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Diazen-1-ium-1,2-diolated nitric oxide donor ester prodrugs of 5-(4-carboxymethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole and its aminosulfonyl 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) wherein an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**13a–b**), or O^2-acetoxymethyl-1-(2-methylpyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**16a–b**), NO-donor moiety was covalently coupled to the COOH group of 5-(4-carboxymethylphenyl)-1-(4-methane(amino) sulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**11a–b**) was synthesized. The percentage of NO released from these diazen-1-ium-1,2-diolates was significantly higher (59.6–74.6% of the theoretical maximal release of 2 molecules of NO/molecule of the parent hybrid ester prodrug) upon incubation in the presence of rat serum, relative to incubation with phosphate buffer (PBS) at pH 7.4 (5.0–7.2% range). These incubation studies suggest that both NO and the AI compound would be released from the parent NONO-coxib upon in vivo cleavage by non-specific *serum* esterases. All compounds were weak inhibitors of the COX-1 isozyme (IC_{50} = 8.1–65.2 μM range) and modest inhibitors of the COX-2 isozyme (IC_{50} = 0.9–4.6 μM range). The most potent parent aminosulfonyl compound **11b** exhibited AI activity that was about sixfold greater than that for aspirin and threefold greater than that for ibuprofen. The ester prodrugs **13b**, **16b** exhibited similar AI activity to that exhibited by the more potent parent acid **11b** when the same oral μmol/kg dose was administered. These studies indicate hybrid ester AI/NO donor prodrugs of this type (NONO-coxibs) constitute 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

Non-steroidal anti-inflammatory drugs (NSAIDs) represent one of the most common classes of medications used worldwide, with an estimated usage of >30 million individuals per day.[1] The beneficial anti-inflammatory (AI) and analgesic effects of these drugs have been linked to inhibition of the inducible cyclooxygenase-2 (COX-2) isozyme, whereas adverse gastrointestinal irritation and ulcerative effects are believed to be mainly due to inhibition of the constitutive cyclooxygenase isoform (COX-1). Therefore, COX-2 selective inhibitors (coxibs) such as celecoxib (Celebrex®, **1**), rofecoxib (Vioxx®, **2**), valdecoxib (Bextra®, **3**), etoricoxib (Arcoxia®, **4**) and lumiracoxib (Prexige®, **5**) have been developed for the long term treatment of patients suffering from chronic pain and inflammation.[2] 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 vasoconstrictory and prothrombotic thromboxane A_2 (TxA_2).[3–5] These two adverse biochemical changes in the COX pathway are believed to be responsible for the increased incidences of high blood pressure and myocardial infarction that ultimately prompted the withdrawal of rofecoxib (Vioxx®) and valdecoxib (Bextra®).[6,7] Nitric oxide (NO) is an effective vasodilation agent that also inhibits platelet aggregation and adhesion.[8] Therefore, NO release provides an attractive method to suppress vascular side effects associated with NSAID use.[9] In this regard, we described novel hybrid ester prodrugs having 2-nitrooxyethyl (**6**), diazen-1-ium-1,2-diolate (**7, 8**)[10,11] and other NO-donor analogs[12] that are effectively cleaved by esterases to release the parent COX-2 inhibitory compound and NO. NO-coxibs that release NO from a nitrooxy group (nitrate

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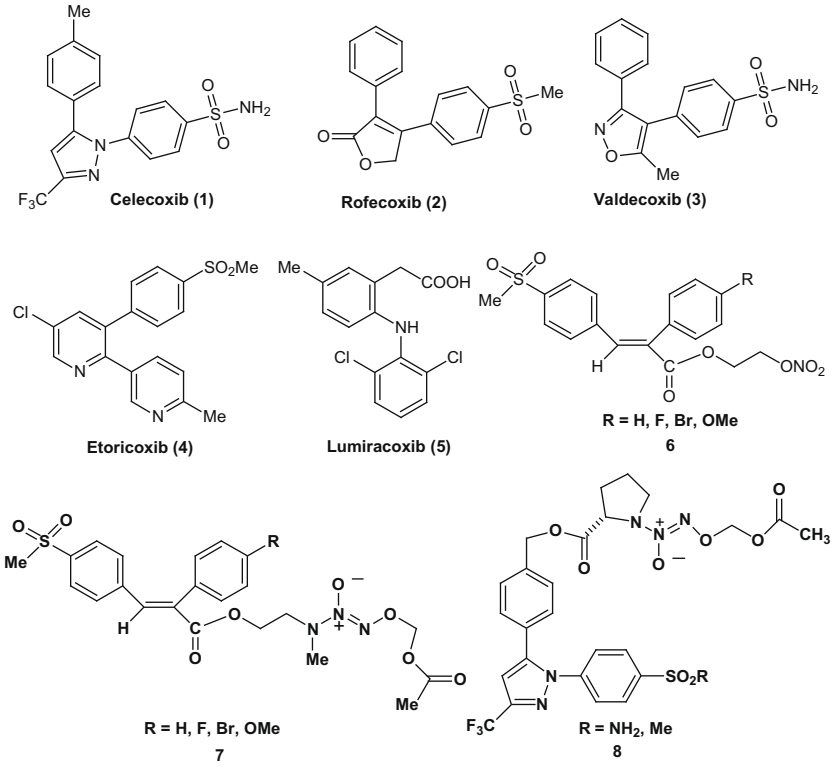


