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Diazen-1-ium-1,2-diolated nitric oxide donor ester prodrugs of 5-(4-hydroxymethylphenyl)-1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole and its methanesulfonyl analog: Synthesis, biological evaluation and nitric oxide release studies

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ABSTRACT

A new class of hybrid nitric oxide-releasing anti-inflammatory (AI) ester prodrugs (NONO-coxibs **12a–b**) wherein an O^2-acetoxymethyl 1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**11**, O^2-acetoxymethyl PROLI/NO) NO-donor moiety was covalently coupled to the bromomethyl group of 5-(4-bromomethylphenyl)-1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**9a**), and its methanesulfonyl analog (**9b**), were synthesized. The diazen-1-ium-1,2-diolate compounds **12a–b** released a low amount of NO upon incubation with phosphate buffer (PBS) at pH 7.4 (6.1–8.2% range). In comparison, the percentage NO released was significantly higher (76–77% 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 **12a–b** were incubated in the presence of rat serum. These incubation studies suggest that both NO and the anti-inflammatory 5-(4-hydroxymethylphenyl)-1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**10a**), and its methanesulfonyl analog (**10b**), would be released from the parent NONO-coxib **12a** or **12b** upon in vivo cleavage by non-specific serum esterases. The hydroxymethyl compounds **10a–b** were weak inhibitors of the cyclooxygenase-1 (COX-1) and COX-2 isozymes (IC_{50} = 3.7–10.5 μM range). However, the hydroxymethyl compounds **10a–b** and the parent NONO-coxibs **12a–b** exhibited good AI activities (ED_{50} = 76.7–111.6 μmol/kg po range) that were greater than that exhibited by the reference drugs aspirin (ED_{50} = 710 μmol/kg po) and ibuprofen (ED_{50} = 327 μmol/kg po), but less than that of celecoxib (ED_{50} = 30.9 μmol/kg po). These studies indicate hybrid ester AI/NO-donor prodrugs (NONO-coxibs) constitutes a plausible drug design concept targeted toward the development of selective COX-2 inhibitory AI drugs that are devoid of adverse cardiovascular effects.

1. Introduction

Selective inhibition of the inducible cyclooxygenase-2 (COX-2) isozyme in the periphery provided a useful drug design concept that resulted in the development of effective anti-inflammatory (AI) drugs that were devoid of adverse gastrointestinal ulcerogenicity frequently associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs).[1] Unfortunately, some selective COX-2 inhibitory drugs that include rofecoxib (**1**) and valdecoxib (**2**) alter the natural balance in the COX pathway (see structures in Fig. 1). In this regard, the amount of the desirable vasodilatory and anti-aggregatory prostacyclin (PGI_2) produced is decreased together with a simultaneous increase in the level of the undesirable prothrombotic thromboxane A_2 (TxA_2).[2–4] These two adverse biochemical changes in the COX pathway are believed to be responsible for increased incidences of high blood pressure and myocardial infarction that ultimately prompted the withdrawal of rofecoxib (Vioxx®) and valdecoxib (Bextra®).[5,6] Nitric oxide (NO) exhibits a number of useful pharmacological actions that include vascular relaxation (vasodilation), and inhibition of platelet aggregation and adhesion.[7] In an earlier study we reported non-ulcerogenic NONO-NSAID ester prodrugs of aspirin (**4**) and indomethacin (**5**) having a NO-donor diazen-1-ium-1,2-diolate (NONOate) moiety that are effectively cleaved by esterases to release the AI drug and NO.[8] Accordingly, attachment of a *N*-diazen-1-ium-1,2-diolate moiety offers a potential drug design concept to circumvent the adverse cardiovascular events associated with the chronic clinical use of highly selective COX-2 inhibitors. We now describe an investigation directed toward the design and synthesis of model

