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A diazen-1-ium-1,2-diolated nitric oxide donor ester prodrug of 3-(4-hydroxymethylphenyl)-4-(4-methanesulfonylphenyl)-5*H*-furan-2-one: Synthesis, biological evaluation and nitric oxide release studies

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ABSTRACT

A novel hybrid nitric oxide-releasing anti-inflammatory (AI) ester prodrug (NONO-coxib **14**) wherein an O^2-acetoxymethyl 1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate (O^2-acetoxymethyl PROLI/NO) NO-donor moiety was covalently coupled to the CH_2OH group of 3-(4-hydroxymethylphenyl)-4-(4-methylsulfonylphenyl)-5*H*-furan-2-one (**12**), was synthesized. The prodrug **14** released a low amount of NO (4.2%) upon incubation with phosphate buffer (PBS) at pH 7.4 which was significantly higher (34.8% of the theoretical maximal release of two molecules of NO/molecule of the parent hybrid ester prodrug) upon incubation in the presence of rat serum. These incubation studies suggest that both NO and the parent compound **12** would be released from the prodrug **14** upon in vivo cleavage by non-specific serum esterases. The prodrug ester **14** is a selective COX-2 inhibitor that exhibited AI activity (ED_{50} = 72.2 mmol/kg po) between that of the reference drugs celecoxib (ED_{50} = 30.9 μmol/kg po) and ibuprofen (ED_{50} = 327 μmol/kg po). The NO donor compound **14** exhibited enhanced inhibition of phenylephrine-induced vasoconstriction of isolated mesenteric arteries compared with that observed under control conditions. 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.

The original drug design concept that selective cyclooxygenase-2 (COX-2) inhibitors would be effective anti-inflammatory (AI) agents showing minimal gastrointestinal (GI) toxicity[1–4] was confirmed with the discovery of celecoxib (**1**),[5] rofecoxib (**2**),[6] and valdecoxib (**3**, see structures in Fig. 1).[7] Accordingly, these drugs preferentially inhibit the inducible COX-2 isozyme that causes inflammation in the periphery rather than the constitutive COX-1 isozyme that provides gastroprotection and maintains vascular homeostasis. However, this apparently safe pharmacological profile shown by selective COX-2 inhibitors was relatively short-lived. Evidence began to accumulate suggesting that highly selective COX-2 inhibitors alter the balance in the COX pathway causing 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 provided a rational explanation for the observed elevation in blood pressure and increased incidences of an adverse cardiovascular thrombotic event such as myocardial infarction.[8] Accordingly, the clinical use of rofecoxib and valdecoxib were subsequently terminated due to adverse cardiovascular effects associated with their use.[9]

Nitric oxide (NO) exhibits a number of biological actions that are similar to those for PGI_2 that encompass a cytoprotective role in GI homeostasis by facilitating mucosal blood flow, and inhibition of platelet aggregation and inflammatory-cell activation.[10–13] It is plausible that these beneficial 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 (**4**) which exhibits anti-inflammatory activity similar to that of valdecoxib with antithrombotic action at higher doses,[14] and the imidazoles (**5a–b**) that exhibit a NO-dependent vasodilator activity.[15] In earlier studies, we described second generation celecoxib (**6**)[16] and indomethacin (**7**)[17] prodrugs having a NONO-donor O^2-(acetoxymethyl)-1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate moiety that are effectively cleaved by esterases to release the parent COX-2 inhibitory AI agent and NO. We now report the synthesis and biological evaluation of a novel hybrid ester prodrug derivative of rofecoxib (**14**) in which the second generation NONO-donor moiety indicated