Figure 1. Chemical structures of the selective cyclooxygenase-2 (COX-2) inhibitors celecoxib (**1**), rofecoxib (**2**), valdecoxib (**3**), etoricoxib (**4**), lumiracoxib **(5**), nitrooxyethyl ester prodrugs (**6**) and diazen-1-ium-1,2-diolated ester prodrugs (**7**, **8**).

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.[13–15] We now report a group of hybrid ester prodrugs in which an (i) O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**13a–b**), or (ii) O^2-acetoxymethyl-1-[2-methylpyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**16a–b**), NO-donor moiety is attached directly to the carboxylic acid group of 5-(4-carboxymethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**11a**), and its aminosulfonyl analog (**11b**). 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.

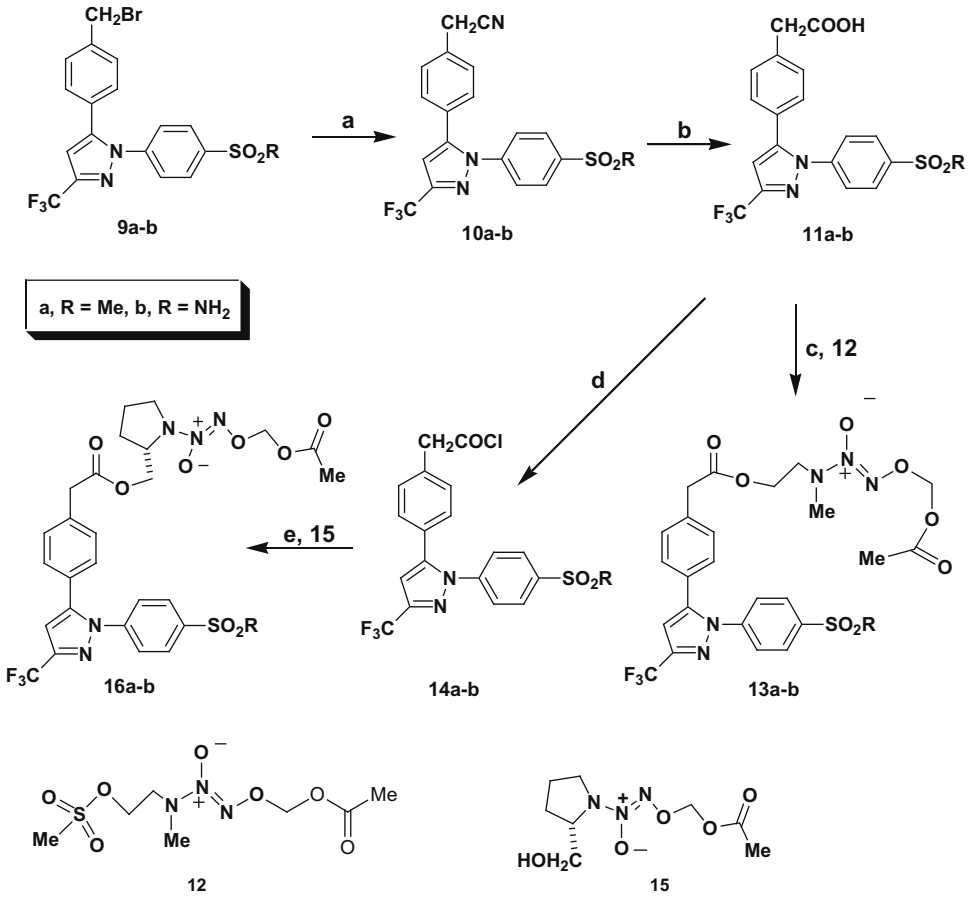
2. Chemistry

The two 5-(4-carboxymethylphenyl)-1-[4-methane(amino)sulfonylphenyl]-3-trifluoromethyl-1*H*-pyrazoles (**11a–b**), and the ester prodrugs **13a–b**, **16a–b**, were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, the bromomethyl compounds **9a–b** were converted to the respective cyanomethyl analogs **10a–b** in high yield (72–91%) upon heating under reflux with NaCN in ethanol for 2 h. Alkaline hydrolysis of the nitrile **10b** bearing a SO_2NH_2 group using aqueous NaOH afforded the corresponding acid **11b** in 72% yield. In contrast, a similar hydrolysis of **10a** having a SO_2Me substituent did not furnish the acid **11a**. The failure of this latter reaction is attributed to the low solubility of the SO_2Me compound **10a** relative to the more soluble SO_2NH_2 compound **10b**. On the other hand, hydrolysis of **10a** using 6 N HCl in dioxane yielded the acid **11a** in 27% yield. Nucleophilic displacement of the mesyloxy group present in the mesylate **12** by the respective sodium salt of the carboxylic acids **11a–b** in hexamethylphosphoramide (HMPA) afforded the target products **13a** (84% yield) and **13b** (32% yield). Elaboration of the carboxylic acids **11a–b** using oxalyl chloride gave the more reactive acid chlorides (**14a–b**) which upon subsequent reaction with a pure enantiomer of O^2-acetoxymethyl-1-(2-hydroxymethylpyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**15**) derived from L-prolinol[24] in dry THF, in the presence of the non-nucleophilic base triethylamine, furnished the two target ester prodrugs **16a–b** in good yield (51–69%).