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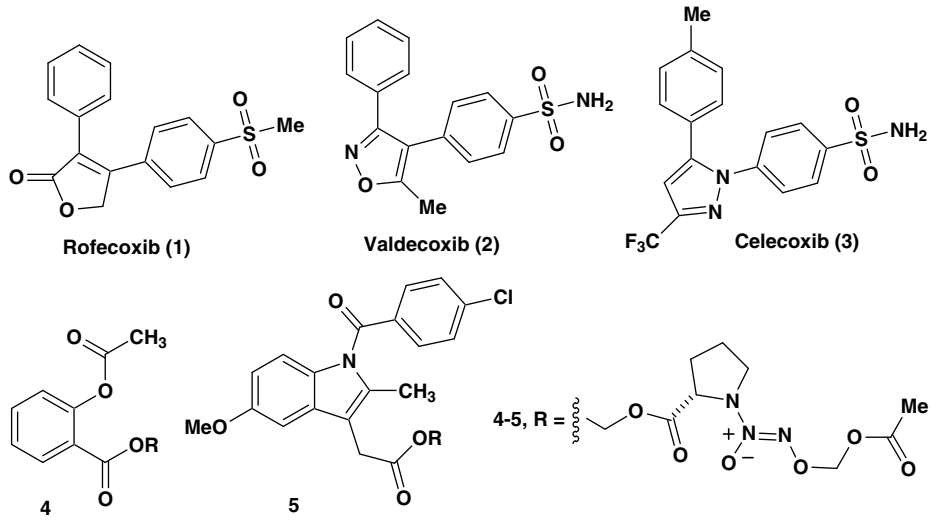
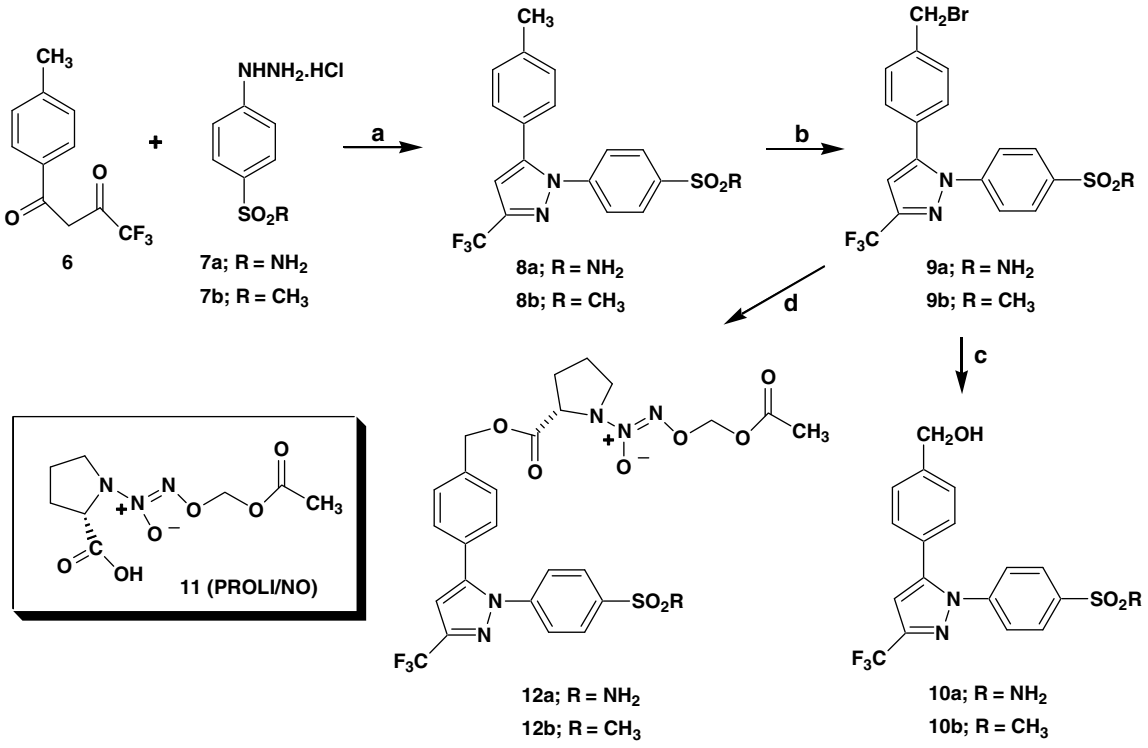


Figure 1. Chemical structures of the selective cyclooxygenase-2 (COX-2) inhibitors rofecoxib (**1**), valdecoxib (**2**) and celecoxib (**3**), and the second-generation O^2-acetoxymethyl-1-(2-carboxypyrrolidin-1-yl)diazenium-1,2-diolate nitric oxide donor (O^2-acetoxymethyl PROLI/NO) prodrug esters of aspirin (**4**) and indomethacin (**5**).

hybrid nitric oxide donor *N*-diazen-1-ium-1,2-diolate ester prodrugs of 5-(4-hydroxymethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**12a**), and its methanesulfonyl analog (**12b**), that may be devoid of adverse cardiovascular effects.

