphenyl H-3, H-5), 7.18 (d, J = 8.1 Hz, 2H, methylphenyl H-2, H-6), 7.57 (d, J = 8.7 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.93 (d, J = 8.7 Hz, 2H, methanesulfonylphenyl H-3, H-5).

5.3. General method for preparation of 1-(4-methanesulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylic acids (9a–c)

The ester **8a**, **8b**, or **8c** (1.40 mmol) was added to a stirred solution comprising THF (50 mL), MeOH (50 mL), and LiOH (50 mL of 2 N). This mixture was stirred for 15 h at 25 °C, NaOH (200 mL of 1N) was added, and the mixture was extracted with EtOAc (200 mL). The aqueous phase was acidified with concentrated HCl (38 mL) to pH 1.0 prior to extraction with EtOAc (300 mL), the organic fraction was dried ($MgSO_4$), filtered, and the solvent was removed in vacuo to furnish the respective acid (**9a**, **9b**, or **9c**) for which the physical and spectral data are listed below.

5.3.1. 1-(4-Methanesulfonylphenyl)-5-phenyl-1*H*-pyrazol-3-carboxylic acid (9a)

Yield, 91%; pale brown powder; mp 181–182 °C; IR (film) 3525–3140 (OH), 2965 (C–H aromatic), 2927 (C–H aliphatic), 1717 (CO_2), 1317, 1154 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 3.09 (s, 3H, SO_2CH_3), 7.14 (s, 1H, pyrazole H-4), 7.26 (m, 3H, phenyl H-3, H-4, H-5), 7.42 (m, 2H, phenyl H-2, H-6), 7.76 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.96 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5); MS 365.04 (M+Na).

5.3.2. 5-(4-Fluorophenyl)-1-(4-methanesulfonylphenyl)-1*H*-pyrazol-3-carboxylic acid (9b)

Yield, 63%; pale brown powder; mp 213–215 °C; IR (film) 3583–3280 (OH), 2970 (C–H aromatic), 2930 (C–H aliphatic), 1704 (CO_2), 1299, 1073 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 3.05 (s, 3H, SO_2CH_3), 6.92 (s, 1H, pyrazole H-4), 7.03 (dd, J = 8.5, 8.5 Hz, 2H, fluorophenyl H-3, H-5), 7.19 (dd, J = 8.5, 3.7 Hz, 2H, fluorophenyl H-3, H-5), 7.49 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.87 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5), 12.98 (br s, 1H, COOH); MS 383.05 (M+Na).

5.3.3. 1-(4-Methanesulfonylphenyl)-5-(4-methylphenyl)-1*H*-pyrazol-3-carboxylic acid (9c)

Yield, 82%; white powder; mp 242–243 °C; IR (film) 3566–3306 (OH), 2964 (C–H aromatic), 2913 (C–H aliphatic), 1700 (CO_2), 1314, 1153 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 2.30 (s, 3H, phenyl CH_3), 3.04 (s, 3H, SO_2CH_3), 6.89 (s, 1H, pyrazole H-4), 7.08 (m, 4H, methylphenyl H-2, H-3, H-5, H-6), 7.49 (d, J = 8.6 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.84 (d, J = 8.6 Hz, 2H, methanesulfonylphenyl H-3, H-5), 12.57 (br s, 1H, COOH); MS 379.01 (M+Na).

5.4. General method for preparation of O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate pyrazole esters (11a–c)

The sodium salt of each acid **9a**, **9b**, or **9c** (R = H, F, Me) was prepared in situ by stirring the acid (**8a**, **8b**, or **8c**, 2.5 mmol) in a suspension of sodium carbonate (0.27 g, 2.5 mmol) and HMPA

(3.5 mL) for 24 h at 25 °C. A solution of O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**10**, 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 from the organic fraction was removed in vacuo. The residue obtained was purified by silica gel column chromatography using EtOAc:hexane (2:1, v/v) as eluent to afford the respective product **11a**, **11b**, or **11c** for which the physical and spectral data are listed below.