3. Results and discussion

Three positions on the structure of celecoxib (**1**) were considered for attachment of an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate, or O^2-acetoxymethyl-1-(2-methylpyrrolidin-1-yl)diazen-1-ium-1,2-diolate, NO donor moiety via an ester linkage. 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.[16] 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.[17] The C-5 *para*-methylphenyl substituent (benzylic carbon) in celecoxib undergoes sequential metabolic biotransformation ($Me \rightarrow CH_2OH \rightarrow CO_2H \rightarrow CO_2$–glucuronide conjugate).[18] Accordingly, we decided based on these structural information to couple the NO donor moiety to a pyrazole ring C-5 *para*-C_6H_4–CH_2COOH group via an ester moiety to prepare the target NONO-coxib hybrid ester prodrugs (**13a–b**, **16a–b**).

In vitro COX-1 enzyme inhibition studies (Table 1) showed that the parent acetic acid (CH_2CO_2H) compounds **11a–b**, and the two types of diazen-1-ium-1,2-diolate derivatives **13a–b** and **16a–b**, like the reference drug celecoxib (COX-1 IC_{50} = 7.7 μM), were weak



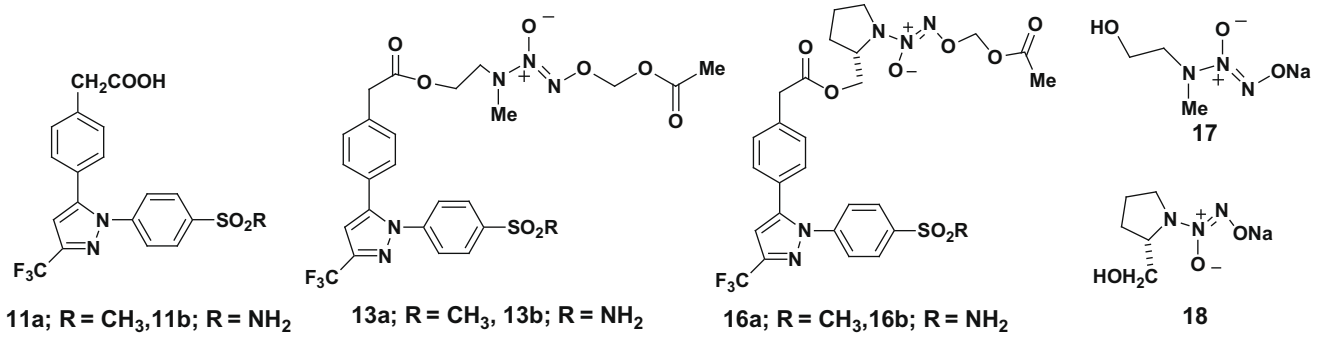
Scheme 1. Reagents and conditions: (a) NaCN, EtOH, reflux, 2 h; (b) HCl, dioxane, reflux, 16 h for **10a**, NaOH, H_2O/THF, reflux, 16 h for **10b**; (c) Na_2CO_3, hexamethylphosphoramide (HMPA), 25 °C, 96 h; (d) $(COCl)_2$, CH_2Cl_2, 25 °C, 12 h; (e) Et_3N, THF, 25 °C, 48 h.

inhibitors of COX-1 (IC_{50} = 8.1–65.2 μM range). In comparison, compounds **11a–b**, **13a–b** and **16a–b** were more potent inhibitors of COX-2 (IC_{50} = 0.9–4.6 μM range) than COX-1 that resulted in a higher selectivity for the COX-2 isozyme (COX-2 selectivity indexes in the 1.8–28.3 range). The differences in COX-2 inhibitory potencies between compounds having SO_2Me and SO_2NH_2 pharmacophores was small. Among the group of compounds **11a–b**, **13a–b** and **16a–b**, the proline diazen-1-ium-1,2-diolate derivatives **16a–b** were the most potent inhibitors of COX-2 (IC_{50} = 0.9 μM) relative to the reference drug celecoxib (COX-2 IC_{50} = 0.12 μM).