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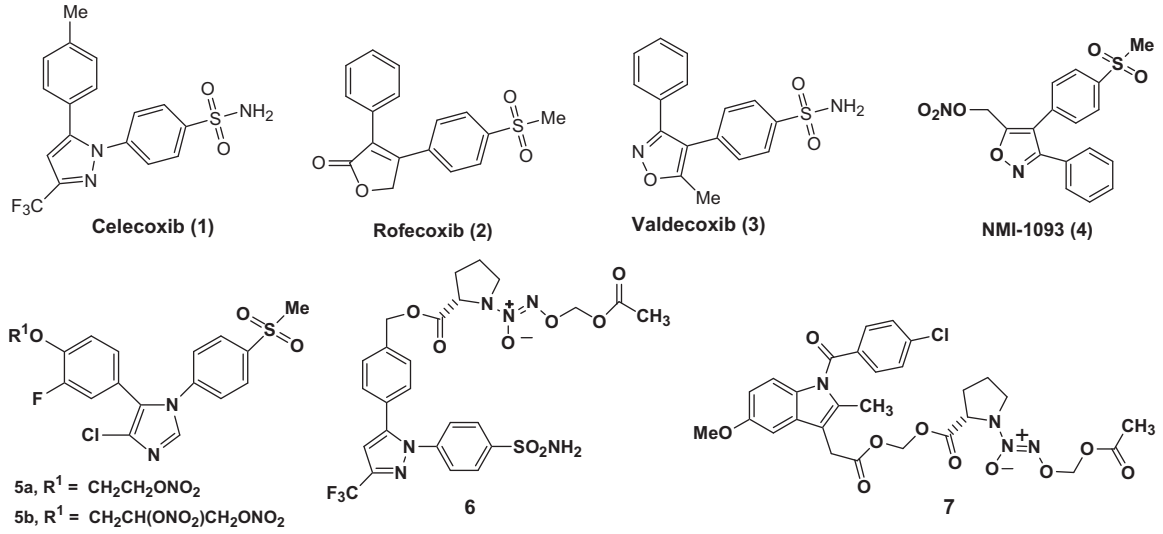
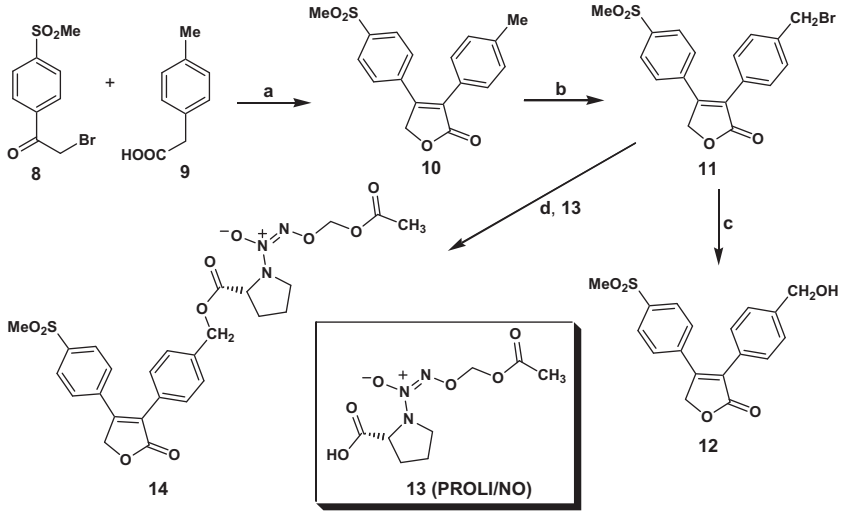


Figure 1. Chemical structures of the selective cyclooxygenase-2 (COX-2) inhibitors celecoxib (**1**), rofecoxib (**2**), valdecoxib (**3**), selective COX-2 inhibitors that possess a nitric oxide donor nitrate (**4–5**) or diazen-1-ium-1,2-diolate (**6**) moiety, and the indomethacin prodrug (**7**) that unlike indomethacin is completely devoid of ulcerogenicity.

above[16,17] is attached directly to the hydroxyl group of 3-(4-hydroxymethylphenyl)-4-(4-methanesulfonylphenyl)-5*H*-furan-2-one (**12**). This hybrid ester prodrug of rofecoxib was designed with the expectation that the anti-platelet aggregation and hypotensive actions of NO that is released from the diazen-1-ium-1,2-diolate moiety would circumvent the adverse thrombotic and hypertensive effects that led to the clinical withdrawal of rofecoxib (Vioxx®).[18]

The 3-(4-hydroxymethylphenyl)-4-(4-methanesulfonylphenyl) -5*H*-furan-2-one (**12**), and the O^2-acetoxymethyl PROLI/NO prodrug (**14**), were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, reaction of the ketone **8** with *para*-tolylacetic acid (**9**) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) afforded the furan-2-one product **10** (63%). Subsequent photochemical promoted bromination of the tolyl methyl group present in compound **10** furnished the benzyl bromide **11** in 41% yield. The bromomethyl compound **11** was converted to the respective hydroxymethyl product **12** in moderate yield (36%) upon heating under reflux in an acetone-water solvent system (13:1, v/v) for 110 h. The target O^2-acetoxymethyl PROLI/NO prodrug ester **14** was synthesized in 30% yield by condensation of the bromomethyl compound **11** with O^2-acetoxymethyl 1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**13**) in the presence of triethylamine in dimethyl sulfoxide.