2. Chemistry

The two 5-(4-hydroxymethylphenyl)-1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**10a**), and its methanesulfonyl analog (**10b**), and the two O^2-acetoxymethyl PROLI/NO prodrugs (**12a–b**), were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, reaction of the dione **6** with either 4-aminosulfonylphenylhydrazine hydrochloride (**7a**), or 4-methylsulfonylphenylhydrazine hydrochloride (**7b**), afforded the respective pyrazole analog **8a** (56%) or **8b** (44%). Subsequent photochemical promoted bromination of the tolyl methyl group present in compounds **8a–b** afforded the respective benzyl bromide derivative **9a**, or **9b**, in 46% yields. The bromomethyl compounds **9a–b** were converted to the respective hydroxymethyl analogs **10a–b** in acceptable yield (54–66%) upon heating under reflux in an acetone/water solvent system (30:4, v/v) for 110 h. The target O^2-acetoxymethyl PROLI/NO prodrug esters (**12a–b**) were synthesized in 45% yields by condensation of the bromomethyl compounds **9a–b** with O^2-acetoxymethyl 1-(2-carboxypyrrolidin-1-



Scheme 1. Reagents and conditions: (a) C_2H_5OH, reflux, 20 h; (b) *N*-bromosuccinimide, benzoyl peroxide, light from a 100-W sun beam lamp, benzene, 25 °C → reflux, 3.5–5 h; (c) acetone, H_2O, reflux, 110 h; (d) O^2-acetoxymethyl 1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**11**), Et_3N, DMSO, 25 °C, 24 h.

yl)diazen-1-ium-1,2-diolate (**11**) in the presence of triethylamine in dimethyl sulfoxide.

3. Results and discussion

Three positions on the structure of celecoxib (**3**) were considered for attachment of an O^2-acetoxymethyl PROLI/NO group via an ester moiety. The *para*-position on the N^1-phenyl ring was not selected since a COX-2 pharmacophore such as a $MeSO_2$ or H_2NSO_2 substituent is required at this location for potent and selective COX-2 inhibitory activity.[9] Although the pyrazole ring C-3 position has very few steric restrictions with respect to COX-2 inhibition properties, the electronegative CF_3 substituent was retained since it generally provides optimal COX-2 potency.[10] The C-5 *para*-methylphenyl substituent (benzylic carbon) in celecoxib provides high selectivity for the COX-2 isozyme. Even though celecoxib undergoes sequential metabolic biotransformation (Me → CH_2OH → CO_2H → CO_2-glucuronide conjugate), it still exhibits potent anti-inflammatory activity.[11] We envisaged, from a synthetic perspective, that a *p*-CH_2Br substituent on the pyrazole C-5 phenyl ring was a suitable substituent for coupling the O^2-acetoxymethyl PROLI/NO moiety. Accordingly, it was decided based on this structural information to couple the O^2-acetoxymethyl PROLI/NO nitric oxide donor moiety (**11**) to a pyrazole ring C-5 *p*-C_6H_4–CH_2Br group via an ester moiety to prepare the target NONO-coxib hybrid ester prodrugs (**12a–b**).

In vitro COX enzyme inhibition studies (Table 1) showed that the hydroxymethyl (CH_2OH) compounds **10a** (COX-1 IC_{50} = 7.1 μM; COX-2 IC_{50} = 3.7 μM) and **10b** (COX-1 IC_{50} = 8.6 μM; COX-2 IC_{50} = 10.5 μM) exhibited similar COX-1 inhibition, but weaker COX-2 inhibition, than the reference drug celecoxib (COX-1 IC_{50} = 7.7 μM; COX-2 IC_{50} = 0.07 μM). In contrast to the hydroxymethyl compounds **10a–b**, which are non-selective COX inhibitors, the PROLI/NO hybrid ester prodrug **12a** was a selective COX-2 inhibitior (COX-1 IC_{50} > 100 μM; COX-2 IC_{50} = 4.9 μM).