The percent NO released from the hybrid ester prodrugs **13a–b**, **16a–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.[19] O^2-substituted-diazen-1-ium-1,2-diolates are stable compounds that hydrolyze slowly even in acidic solution.[20] Consistent with these observations, when compounds **13a–b**, **16a–b** were incubated for 1.5 h in PBS at pH 7.4, the percentage of NO released varied from 5.0% to 7.2% which is indicative of slow NO release.[21] In contrast, the effect of non-specific esterases present in rat serum on the NO release properties of compounds **13a–b**, **16a–b** was substantially higher (59.6–74.6% 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 **13a–b**, **16a–b** can not 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 (**17**) and O^2-sodium 1-[2-(hydroxymethyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**18**), which do not possess an ester group that requires prior ester cleavage, released 84.5–85% of the theoretical maximal release of two molecules of NO/molecule of the parent NO-donor compound in both PBS and serum. Two plausible pathways for the ester hydrolysis of hybrid O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino) diazen-1-ium-1,2-diolate ester prodrugs and the subsequent release of acetic acid, formaldehyde, two molecules of NO, and 2-(*N*-methylamino)ethanol were described in an earlier publication.[22] Similar esterase cleavage and NO release pathways, that are applicable to the O^2-acetoxymethyl-1-[2-(methyl)pyrrolidin-1-yl)]diazen-1-ium-1,2-diolate esters (**16a–b**), are illustrated in Figure 2. Prodrugs **16a–b** were designed (i) such that the carboxyl group of the parent compounds **11a–b** is covalently attached directly to the alcohol substituent of the diazenium-1,2-diolate (**15**), and (ii) subsequent cleavage of the ester groups and release of NO would furnish the parent COX-2 inhibitor (**11a–b**) and the natural amino alcohol L-prolinol.

The anti-inflammatory (AI) activities exhibited by the 5-(4-carboxymethylphenyl)-1-[4-methane(amino)sulfonylphenyl]-3-trifluoromethyl-1*H*-pyrazoles (**11a–b**) were determined using a carrageenan-induced rat foot paw edema model (see data in Table 1). The carboxymethyl compound **11a** exhibited moderate AI activity (ED_{50} = 215.8 μmol/kg po) that was greater than that exhibited by the reference drugs aspirin (ED_{50} = 714 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 more potent aminosulfonyl analog **11b** exhibited higher AI activity (ED_{50} = 113.8 μmol/kg po) that

Table 1
In vitro COX-1 and COX-2 inhibition, percent (%) nitric oxide release and anti-inflammatory (AI) data for 5-(4-carboxymethylphenyl)-1-(4-methane(amino)sulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazoles (**11a–b**), diazeniumdiolate esters (**13a–b**, **16a–b**), O^2-sodium 1-[*N*-(hydroxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**17**), O^2-sodium 1-[2-(hydroxymethyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**18**) and the reference drugs, celecoxib, aspirin and ibuprofen



Compound	IC_{50}[a] (μM)		COX-2 S.I.[b]	% NO released[c]		AI activity[f]	
	COX-1	COX-2		PBS[d]	Serum[e]	ED_{50} (μmol/kg)	% Inhibition (113.8 μmol/kg)
11a	65.2	2.3	28.3	—	—	215.8	—
11b	8.1	1.8	4.5	—	—	113.8	50.0
13a	8.2	4.6	1.8	6.9	72.2	—	—
13b	35.4	3.5	10.1	7.2	74.6	—	55.8
16a	11.8	0.9	13.1	5.0	62.9	—	—
16b	13.4	0.9	14.4	5.4	59.6	—	46.5
17	—	—	—	84.5	84.8	—	—
18	—	—	—	84.5	85.0	—	—
Celecoxib	7.7	0.12	110	—	—	30.9	—
Aspirin	0.3	2.4[g]	0.13	—	—	714	—
Ibuprofen	2.9	1.1[g]	2.64	—	—	327	—

[a] The in vitro test compound concentration required to produce 50% inhibition of ovine COX-1 or human recombinant COX-2. The result (IC_{50}, μM) is the mean of two determinations acquired using the enzyme immuno assay kit (Catalog No. 560131, Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.

[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

[c] 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 (**13a–b**, **16a–b**, **17** and **18**). 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] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as the% inhibition of inflammation at 3 h after oral administration of the test compound at the specified dose (μmol/kg).

[g] Data acquired using ovine COX-2 (Catalog No. 56101, Cayman Chemical Inc.).