Three positions on the structure of rofecoxib (**2**) were considered for attachment of an O^2-acetoxymethyl-1-(2-methylpyrrolidin-1-yl)diazen-1-ium-1,2-diolate (PROLI/NO) NO donor moiety via an ester linkage. The $MeSO_2$ COX-2 pharmacophore must be present at the *para*-position on the C-4 phenyl ring since the corresponding C-3 regioisomer is inactive. A substituent (halogen, methyl, methoxy) at the *para*-position of the C-3 phenyl generally has little effect on COX-2 potency although it may result in a moderate increase in COX-1 potency thereby reducing the COX-2 selectivity index.[6] Although the C-5 position of the furan-2-one central ring tolerates small alkyl and/or hydroxyl substituents,[19,20] it was anticipated that a large PROLI/NO moiety and opening of the lactone ring from a synthetic perspective may be deterrents. Accordingly, we decided based on this structure-activity information to couple the PROLI/NO donor moiety (**13**) to the C-3 *para*-C_6H_4-CH_2OH moiety present in the



Scheme 1. Reagents and conditions: (a) CH_3CN, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 0 °C, 4 h; (b) *N*-bromosuccinimide, benzoyl peroxide, light from a 100-W sun beam lamp, benzene, reflux, 5 h; (c) acetone, H_2O, reflux, 110 h; (d) Et_3N, DMSO, 25 °C, 36 h.

benzyl alcohol **12** via an ester moiety to prepare the target PROLI/NO hybrid ester prodrug **14**.

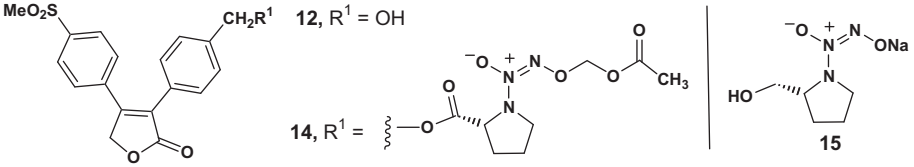
In vitro COX enzyme inhibition studies (Table 1) showed that the hydroxymethyl (CH_2OH) compound **12** (COX-1 IC_{50} = 33.1 μM; COX-2 IC_{50} = 4.2 μM) showed weaker COX-2 inhibitory activity compared to the reference drugs rofecoxib (IC_{50} = 0.5 μM) and celecoxib (IC_{50} = 0.07 μM). The observation that the hydroxymethyl compound **12** is a weak COX-1 inhibitor is consistent with literature data indicating that incorporation of a small substituent at the *para*-position of the C-3 phenyl ring in rofecoxib (**2**, COX-1 IC_{50} > 100 μM) increases COX-1 inhibition.[6] In comparison to the parent hydroxymethyl compound **12**, which showed a COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}) of 7.9, the PROLI/NO hybrid ester prodrug **14** was a highly selective COX-2 inhibitior (COX-1 IC_{50} > 100 μM; COX-2 IC_{50} = 4.9 μM).

The percent NO released from the PROLI/NO hybrid ester prodrug **14** 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 manipulation such as attachment of an alkyl substituent to the O^2-position[21] that furnishes stable O^2-substituted-diazen-1-ium-1,2-diolates that hydrolyze slowly even in acidic solution.[22] Consistent with these observations, when the PROLI/NO prodrug **14** was incubated for 1.5 h in PBS at pH 7.4, the percentage of NO released was 4.2% which is indicative of slow NO release.[23] On the other hand, the effect of non-specific esterases present in rat serum with respect to NO release from **14** was substantially higher (38.4%). In this regard, non-specific serum esterases present in rat serum cleave the hybrid prodrug ester more effectively than PBS at pH 7.4. From a mechanistic perspective, it is not possible for the hybrid ester prodrug **14** to 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-[2-(hydroxymethyl)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**15**), which does not possess an ester group that requires prior ester cleavage, released 84.5 to 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-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate ester prodrugs and the subsequent release of acetic acid, formaldehyde, two molecules of NO, and the natural amino acid L-proline were described in an earlier publication.[17] Similar esterase cleavage and NO release pathways for the ester prodrug **14** are illustrated in Figure 2. The prodrug **14** was designed (i) such that the $-CH_2OH$ group of the parent compound **12** is covalently attached directly to the CO_2H substituent of the diazenium-1,2-diolate (**13**), and (ii) subsequent cleavage of the ester groups and release of NO would furnish the parent COX-2 inhibitor (**12**) and the non-toxic natural amino acid L-proline.