5.4.1. O^2-Acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate 1-(4-methanesulfonylphenyl)-5-phenyl-1*H*-pyrazol-3-carboxylate (11a)

Yield, 39%; white powder; mp 95–97 °C; IR (film) 2970 (C–H aromatic), 2920 (C–H aliphatic), 1744 (CO_2), 1322, 1148 (SO_2), 1226, 1070 (N═N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.09 (s, 3H, $COCH_3$), 3.08 (s, 3H, CH_3SO_2), 3.20 (s, 3H, NCH_3), 3.86, (t, J = 5.7 Hz, 2H, CH_2N), 4.60 (t, J = 5.7 Hz, 2H, CO_2CH_2), 5.77 (s, 2H, OCH_2O), 7.05 (s, 1H, pyrazole H-4), 7.25 (m, 3H, phenyl H-3, H-4, H-5), 7.40 (m, 2H, phenyl H-2, H-6), 7.56 (d, J = 9.1 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.95 (d, J = 9.1 Hz, 2H, methanesulfonylphenyl H-3, H-5); MS 554.07 (M+Na). Anal. Calcd for $C_{23}H_{25}N_5O_8S$: C, 51.97; H, 4.74; N, 13.18. Found: C, 51.71; H, 4.99; N, 12.94.

5.4.2. O^2-Acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate 5-(4-fluorophenyl)-1-(4-methanesulfonylphenyl)-1*H*-pyrazol-3-carboxylate (11b)

Yield, 22%; white powder; mp 120–122 °C; IR (film) 2972 (C–H aromatic), 2928 (C–H aliphatic), 1730 (CO_2), 1317, 1157 (SO_2), 1221, 1065 (N═N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.09 (s, 3H, $COCH_3$), 3.08 (s, 3H, CH_3SO_2), 3.19 (s, 3H, NCH_3), 3.85 (t, J = 5.7 Hz, 2H, CH_2N), 4.60 (t, J = 5.7 Hz, 2H, CO_2CH_2), 5.77 (s, 2H, OCH_2O), 7.03 (s, 1H, pyrazole H-4), 7.09 (dd, J = 8.5, 8.5 Hz, 2H, fluorophenyl H-3, H-5), 7.24 (dd, J = 8.5, 3.7 Hz, 2H, fluorophenyl H-2, H-6), 7.55 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.96 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5); MS 572.01 (M+Na). Anal. Calcd for $C_{23}H_{24}FN_5O_8S$: C, 50.27; H, 4.40; N, 12.74. Found: C, 50.28; H, 4.47; N, 12.53.

5.4.3. O^2-Acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate 1-(4-methanesulfonylphenyl)-5-(4-methylphenyl)-1*H*-pyrazol-3-carboxylate (11c)

Yield, 28%; white crystals; mp 75–77 °C; IR (film) 2970 (C–H aromatic), 2920 (C–H aliphatic), 1738 (CO_2), 1318, 1153 (SO_2), 1221, 1065 (N═N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.10 (s, 3H, $COCH_3$), 2.39 (s, 3H, phenyl CH_3), 3.08 (s, 3H, CH_3SO_2), 3.20 (s, 3H, NCH_3), 3.86, (t, J = 5.5 Hz, 2H, CH_2N), 4.59 (t, J = 5.5 Hz, 2H, CO_2CH_2), 5.77 (s, 2H, OCH_2O), 7.01 (s, 1H, pyrazole H-4), 7.11 (d, J = 8.3 Hz, 2H, methylphenyl H-3, H-5), 7.18 (d, J = 8.3 Hz, 2H, methylphenyl H-2, H-6), 7.56 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.94 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5),; MS 546.07 (M+1). Anal. Calcd for $C_{24}H_{27}N_5O_8S.1/2H_2O$: C, 51.98; H, 5.00; N, 12.63. Found: C, 51.85; H, 4.90; N, 12.94.

5.5. Nitric oxide release assays

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, 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–c**), and the reference compound O^2-sodium 1-[*N*-(2-hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**12**) using the reported procedures.[32]

5.6. In vivo anti-inflammatory assay

The test compounds **9a–c**, and the reference drugs celecoxib and aspirin, were evaluated using the in vivo carrageenan-induced foot paw edema model reported previously.[33]

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