Table 1
In vitro COX-1/COX-2 enzyme inhibition, in vivo anti-inflammatory activity, and in vitro nitric oxide release data for the 5-(4-hydroxymethylphenyl)-1-[4-methane(amino)sulfonylphenyl]-3-trifluoromethyl-1*H*-pyrazoles (**10a–b**) and the diazen-1-ium-1,2-diolate prodrug esters (**12a–b**)

Compound	COX-1 IC_{50}[a] (μM)	COX-2 IC_{50}[a] (μM)	AI activity[b] ED_{50} (μmol/kg)	% NO released[c]	
				PBS[d]	Serum[e]
10a	7.1	3.7	77.1	—	—
10b	8.6	10.5	111.6	—	—
12a	>100	4.9	94.3	8.2	76.0
12b	ND[f]	ND	76.7	6.1	77.0
Celecoxib	7.7	0.07	30.9	—	—
Aspirin	0.3	2.4	710	—	—
Ibuprofen	2.9	1.1	327	—	—

[a] The in vitro test compound concentration required to produce 50% inhibition of COX-1 or COX-2. The result (IC_{50}, μM) is the mean 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] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as the ED_{50} value (μmol/kg) at 3 h after oral administration (n = 3) of the test compound.

[c] Percent of nitric oxide released based on a theoretical maximum release of 2 mol of nitric oxide/mol of the diazen-1-ium-1,2-diolate test compound (**12a–b**). The result is the mean value of three measurements (n = 3) where variation from the mean% value was ⩽0.5%.

[d] A solution of the test compound (2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer containing a small amount of DMSO at pH 7.4),[17] 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 containing a small amount of DMSO at pH 7.4 to which 90 μL rat serum had been added),[17] was incubated at 37 °C for 1.5 h.

[f] ND = not determined.

The percent NO released from the hybrid ester PROLI/NO prodrugs (**12a–b**) 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). The rate of NO release from diazen-1-ium-1,2-diolates can be controlled by chemical modification such as attachment of an alkyl substituent to the O^2-position.[12] O^2-Substituted-diazen-1-ium-1,2-diolates are stable compounds that hydrolyze slowly even in acidic solution.[13] Consistent with these observations, when compounds **12a–b** were incubated for 1.5 h in PBS at pH 7.4, the percentage of NO released varied from 6.1% to 8.2% which is indicative of slow NO release. In contrast, the effect of non-specific esterases present in rat serum on the NO release properties of compounds **12a–b** was substantially higher (76–77% 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 **12a–b** can not release NO prior to cleavage of the acetoxy moiety present in the terminal O^2-acetoxymethyl- PROLI/NO moiety. Two plausible pathways for the ester hydrolysis of hybrid PROLI/NO ester prodrugs and the subsequent release of acetic acid, formaldehyde, two molecules of NO, and L-proline was described in an earlier publication.[8] The hybrid ester NO-donor prodrugs **12a–b** 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 release formaldehyde to furnish the free diazen-1-ium-1,2-diolate compound that can subsequently fragment to release two molecules of NO. On the other hand, cleavage of the second ester group attached to the C-5 position of the pyrazole ring, that releases the hydroxymethyl compounds **10a–b**, can occur either prior to, or after, NO release has occurred.