Vascular studies showed that none of the COX-2 inhibitors (rofecoxib, compound **12**, and compound **14**) altered the potency (IC_{50}) of the vasoconstrictive response to phenylephrine (PE) compared with the control vessels (Table 2). On the other hand, the slope of the PE concentration-response curve for the NO donor compound **14** was very much steeper than that of the other drugs (Figure 3), such that the IC_{20} of this inhibitor was significantly higher than that of the hydroxymethyl compound **12**, or indeed of rofecoxib (Table 2). The maximum responses of the COX-2 inhibitors were all significantly lower than those of the control vessels and, although the maximum response of the NO donor compound **14** tended to be the lowest of the groups, this failed to reach significance compared with the two inhibitors. The IC_{50}'s for ACh-induced relaxation of pre-constricted vessels was not altered by either rofecoxib or the hydroxymethyl compound **12** although maximal relaxation was enhanced since the COX-2 inhibitor-treated vessels relaxed to a greater extent than did the control vessels (Table 3).

As anticipated, the novel COX-2 inhibitor NO donor compound **14** exhibited enhanced inhibition of PE-induced vasoconstriction of isolated mesenteric arteries compared with that observed under control conditions, after incubations with rofecoxib, and after incubation with its parent hydroxymethyl compound **12**. This was manifest, not as a change in IC_{50}, but rather as a marked inhibition of PE-induced vasoconstriction at the lower doses of PE (IC_{20}). This

Table 1
In vitro COX-1/COX-2 enzyme inhibition, in vivo anti-inflammatory activity, and *in vitro* nitric oxide release data for the 3-(4-hydroxymethylphenyl)-4-(4-methanesulfonylphenyl)-5*H*-furan-2-one (**12**), the diazen-1-ium-1,2-diolate prodrug ester (**14**), the reference drugs rofecoxib, celecoxib, ibuprofen, and the diazen-1-ium-1,2-diolate (**15**)



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]
12	33.1	4.2	112.9	—	—
14	>100	2.4	72.2	4.2	38.4
15	—	—	—	84.5	85.0
Rofecoxib	>100	0.5	4.8[f]	—	—
Celecoxib	7.7	0.07	30.9	—	—
Ibuprofen	2.9	1.1	326.7	—	—

[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 of the test compound.

[c] Percent of nitric oxide released based on a theoretical maximum release of 2 mol of nitric oxide/mole of the diazen-1-ium-1,2-diolate test compounds (**14, 15**). The result is the mean value of 3 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), was incubated at 37 °C for 1.5 h.

[f] Literature data (*J. Pharmacol. Exp. Ther.* **1999**, *290*, 551).

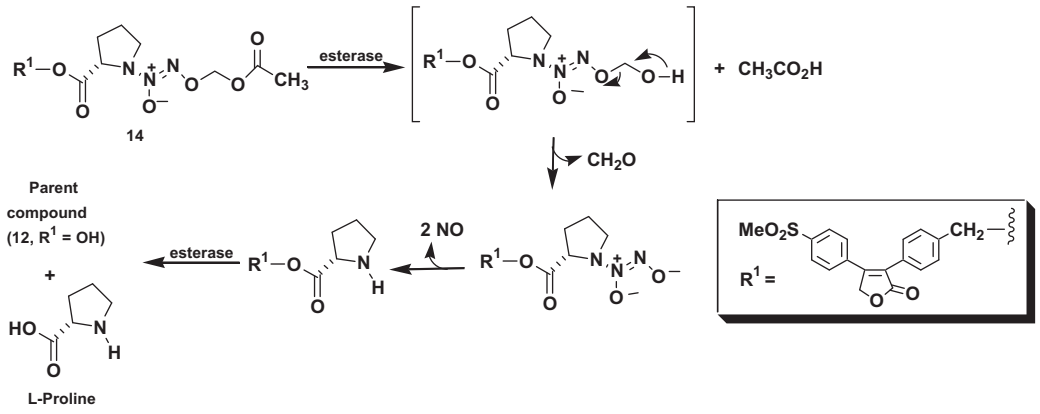


Figure 2. Theoretical metabolic activation (esterase hydrolysis) and nitric oxide release from the O^2-(acetoxymethyl)-1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate ester prodrug (**14**). The sequence in which ester cleavage and nitric oxide release may also occur in a reverse order.