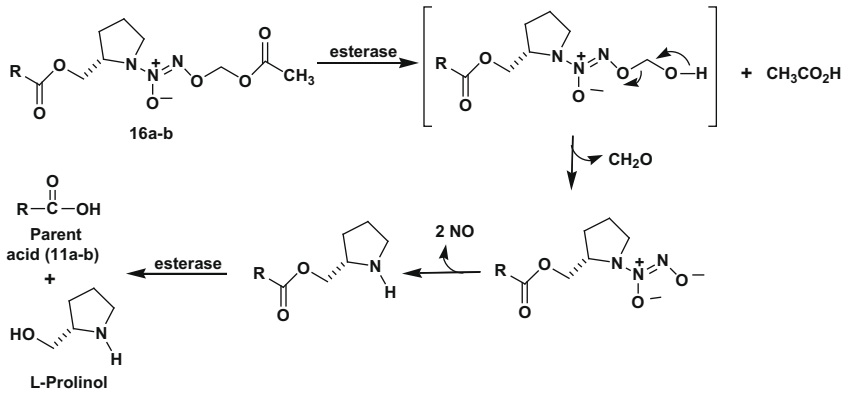


Figure 2. Theoretical metabolic activation (esterase hydrolysis) and nitric oxide release from the O^2-acetoxymethyl-1-[2-methylpyrrolidin-1-yl]diazen-1-ium-1,2-diolate esters (**16a–b**). The sequence in which ester cleavage and nitric oxide release may also occur in a reverse order.

was about sixfold greater than that exhibited by aspirin and threefold greater than that exhibited by the ibuprofen. The ester prodrugs **13b**, **16b** exhibited similar in vivo anti-inflammatory activity (55.8%, 46.5% inhibition, respectively) to that exhibited by the more potent parent acid **11b** (50.0% inhibition) when the same oral dose (113.8 μmol/kg) was administered. These similarities in oral AI activity between the parent acid **11b** and the corresponding NONO-coxib esters **13b** and **16b** suggest that **13a–b** and **16a–b** (i) act as classical prodrugs that require metabolic activation by esterase-mediated hydrolysis, and (ii) the AI data provides credence for the drug design concept that covalent attachment of a NO donor moiety directly to a suitably positioned *para*-C_6H_4–

CH_2COOH group present 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) (**13a–b, 16a–b**) in which an O^2-acetoxymethyl 1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate or O^2-acetoxymethyl-1-(2-methylpyrrolidin-1-yl)diazen-1-ium-1,2-diolate, NO-donor moiety was covalently coupled to the COOH group of 5-(4-carboxymethylphenyl)-1-(4-methane(amino)sulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazoles (**11a–b**) were synthesized for evaluation as COX-1/COX-2 isozyme inhibitors, NO donors, and as AI agents. Structure–activity and biological stability studies showed that these ester prodrugs (i) retain COX-1 and COX-2 inhibitory activity with moderate selectivity for the COX-2 isozyme, (ii) are relatively stable in phosphate-buffered saline at pH 7 where NO release is in the 5.0–7.2% range, (iii) undergo extensive cleavage of the terminal acetoxy group by rat serum esterase(s) that is followed by a significant release of NO in the 59.6–74.6% range, and (iv) the relatively potent AI activity exhibited by the carboxymethyl compound **11b** (ED_{50} = 113.8 μmol/kg po range) and the approximate equipotency of its hybrid ester prodrugs **13b**, **16b** support the drug design concept that covalent attachment of the NO donor moiety directly to a suitably positioned *para*-C_6H_4–CH_2COOH 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 and ^{13}C NMR spectra were measured on a Bruker AM-300 spectrometer in $CDCl_3$, or $CDCl_3$ + DMSO-d_6, with TMS as the internal standard. Nominal mass, positive polarity, electrospray spectra were acquired using a Waters Micromass ZQ mass spectrometer. 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. 5-(4-Bromomethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**9a**),[11] 4-[5-(4-bromomethylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonamide (**9b**)[11] O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**12**)[23], and O^2-acetoxymethyl-1-(2-hydroxymethylpyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**15**)[24] were prepared according to literature procedures.

5.1. 5-(4-Cyanomethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (10a)

A solution of the bromomethyl compound **9a** (2.3 g, 5 mmol) and NaCN (0.368 g, 7.5 mmol) in ethanol (50 mL) was heated under reflux for 2 h. After removal of the solvent in vacuo, the residue was dissolved in EtOAc (50 mL), this solution was washed with water, the organic layer was dried (Na_2SO_4), and the solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography using EtOAc/hexane (1:1, v/v) as eluent to give **10a** as a yellow powder (1.84 g, 91%): mp 171–174 °C; IR (film) 3024 (CN), 3030 (C–H aromatic), 2928 (C–H aliphatic), 1318, 1154 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.08 (s, 3H, SO_2CH_3), 3.81 (s, 2H, CH_2CN, 6.81 (s, 1H, pyrazole H-4), 7.27 (dd, J = 6.1, 1.8 Hz, 2H, cyanomethylphenyl H-3, H-5), 7.39 (dd, J = 6.1, 1.8 Hz, 2H, cyanomethylphenyl H-2, H-6), 7.53 (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); MS 406.0 (M+1).