The anti-inflammatory (AI) activities exhibited by the 5-(4-hydroxymethylphenyl)-1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**10a**, R = NH_2) and its methanesulfonyl analog (**10b**, R = CH_3) that would be released upon cleavage of the ester group attached at the C-5 position of the pyrazole ring, and the hybrid PROLI/NO ester prodrugs **12a–b** were determined using a carrageenan-induced rat foot paw edema model (see data in Table 1). Compounds **10a–b** and **12a–b** exhibited AI activities (ED_{50} = 76.7–111.6 μmol/kg po range) that were greater than that exhibited by the reference drugs aspirin (ED_{50} = 710 μmol/kg po) and ibuprofen (ED_{50} = 327 μmol/kg po), but less than that of celecoxib (ED_{50} = 30.9 μmol/kg po). The relative AI potency profile with respect to the SO_2NH_2 and SO_2Me substituents was variable with SO_2NH_2 (**10a**) > SO_2Me (**10b**) for the hydroxymethyl compounds, but SO_2Me (**12b**) > SO_2NH_2 (**12a**) for the parent PROLI/NO prodrug esters. A comparison of AI potency profiles showed that hydroxymethyl compound **10a** was 1.22-fold more potent than the PROLI/NO prodrug (**12a**). In contrast, the PROLI/NO prodrug **12b** was 1.45-fold more potent than the hydroxymethyl compound **10b**. These AI data provide credence for the drug design concept that covalent attachment of a PROLI/NO-donor moiety directly to a suitably positioned *p*-C_6H_4–CH_2Br group in a selective COX-2 inhibitor offers a potential method to circumvent adverse cardiovascular effects.

4. Conclusions

A novel type of hybrid ester prodrugs (NONO-coxibs) (**12a–b**) in which an O^2-acetoxymethyl PROLI/NO nitric oxide donor moiety is attached to the CH_2Br group of 5-(4-bromomethylphenyl)-1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**9a**), and its methanesulfonyl analog (**9b**) were synthesized for comparative biological evaluation. COX-1/COX-2 inhibition, NO release, and AI studies showed that (i) the 5-(4-hydroxymethylphenyl)-1-(4-

aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**10a**), or its methanesulfonyl analog (**10b**) that would be released after ester hydrolysis are weak non-selective inhibitors of the COX-1 and COX-2 isozymes, (ii) the parent NONO-coxib prodrugs (**12a–b**) are relatively stable in phosphate-buffered saline at pH 7 where NO release is in the 6.1–8.2% range, (iii) the hybrid PROLI/NO ester prodrugs (**12a–b**) undergo extensive cleavage of the terminal acetoxy group by rat serum esterase(s) that is followed by a significant release of NO in the 76–77% range, and (iv) the relatively potent AI activity exhibited by the hydroxymethyl compounds **10a–b** (ED_{50} = 77.1–111.6 μmol/kg po range) supports the drug design concept that covalent attachment of the second-generation PROLI/NO-donor moiety directly to a suitably positioned *p*-C_6H_4–CH_2Br group present in a selective COX-2 inhibitor offers a rational approach to circumvent adverse cardiovascular 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. Microanalyses were performed for C, H, N (Micro Analytical 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. 1-(4-Methylphenyl)-4,4,4-trifluorobutan-1,3-dione (**6**),[9] 4-sulfamoylphenylhydrazine hydrochloride (**7a**)[14], 4-methylsulfonylphenylhydrazine hydrochloride (**7b**)[15] and O^2-acetoxymethyl 1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**11**)[8] were prepared according to literature procedures.

5.1. 4-[5-(4-Methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonamide (8a)

4-Sulfamoylphenylhydrazine hydrochloride (**7a**, 0.980 g, 4.4 mmol) was added to a stirred solution of the dione **6** (0.921 g, 4.0 mmol) in EtOH (50 mL), and the reaction was allowed to proceed at reflux for 20 h with stirring. After cooling to 25 °C, the solvent was removed in vacuo. The residue was dissolved in EtOAc (25 mL), washed with water and then brine, the EtOAc fraction was dried ($MgSO_4$), filtered, and the solvent was removed in vacuo to furnish **8a** (0.91 g, 56%) as a white powder; mp 157–159 °C (lit.[9] mp 157–159 °C); IR (film) 3358, 3263 (NH_2), 2979 (C–H aromatic), 2920 (C–H aliphatic), 1339, 1169 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H, C*H*$_3$), 4.92 (br s, 2H, N*H*$_2$), 6.75 (s, 1H, pyrazole H-4), 7.12 (d, *J* = 7.9 Hz, 2H, methylphenyl H-3, H-5), 7.19 (d, *J* = 7.9 Hz, 2H, methylphenyl H-2, H-6), 7.49 (dd, *J* = 6.8, 1.9 Hz, 2H, sulfamoylphenyl H-3, H-5), 7.92 (dd, *J* = 6.8, 1.9 Hz, 2H, sulfamoylphenyl H-2, H-6).