Table 2
Effect of rofecoxib, compound **12** and compound **14** on PE-induced constriction of isolated small mesenteric arteries

Compound	IC_{50} (M)	IC_{20} (M)	Maximum response (g/mm)
Control (n = 11)	$1.48 \pm 0.10 \times 10^{-6}$	0.91 ± 0.08	2.13 ± 0.23
Rofecoxib (n = 13)	$1.66 \pm 0.14 \times 10^{-6}$	0.95 ± 0.07	1.32 ± 0.14*
12 (n = 11)	$1.68 \pm 0.15 \times 10^{-6}$	1.01 ± 0.07	1.51 ± 0.14*
14 (n = 9)	$1.75 \pm 0.06 \times 10^{-6}$	1.37 ± 0.04#	1.17 ± 0.05*

* Significant difference from control group ($P < 0.05$).
\# Significant difference from compound **12** ($P < 0.05$).

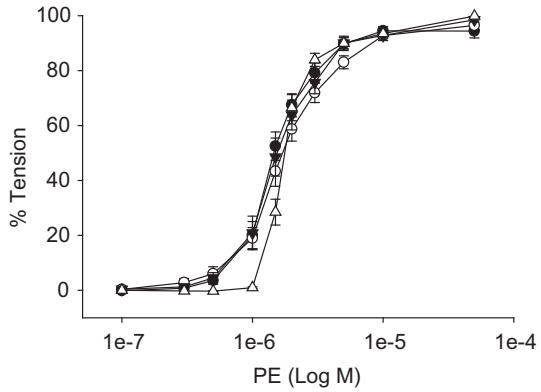


Figure 3. Effect of COX-2 inhibitors on concentration-response curve of isolated mesenteric arteries to PE. Control: filled circles (n = 11); Rofecoxib: open circles (n = 13); Compound **12**: filled triangles (n = 11); Compound **14**: open triangles (n = 14). Vertical bars delineate standard error of mean.

Table 3
Effect of rofecoxib and compound **12** on ACh-induced relaxation of pre-constricted (PE EC_{80}) isolated small mesenteric arteries

Compound	IC_{50} (M)	Maximum response (g/mm)
Control (n = 11)	$5.9 \pm 1.6 \times 10^{-8}$	0.38 ± 0.01
Rofecoxib (n = 13)	$8.8 \pm 1.6 \times 10^{-8}$	0.23 ± 0.02*
12 (n = 11)	$9.5 \pm 1.6 \times 10^{-8}$	0.30 ± 0.04*

* Significant difference from control group ($P < 0.05$).

observation is consistent with the fact that the amount of NO released from the NO donor prodrug **14** in this assay will be low since the mesenteric artery preparation does not contain the required esterase enzyme to release the vasorelaxant NO.

There have been very few studies of the effects of COX-2 inhibitors on vascular reactivity of isolated resistance blood vessels. Brueggeman et al[24] found that celecoxib, but not rofecoxib, dilated preconstricted small mesenteric arteries. They attributed the vasodilatory activity of celecoxib to enhancement of KCNQ potassium currents and suppression of L-type voltage-sensitive calcium currents. There were however significant methodological differences between that study and our own in that their vessels were pre-constricted with vasopressin and studied by pressure myography, whereas ours were preconstricted with PE and evaluated by isometric wire myography. Other studies have also reported enhanced vasorelaxation in the presence of celecoxib: in KCl-pre-constricted vessels from celecoxib-fed animals hypertensive rats,[25] and in coronary vessels of celecoxib-infused guinea pig Langendorff hearts.[26] In neither of these two aforementioned studies did the authors find rofecoxib altered vascular tone. We, on the other hand, found that rofecoxib reduced maximal PE-induced vasoconstriction and enhanced maximal ACh-induced vasorelaxation. We suggest that the unique conditions under which we conducted our studies allowed this activity to be demonstrated. Furthermore, our in vitro studies demonstrate that coupling of the parent hydroxymethyl drug **12** to an NO-donor moiety produces a prodrug **14** which, when preincubated with isolated small arteries, significantly impairs PE-induced vasoconstriction.