5.2. 4-[5-(4-Cyanomethylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonamide (10b)

The title compound **10b** was synthesized, using a similar procedure to that described for the preparation of **10a**, in 72% yield as a white powder; mp 82–84 °C; IR (film) 3348, 3264 (NH_2), 3025 (CN), 3027 (C–H aromatic), 2965 (C–H aliphatic), 1339, 1163 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.81 (s, 2H, CH_2CN, 4.91 (br s, 2H, NH_2, exchanges with D_2O), 6.80 (s, 1H, pyrazole H-4), 7.27 (d, J = 8.6 Hz, 2H, cyanomethylphenyl H-3, H-5), 7.38 (d, J = 8.6, 2H, cyanomethylphenyl H-2, H-6), 7.48 (dd, J = 6.7, 1.9 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.94 (dd, J = 6.7, 1.9 Hz, 2H, aminosulfonylphenyl H-3, H-5); MS 407.02 (M+1), 428.97 (M+Na).

5.3. 5-(4-Carboxymethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyraz-ole (11a)

A solution of 6 N HCl (35 mL) was added to a solution of 5-(4-cyanomethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (**10a**, 1.22 g, 3 mmol) in dioxane (15 mL) and the reaction was allowed to proceed at reflux for 16 h. After cooling to 25 °C, the solvent was removed in vacuo, the residue was mixed with cold water (10 mL), and this mixture was extracted with EtOAc (2 × 25 mL). The organic layer was extracted with 1 N NaOH (2 × 15 mL), the aqueous NaOH layer was acidified with 1 N HCl to pH 1, and the acidic mixture was extracted with EtOAc (2 × 25 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography using EtOAc/hexane (2:1, v/v) as eluent to furnish the product that was recrystallized from EtOAc/hexane to give **11a** as a white powder (0.34 g, 27%): mp 168–170 °C; IR (film) 3350 (broad OH), 3027 (C–H aromatic), 2963 (C–H aliphatic), 1733 (CO_2), 1320, 1156 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.08 (s, 3H, SO_2CH_3), 3.72 (s, 2H, CH_2CO_2H, 6.79 (s, 1H, pyrazole H-4), 7.22 (d, J = 8.2 Hz, 2H, benzyl H-3, H-5), 7.34 (d, J = 8.2 Hz, 2H, benzyl H-2, H-6), 7.55 (d, J = 8.8 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.96 (d, J = 8.8 Hz, 2H, methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 40.5, 44.5, 104.1, 120.9, 125.7, 127.6, 128.6, 129.0, 130.2, 134.9, 139.9, 143.3, 144.1, 144.6, 176.2; MS 425.08 (M+1).

5.4. 5-(4-Carboxymethylphenyl)-1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (11b)

A solution of 1 N NaOH (18 mL, 18 mmol) was added to a solution of 4-[5-(4-cyanomethylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonamide (**10b**, 1.22 g, 3 mmol) in THF (5 mL), and the resulting mixture was heated under reflux for 16 h. After cooling to 25 °C, the solvent was removed in vacuo, the residue was diluted with cold water (10 mL), the aqueous alkaline layer was washed with ether (10 mL), the aqueous alkaline layer was acidified with 1 N HCl to pH 1, and the mixture was extracted with EtOAc (2 × 25 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was recrystallized from EtOAc/hexane to give **11b** as yellow crystals (0.92 g, 72%): mp 145–148 °C; IR (film) 3352, 3254 (NH_2), 3030 (C–H aromatic), 2926 (C–H aliphatic), 1717 (CO_2), 1339, 1160 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.72 (s, 2H, CH_2CO_2H), 5.18 (br s, 2H, NH_2, exchanges with D_2O), 6.77 (s, 1H, pyrazole H-4), 7.20 (d, J = 7.9 Hz, 2H, benzyl H-3, H-5), 7.30 (d, J = 7.9 Hz, 2H, benzyl H-2, H-6), 7.45 (dd, J = 6.7, 1.9 Hz, 2H, aminosulfonylphenyl

H-2, H-6), 7.88 (d, J = 6.7, 1.9 Hz, 2H, aminosulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 40.1, 105.4, 120.3, 124.4, 126.2, 126.3, 128.0, 129.2, 135.4, 140.6, 142.3, 142.8, 144.0, 171.9; MS 426.07 (M+1).

5.5. General method for preparation of the O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino) diazen-1-ium-1,2-diolate esters (13a–b)

Sodium carboxylates of the respective acids **11a–b** 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 24 h at 25 °C. A solution of O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino]-diazen-1-ium-1,2-diolate (**12**, 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 eluent. Physical and spectral data for **13a–b** are listed below.