5.2. 1-(4-Methanesulfonylphenyl)-5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazole (8b)

The title compound **8b** was synthesized using the same procedure described for the preparation of **8a**, by using 4-methylsulfonylphenylhydrazine hydrochloride (**7b**) in place of **7a**, in 44% as a yellow powder; IR (film) 3025 (C–H aromatic), 2926 (C–H aliphatic), 1320, 1157 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H, phenyl C*H*$_3$), 3.07 (s, 3H, SO_2C*H*$_3$), 6.76 (s, 1H, pyrazole H-4), 7.12 (d, *J* = 8.1 Hz, 2H, methylphenyl H-3, H-5), 7.20 (d, *J* = 8.1 Hz, 2H, methylphenyl H-2, H-6), 7.54 (d, *J* = 7.9 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.95 (d, *J* = 7.9 Hz, 2H, methanesulfonylphenyl H-3, H-5). This ^{1}H NMR spectral data for **8b** is identical to the previously reported data.[10]

5.3. 4-[5-(4-Bromomethylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonamide (9a)

N-Bromosuccinimide (1.32 g, 7.43 mmol) was added to a stirred solution of **8a** (3.02 g, 7.43 mmol) in benzene (100 mL). Benzoyl peroxide (0.177 g, 0.73 mmol) was added and the reaction mixture was then irradiated with light from a 100-W sun beam lamp for 3.5 h. After cooling to 25 °C, the reaction mixture was filtered, the filtrate was washed with water and then brine, the filtrate was dried (Na_2SO_4), filtered, and the solvent was removed from the filtrate in vacuo. The residue was purified by silica gel column chromatography using a gradient of EtOAc/hexane (1:9, v/v) to EtOAc/hexane (3:7, v/v) as the eluent to give **9a** (1.57 g, 46%) as a white powder: mp 154–156 °C; IR (film) 3370, 3268 (NH_2), 2965 (C–H aromatic), 2925 (C–H aliphatic), 1345, 1165 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.50 (s, 2H, C*H*$_2$Br), 4.92 (br s, 2H, N*H*$_2$), 6.80 (s, 1H, H-4), 7.22 (d, *J* = 8.0 Hz, 2H, bromomethylphenyl H-3, H-5), 7.42 (d, *J* = 8.0 Hz, 2H, bromomethylphenyl H-2, H-6), 7.50 (dd, *J* = 6.7, 2.4 Hz, 2H, sulfamoylphenyl H-3, H-5), 7.94 (dd, *J* = 6.7, 2.4 Hz, 2H, sulfamoylphenyl H-2, H-6); ^{13}C NMR ($CDCl_3$) δ 32.2, 106.7, 120.9, 125.5, 127.5, 128.5, 129.1, 129.7, 139.3, 141.6, 142.2, 143.5, 144.4.

5.4. 5-(4-Bromomethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (9b)

N-Bromosuccinimide (1.32 g, 7.43 mmol) was added to a stirred solution of **8b** (3.01 g, 7.43 mmol) in benzene (100 mL). Benzoyl peroxide (0.177 g, 0.73 mmol) was added and the reaction mixture was irradiated with light from a 100-W sun beam lamp while heating at reflux for 4.5 h. The product **9b** was isolated and purified by silica gel column chromatography using a procedure similar to that described for **9a**. Product **9b** (1.57 g, 46%) was obtained as a yellow powder: mp 139–142 °C; IR (film) 2965 (C–H aromatic), 2928 (C–H aliphatic), 1318, 1156 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.07 (s, 3H, SO_2C*H*$_3$), 4.50 (s, 2H, C*H*$_2$Br), 6.81 (s, 1H, pyrazole H-4), 7.24 (d, *J* = 8.3 Hz, 2H, bromomethylphenyl H-3, H-5), 7.43 (d, *J* = 8.3 Hz, 2H, bromomethylphenyl H-2, H-6), 7.55 (dd, *J* = 6.7, 1.7 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.97 (dd, *J* = 6.7, 1.7 Hz, 2H, methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 32.1, 44.4, 106.9, 120.9, 125.7, 128.4, 128.6, 129.1, 129.7, 139.4, 140.1, 143.2, 144.3, 144.4.