The AI activities exhibited by the parent rofecoxib CH_2OH derivative **12**, and the O^2-acetoxymethyl-protected PROLI/NO prodrug **14**, were determined using a carrageenan-induced rat foot paw edema model (see data in Table 1). Upon oral administration to rats, both compounds produced a significant AI activity with the prodrug **14** (ED_{50} = 72.2 μmol/kg po) showing a greater AI potency than the parent compound **12** (ED_{50} = 112.9 μmol/kg po). Plausible explanations for the greater potency shown by the prodrug **14** include differences in their absorption, biodistribution and/or pharmacodynamic, profiles. In comparison, both compounds **12** and **14** were more potent than the reference drug ibuprofen (ED_{50} = 326.7 μmol/kg po), but less potent than the reference drug celecoxib (ED_{50} = 30.9 μmol/kg po).

In conclusion, the hitherto-unknown ester prodrug O^2-acetoxymethyl 1-[2-[4-(4-methanesulfonylphenyl)-*5H*-furan-2-on-3-yl) phenylmethoxy-carbonyl]pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**14**) was synthesized[27] for evaluation as a selective COX-2 inhibitor,[28] NO donor,[29] vascular relaxant,[30] and AI[31] agent. Structure-activity and biological stability studies showed that the NO donor prodrug **14** (i) like rofecoxib is a selective COX-2 inhibitor, (ii) is relatively stable in phosphate-buffered saline at pH 7 where NO release is low (4.2%), (iii) undergoes extensive cleavage of the terminal acetoxy group by rat serum esterase(s) that is followed by a

significant release of NO (38.4%), (iv) upon preincubation with isolated small arteries, significantly impairs PE-induced vasoconstriction, and (v) the relatively potent AI activity exhibited by this ester prodrug **14** support the drug design concept that covalent attachment of the NO donor moiety directly to a suitably positioned CH_2OH group present in a selective COX-2 inhibitor such as rofecoxib offers a rational drug design approach to circumvent adverse thrombotic and hypertensive effects associated with chronic use of selective COX-2 inhibitors.