5.5.1. O^2-Acetoxymethyl-1-{*N*-[2-(2-(4-(1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazol-5-yl)phenyl)acetoxy)ethyl]-*N*-methylamino}diazen-1-ium-1,2-diolate (13a)

Yield, 84%; pale yellow gum; IR (film) 3026 (C–H aromatic), 2963 (C–H aliphatic), 1734, 1718 (CO_2), 1318, 1156 (SO_2), 1236, 1099 (N=N–O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 3H, COCH_3), 3.07 (s, 3H, SO_2CH_3), 3.08 (s, 3H, NCH_3), 3.67 (t, J = 5.2 Hz, 2H, CH_2N), 3.68 (s, 2H, CH_2COO, 4.33 (t, J = 5.2 Hz, 2H, CO_2CH_2), 5.79 (s, 2H, OCH_2O), 6.79 (s, 1H, pyrazole H-4), 7.21 (d, J = 8.5 Hz, 2H, benzyl H-3, H-5), 7.32 (d, J = 8.5 Hz, 2H, benzyl 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 636.01 (M+Na); Anal. Calcd for $C_{25}H_{26}F_3N_5O_8S{\cdot}1/3H_2O$: C, 48.47; H, 4.34; N, 11.30. Found: C, 48.12; H, 4.55; N, 11.68.

5.5.2. O^2-Acetoxymethyl-1-{*N*-[2-(2-(4-(1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazol-5-yl)phenyl)acetoxy)ethyl]-*N*-methylamino}diazen-1-ium-1,2-diolate (13b)

Yield, 36%; pale yellow gum; IR (film) 3352, 3259 (NH_2), 3024 (C–H aromatic), 2965 (C–H aliphatic), 1734, 1718 (CO_2), 1342, 1164 (SO_2), 1240, 1100 (N=N–O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3H, COCH_3), 3.04 (s, 3H, NCH_3), 3.65 (t, J = 5.2 Hz, 2H, CH_2N), 3.67 (s, 2H, CH_2COO, 4.32 (t, J = 5.2 Hz, 2H, CO_2CH_2), 5.11 (br s, 2H, NH_2, exchanges with D_2O), 5.76 (s, 2H, OCH_2O), 6.77 (s, 1H, pyrazole H-4), 7.20 (d, J = 8.0 Hz, 2H, benzyl H-3, H-5), 7.30 (d, J = 8.0 Hz, 2H, benzyl H-2, H-6), 7.47 (dd, J = 7.1, 2.5 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.94 (d, J = 7.1, 2.5 Hz, 2H, aminosulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 20.8, 40.8, 40.9, 52.5, 61.4, 87.2, 106.5, 121.0, 125.5, 127.5, 127.6, 129.0, 130.0, 135.2, 141.8, 142.1, 143.8, 144.6, 169.6, 170.5; MS 637.08 (M+Na); Anal. Calcd for $C_{24}H_{25}F_3N_6O_8S$: C, 46.91; H, 4.10; N, 13.68. Found: C, 47.31; H, 4.39; N, 13.48.

5.6. General method for preparation of acid chlorides (14a–b)

Oxalyl chloride (0.3 mL, 3.4 mmol) was added drop wise to a solution of the respective acid **11a–b** (2.8 mmol) in dry CH_2Cl_2 (20 mL) under argon at 25 °C. The reaction mixture was stirred for 12 h at 25 °C, the solvent was removed in vacuo, the crude product was washed with hexane (3 × 25 mL), and dried under vacuum to give **14a** or **14b**. Physical and spectral data for **14a–b** are listed below.

5.6.1. 5-(4-Chlorocarbonylmethylphenyl)-1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (14a)

Yield, 90%; pale yellow solid; IR (film) 3028 (C–H aromatic), 2964 (C–H aliphatic), 1794 (CO), 1322, 1157 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.08 (s, 3H, SO_2CH_3), 4.20 (s, 2H, CH_2CO), 6.81 (s, 1H, pyrazole H-4), 7.26 (d, J = 8.5 Hz, 2H, benzyl H-3, H-5), 7.32 (d, J = 8.5 Hz, 2H, benzyl H-2, H-6), 7.54 (dd, J = 6.7, 1.8 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.96 (dd, J = 6.7, 1.8 Hz, 2H, methanesulfonylphenyl H-3, H-5); MS 443.04 (M), 445.04 (M+2).

5.6.2. 5-(4-Chlorocarbonylmethylphenyl)-1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazole (14b)

Yield, 89%; pale yellow solid; IR (film) 3340, 3270 (NH_2), 3064 (C–H aromatic), 2924 (C–H aliphatic), 1792 (CO), 1337, 1162 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.19 (s, 2H, CH_2CO), 4.88 (br s, 2H, NH_2, exchanges with D_2O), 6.80 (s, 1H, pyrazole H-4), 7.25 (d, J = 8.3 Hz, 2H, benzyl H-3, H-5), 7.31 (d, J = 8.3 Hz, 2H, benzyl H-2, H-6), 7.48 (d, J = 8.8 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.91 (d, J = 8.8 Hz, 2H, aminosulfonylphenyl H-3, H-5); MS 444.04 (M), 446.04 (M+2).