5.5. 4-[5-(4-Hydroxymethylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonamide (10a)

A solution of the bromomethyl compound **9a** (1.16 g, 2.5 mmol) in acetone (30 mL) and water (4 mL) was refluxed for 110 h. After removal of the solvents in vacuo, the residue was dissolved in EtOAc, the EtOAc fraction was dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography using EtOAc/hexane (1:1, v/v) as eluent to give the alcohol **10a** as a pale yellow solid (0.536 g, 54%): mp 82–84 °C; IR (film) 3360, 3261 (NH_2), 2959 (C–H aromatic), 2919 (C–H aliphatic), 1340, 1159 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.75 (br s, 1H, O*H*, exchanges with D_2O), 4.76 (s, 2H, C*H*$_2$), 4.93 (br s, 2H, N*H*$_2$, exchanges with D_2O), 6.79 (s, 1H, pyrazole H-4), 7.24 (d, *J* = 7.9 Hz, 2H, hydroxymethylphenyl H-3, H-5), 7.43 (d, *J* = 7.9 Hz, 2H, hydroxymethylphenyl H-2, H-6), 7.48 (d, *J* = 8.9 Hz, 2H, sulfamoylphenyl H-3, H-5), 7.92 (d, *J* = 8.9 Hz, 2H, sulfamoylphenyl H-2, H-6); ^{13}C NMR ($CDCl_3$) δ 64.4, 106.5, 120.9, 125.5, 127.4, 127.5, 127.7, 129.0, 141.5, 142.3, 142.4, 144.1, 144.9; MS 398.08 (M+1).

5.6. 5-(4-Hydroxymethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (10b)

The title compound **10b** was synthesized and the product was purified using a procedure similar to that described for the preparation of **10a** using the bromomethyl compound **9b** in place of **9a**. The product **10b** was obtained in 66% yield as a yellow powder: mp 159–160 °C; IR (film) 3592–3196 (O–H), 2962 (C–H aromatic), 2926 (C–H aliphatic), 1319, 1153 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.71 (br s, 1H, O*H*, exchanges with D_2O), 3.08 (s, 3H, SO_2CH_3), 4.77 (s, 2H, CH_2), 6.70 (s, 1H, pyrazole H-4), 7.25 (d, J = 7.9 Hz, 2H, hydroxymethylphenyl H-3, H-5), 7.41 (d, J = 7.9 Hz, 2H, hydroxymethylphenyl H-2, H-6), 7.55 (dd, J = 7.1, 1.9 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.62 (dd, J = 7.1, 1.9 Hz, 2H, methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 44.5, 64.5, 106.7, 120.9, 125.7, 127.4, 127.6, 128.5, 129.0, 139.9, 142.6, 143.3, 144.3, 144.9; MS 397.04 (M+1).

5.7. *O*^{2}-Acetoxymethyl 1-{2-[4-(1-(4-sulfamoylphenyl)-3-trifluoromethyl-1*H*-pyrazol-5-yl)phenylmethoxycarbonyl]pyrrolidin-1-yl}diazen-1-ium-1,2-diolate (12a)