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27. Experimental procedures and spectral data for compounds **10**–**14**.
General: Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Unless otherwise noted, 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 $CDCl_3$, DMSO-d_6, or $CDCl_3$ + DMSO-d_6 with TMS as the internal standard. Microanalyses were performed for C, H, N (MicroAnalytical Service Laboratory, Department of Chemistry, University of Alberta). Nominal mass, positive polarity, electrospray, spectra were acquired using a Water's Micromass ZQ 4000 mass spectrometer. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70–230 mesh). 2-Bromo-4′-(methylsulphonyl)acetophenone (**8**)[32] and O^2-acetoxymethyl 1-(2-carboxypyrrolidin-1-yl)diazen-1-ium-1,2-diolate (**13**)[17] were prepared according to literature procedures. All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification. The in vivo anti-inflammatory assay was carried out using a protocol approved by the Health Sciences Animal Welfare Committee at the University of Alberta.
3-(para-Tolyl)-4-(4-methanesulfonylphenyl)-5H-furan-2-one (**10**): Triethyl amine (0.7 mL, 5 mmol) was added drop wise to a mixture of 2-bromo-4′-(methylsulphonyl)acetophenone (**8**, 0.61 g, 2.25 mmol) and *para*-tolylacetic acid (**9**, 0.3 g, 2 mmol) in acetonitrile (8 mL) under argon. The resulting mixture was maintained at 25 °C for 1 hour with stirring prior to cooling to 0 °C. DBU (0.58 mL, 3.88 mmol) was added, the mixture was stirred for 2 h at 0 °C, a solution of 1 N HCl (7 mL) was added, and the mixture was extracted with EtOAc (3 × 50 mL). The organic extract was dried (Na_2SO_4), the solvent was removed in vacuo, and the residue was purified by elution from a silica gel column using EtOAc-hexane (2:1, v/v) as eluant to furnish **10** (0.41 g, 63%) as a yellow powder: mp 174–176 °C; IR (film) 3030 (C–H aromatic), 2929 (C–H aliphatic), 1750 (CO), 1315, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.39 (s, 3H, CH_3), 3.08 (s, 3H, SO_2CH_3), 5.19 (s, 2H, furanone CH_2), 7.21 (d, J = 7.9 Hz, 2H, tolyl H-3, H-5), 7.30 (d, J = 7.9 Hz, 2H, tolyl H-2, H-6), 7.54 (dd, J = 6.7, 1.8 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.94 (d, J = 6.7, 1.8 Hz, 2H, methanesulfonylphenyl H-3, H-5); MS m/z (ES$^+$) 329.08, $C_{18}H_{17}O_4S$ (M+H) requires 329.38; 350.96, $C_{18}H_{16}O_4SNa$ (M+Na) requires 351.08.
3-(4-Bromomethylphenyl)-4-(4-methanesulfonylphenyl)-5H-furan-2-one (**11**): *N*-Bromosuccinimide (0.641 g, 3.6 mmol) was added to a stirred solution of **10** (0.985 g, 3.0 mmol) in benzene (40 mL). Benzoyl peroxide (0.73 g, 0.3 mmol) was added and the reaction mixture was then irradiated with light from a 100-watt sun beam lamp for 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 (1:8, v/v) to EtOAc/hexane (1:1, v/v) as eluent to give **11** (0.501 g, 41%) as a yellow powder: mp 160–163 °C; IR (film) 3030 (C–H aromatic), 2929 (C-H aliphatic), 1755 (CO), 1310, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.08 (s, 3H, SO_2CH_3), 4.50 (s, 2H, CH_2Br), 5.21 (s, 2H, furanone CH_2), 7.42 (m, 4H, bromomethylphenyl H-2, H-3, H-5, H-6), 7.53 (dd, J = 6.7, 1.8 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.95 (d, J = 6.7, 1.8 Hz, 2H, methanesulfonylphenyl H-3, H-5); MS m/z 406.96, $C_{18}H_{16}{}^{79}BrO_4S$ (M+H) requires 407.28; 408.93, $C_{18}H_{16}{}^{81}BrO_4S$ (M+H) requires 409.28; 428.91, $C_{18}H_{15}{}^{79}BrO_4SNa$ (M+Na) requires 429.28; 430.88, $C_{18}H_{15}{}^{81}BrO_4SNa$ (M+Na) requires 431.28.
3-(4-Hydroxymethylphenyl)-4-(4-methanesulfonylphenyl)-5H-furan-2-one (**12**): A solution of the bromomethyl compound **11** (102 mg, 0.25 mmol) in acetone (4 mL) and water (0.3 mL) was refluxed for 110 h. After removal of the solvents in vacuo, the residue was dissolved in EtOAc (20 mL), 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 (3:1, v/v) as eluent to afford the alcohol **12** as a yellow powder (31 mg, 36%); mp 68–70 °C; IR (film) 3623–3184 (OH), 3030 (C-H aromatic), 2925 (C-H aliphatic), 1750 (CO), 1305, 1148 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.76 (br s, 1H, O*H*, exchangeable with D_2O), 3.08 (s, 3H, SO_2CH_3), 4.75 (s, 2H, CH_2OH), 5.20 (s, 2H, furanone CH_2), 7.39–7.43 (m, 4H, hydroxymethylphenyl H-2, H-3, H-5, H-6), 7.53 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.93 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5); MS m/z 344.98, $C_{18}H_{17}O_5S$ (M + H) requires 345.38; 366.99, $C_{18}H_{16}O_5SNa$ (M + Na) requires 367.38.
O^2-Acetoxymethyl 1-[2-[4-(4-(4-methanesulfonylphenyl)-5H-furan-2-on-3-yl)phenylmethoxy-carbonyl]pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (**14**): A solution of the bromomethyl compound **11** (273 mg, 0.67 mmol) in DMSO (1 mL) and Et_3N (0.08 mL, 0.67 mmol) was stirred at 25 °C for 5 minutes. A solution of compound **13** (166 mg, 0.67 mmol) in DMSO (1 mL) was added and the reaction was allowed to proceed for 36 h at 25 °C with stirring. Ethyl acetate (30 mL) was added to dilute the reaction mixture, the organic phase was washed with water (5 × 10 mL), dried ($MgSO_4$), and the solvent from the organic fraction was removed in vacuo. The residue was purified by silica gel column chromatography using EtOAc-hexane (2:1, v/v) as eluent to give **14** as a white powder (115 mg, 30%): mp 75–77 °C; IR (film) 3030 (C-H aromatic), 2920 (C-H aliphatic), 1755 (CO), 1311, 1148 (SO_2), 1221, 1086 (N = N-O) cm^1; ^{1}H NMR ($CDCl_3$) δ 2.06–2.10 (m, 3H, pyrrolidin-1-yl H-3, H-4, H′-4), 2.08 (s, 3H, $COCH_3$), 2.30–2.39 (m, 1H, pyrrolidin-1-yl H′-3), 3.10 (s, 3H, SO_2CH_3), 3.73–3.81 (m, 1H, pyrrolidin-1-yl H-5), 3.85–3.93 (m, 1H, pyrrolidin-1-yl H′-5), 4.66 (dd, J = 8.5, 3.2 Hz, 1H, pyrrolidin-1-yl H-2), 5.19 (d, J = 12.8 Hz, 1H, -C*H*H′OCO), 5.21 (s, 2H, furanone CH_2), 5.26 (d, J = 12.8, 1H, CH*H*′OCO), 5.70 (d,