5.7. General procedure for the synthesis of O^2-acetoxymethyl-1-[2-methylpyrrolidin-1-yl] diazen-1-ium-1,2-diolate esters (16a–b)

The respective acid chloride **14a** or **14b** (1 mmol), O^2-acetoxymethyl-1-(2-hydroxymethylpyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**15**, 1 mmol) and triethylamine (1 mmol) were dissolved in dry THF (10 mL), and the reaction was allowed to proceed at 25 °C for 48 h with stirring. The precipitate (triethylammonium chloride) was filtered off and the solvent was removed in vacuo. The residue was dissolved in dichloromethane and washed with water, the organic layer was dried (Na_2SO_4), the solvent was evaporated under vacuum, and the product was purified by silica gel column chromatography using EtOAc/hexane (2:1, v/v) as eluent. Physical and spectral data for **16a–b** are listed below.

5.7.1. O^2-Acetoxymethyl-1-{2-[2-(4-(1-(4-methanesulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazol-5-yl)phenyl)acetoxymethyl]pyrrolidin-1-yl}diazen-1-ium-1,2-diolate (16a)

Yield, 69%; pale yellow powder; mp 52–54 °C; $[\alpha]_D^{21.0} = -36.0$ (1.0100, $CHCl_3$); IR (film) 3065 (C–H aromatic), 2934 (C–H aliphatic), 1755, 1735 (CO_2), 1321, 1156 (SO_2), 1241, 1098 (N=N–O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.80–2.09 (m, 4H, pyrrolidin-1-yl H-3, H-4), 2.10 (s, 3H, COCH_3), 3.07 (s, 3H, SO_2CH_3), 3.52–3.73 (m, 2H, pyrrolidin-1-yl H-5), 3.76 (s, 2H, CH_2COO), 4.19–4.31 (m, 3H, pyrrolidin-1-yl H-2, COOCH_2), 5.73 (d, J = 7.3 Hz, 1H, –OCHH′OAc), 5.76 (d, J = 7.3 Hz, 1H, OCHH'OAc), 6.77 (s, 1H, pyrazole H-4), 7.23 (d, J = 8.2 Hz, 2H, benzyl H-3, H-5), 7.31 (d, J = 8.2 Hz, 2H, benzyl H-2, H-6), 7.54 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.94 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 20.9, 22.8, 26.9, 40.8, 44.4, 52.6, 59.7, 65.9, 87.3, 106.7, 120.9, 125.7, 127.4, 128.5, 129.0, 130.1, 135.5, 140.0, 143.3, 144.1, 144.7, 169.4, 170.5; MS 662.04 (M+Na); Anal. Calcd for $C_{27}H_{28}F_3N_5O_8S$: C, 50.70; H, 4.41; N, 10.95. Found: C, 51.30; H, 4.64; N, 10.41.

5.7.2. O^2-Acetoxymethyl-1-{2-[2-(4-(1-(4-aminosulfonylphenyl)-3-trifluoromethyl-1*H*-pyrazol-5-yl)phenyl)acetoxymethyl]pyrrolidin-1-yl}diazen-1-ium-1,2-diolate (16b)

Yield, 51%; white powder; mp 61–63 °C; $[\alpha]_D^{21.0} = -23.0$ (1.0200, $CHCl_3$); IR (film) 3341, 3271 (NH_2), 3065 (C–H aromatic), 2934 (C–H aliphatic), 1758, 1737 (CO_2), 1341, 1168 (SO_2), 1239, 1101 (N═N–O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.82–2.05 (m, 4H, pyrrolidin-1-yl H-3, H-4), 2.11 (s, 3H, $COCH_3$), 3.52–3.63 (m, 2H, pyrrolidin-1-yl H-5), 3.68 (s, 2H, CH_2COO), 4.17–4.32 (m, 3H, pyrrolidin-1-yl H-2, CO_2CH_2), 5.14 (br s, 2H, NH_2, exchanges with D_2O), 5.73 (d, $J = 7.3$ Hz, 1H, -OC*H*H′OAc), 5.75 (d, $J = 7.3$ Hz, 1H, OCH*H*′OAc), 6.77 (s, 1H, pyrazole H-4), 7.19 (d, $J = 7.9$ Hz, 2H, benzyl H-3, H-5), 7.29 (d, $J = 7.9$ Hz, 2H, benzyl H-2, H-6), 7.45 (dd, $J = 6.5$, 2.5 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.91(d, $J = 6.5$, 2.5 Hz, 2H, aminosulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 20.9, 22.8, 27.9, 40.9, 52.6, 59.7, 65.7, 87.2, 106.5, 121.0, 125.4, 127.5, 127.6, 129.0, 129.7, 135.4, 141.8, 142.1, 143.8, 144.6, 169.7, 170.5; MS 662.92 (M+Na).

6. Cyclooxygenase inhibition assays

The ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and human recombinant COX-2 (IC_{50} value, μM) was determined using an enzyme immuno assay (EIA) kit (catalog no. 560131, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method.[25]

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 (**13a–b, 16a–b, 17–18**) using the reported procedures.[26]

8. In vivo anti-inflammatory assay

The test compounds **11a–b**, **13a–b, 16a–b** and the reference drugs celecoxib, aspirin and ibuprofen were evaluated using the in vivo carrageenan-induced foot paw edema model reported previously.[27]

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