A solution of compound **11** (0.20 g, 0.8 mmol) in DMSO (1 mL) and triethylamine (0.1 mL, 0.8 mmol) was stirred at 25 °C for 5 min. A solution of compound **9a** (0.368 g, 0.8 mmol) in DMSO (1 mL) was added and the reaction was allowed to proceed for 24 h at 25 °C with stirring. Ethyl acetate (50 mL) was added to dilute the reaction mixture, the organic phase was washed with water (5× 15 mL), dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography using EtOAc/hexane (1:1, v/v) as eluent to give **12a** as a white powder (0.226 g, 45%): mp 74–76 °C; $[\alpha]_D^{21.0}$ +35.1 (1.0000, $CHCl_3$); IR (film) 3377, 3260 (NH_2), 2963 (C–H aromatic), 2913 (C–H aliphatic), 1749 (CO_2), 1344, 1164 (SO_2), 1273, 1135 (N═N–O) cm^1; ^1H NMR ($CDCl_3$) δ 2.06–2.13 (m, 3H, pyrrolidin-1-yl H-3, H-4, H′-4), 2.12 (s, 3H, $COCH_3$), 2.32–2.39 (m, 1H, pyrrolidin-1-yl H′-3), 3.68–3.77 (m, 1H, pyrrolidin-1-yl H-5), 3.83–3.91 (m, 1H, pyrrolidin-1-yl H′-5), 4.62 (dd, J = 8.5, 3.8 Hz, 1H, pyrrolidin-1-yl H-2), 5.15 (d, J = 12.9 Hz, 1H, –C*H*H′OCO), 5.22 (br s, 2H, NH_2, exchanges with D_2O), 5.29 (d, J = 12.9, 1H, CH*H*′OCO), 5.68 (s, 2H, OCH_2O), 6.80 (s, 1H, pyrazole H-4), 7.22 (d, J = 8.0 Hz, 2H, benzyl H-3, H-5), 7.36 (d, J = 8.0 Hz, 2H, benzyl H-2, H-6), 7.40 (d, J = 6.8 Hz, 2H, sulfamoylphenyl H-2, H-6), 7.94 (d, J = 6.8 Hz, 2H, sulfamoylphenyl H-3, H-5); MS 627.15 (M+1); ^{13}C NMR ($CDCl_3$) δ 20.8, 22.4, 27.8, 50.8, 61.6, 66.2, 87.1, 106.4, 120.9, 125.4, 127.5, 128.4, 128.6, 129.1, 137.0, 141.9, 143.5, 144.3, 144.5, 169.8, 170.9. Anal. Calcd for $C_{25}H_{25}F_3N_6O_8S{\cdot}1/2H_2O$: C, 47.25; H, 4.12; N, 13.12. Found: C, 47.87; H, 4.96; N, 12.54.

5.8. *O*^{2}-Acetoxymethyl 1-{2-[4-(1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazol-5-yl)phenylmethoxycarbonyl]-pyrrolidin-1-yl}diazen-1-ium-1,2-diolate (12b)

The title compound **12b** was synthesized using the same procedure described for the preparation of **12a**, except that **9b** was used in place of **9a**. Product **12b** was obtained in 45% yield as a yellow solid: mp 66–68 °C; $[\alpha]_{21.0}^{D}$ +1.74 (1.0000, $CHCl_3$); IR (film) 2962 (C–H aromatic), 2927 (C–H aliphatic), 1755 (CO_2), 1344, 1159 (SO_2), 1238, 1102 (N═N–O) cm^1; ^1H NMR ($CDCl_3$) δ 2.07–2.16 (m, 3H, pyrrolidin-1-yl H-3, H-4, H′-4), 2.14 (s, 3H, $COCH_3$), 2.31–2.42 (m, 1H, pyrrolidin-1-yl H′-3), 3.09 (s, 3H, SO_2CH_3), 3.73–3.79 (m, 1H, pyrrolidin-1-yl H-5), 3.89–3.95 (m, 1H, pyrrolidin-1-yl H′-5), 4.64 (dd, J = 9.1, 3.8 Hz, 1H, pyrrolidin-1-yl H-2), 5.17 (d, J = 12.8 Hz, 1H, –C*H*H′OCO), 5.28 (d, J = 12.8, 1H, CH*H*′OCO), 5.71 (d, J = 7.4 Hz, 1H, –OC*H*H′O–), 5.74 (d, J = 7.4, 1H, –OCH*H*′O–), 6.80 (s, 1H, pyrazole H-4), 7.25 (d, J = 8.5 Hz, 2H, benzyl H-3, H-5), 7.38 (d, J = 8.50 Hz, 2H, benzyl H-2, H-6), 7.88 (d, J = 9.2 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.97 (d, J = 9.2 Hz, 2H, methanesulfonylphenyl H-3, H-5); MS 626.15 (M+1). Anal. Calcd for $C_{26}H_{26}F_3N_5O_8S{\cdot}1/4H_2O$: C, 49.57; H, 4.24; N, 11.12. Found: C, 49.92; H, 4.64; N, 10.74.

6. Cyclooxygenase 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 No. 560101, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method.[16]

7. 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 (**12a–b**) using the reported procedures.[17]

8. In vivo anti-inflammatory assay

The test compounds **10a–b, 12a–b**, and the reference drugs celecoxib, ibuprofen and aspirin were evaluated using the in vivo carrageenan-induced foot paw edema model reported previously.[18]

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