J = 7.4, 1H, OC*H*H′O), 5.73 (d, *J* = 7.4, 1H, OCH*H*′O), 7.41 (m, 4H, benzyl H-2, H-3, H-5, H-6), 7.52 (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 *m*/*z* 595.93, $C_{26}H_{27}N_3O_{10}SNa$ (M + Na) requires 596.14. Anal. Calcd for $C_{26}H_{27}N_3O_{10}S.1/7H_2O$: C, 54.24; H, 4.78; N, 7.30. Found: C, 54.70; H, 5.23; N, 6.84.

28. *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 (Rao, P. N. P.; Amini, M.; Li, H.; Habeeb, A.; Knaus, E. E. *J. Med. Chem.* **2003,** *46*, 4872).
29. *Nitric oxide release assays:* In vitro nitric oxide release, upon incubation of the test compound at 37 °C for 1.5 hour 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 (**14–15**) using the reported procedures (Velázquez, C.; Vo, D.; Knaus, E. E. *Drug Dev. Res.* **2003**, *60*, 204).
30. *Preparation of isolated vessels:* Adult male rats (Long Evans) were decapitated and a segment of the small intestine and attached mesentery was isolated (~10 cm from the ileal-cecal junction). Second order vessels, <250 mm in diameter and ~2 mm in length, were dissected out in cold (0–4 °C) HEPES-buffered phosphate saline (Concentration in mmol L^{-1}: 142 NaCl, 4.7 KCl, 1.17 $MgSO_4$, 1.56 $CaCl_2$, 1.18 K_2PO_4, 10 HEPES and 5.5 glucose, @ pH 7.4).
Vascular reactivity: Isolated mesenteric arteries were mounted on an isometric wire myograph system (Kent Scientific, Litchfield, CA, USA) and pretreated with rofecoxib (1 μM), Compound **12** (1 μM), compound **14** (1 μM) or DMSO for 45 min. Constrictive responses to phenylephrine (PE, 10^{-7}–5×10^{-5} M) were then measured as previously described (Andrew, P. S.; Kaufman, S. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2003,** *284*, R567). Separate arterial segments were pretreated with the inhibitors, and constricted with PE at a submaximal dose of 1 μM (EC_{80}). After reaching a plateau contraction, cumulative concentration response curves to acetylcholine (ACh, 10^{-10} to 10^{-4} M) were obtained to evaluate endothelium-dependent relaxation (Tawfik, H. E.; Cena, J.; Schulz, R.; Kaufman, S. *Am. J. Physiol. Heart Circ. Physiol.* **2008**, *295*, H1736).
Statistical analysis: Between groups variation was assessed using one-way ANOVA, followed by post hoc analysis with the Student-Newman-Keuls test..
31. *In vivo anti-inflammatory assay*: The test compounds **12**, **14**, and the reference drugs celecoxib and ibuprofen were evaluated using the in vivo carrageenan-induced rat foot paw edema model reported previously (Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544).
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