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Celecoxib analogs possessing a *N*-(4-nitrooxybutyl)piperidin-4-yl or *N*-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridin-4-yl nitric oxide donor moiety: Synthesis, biological evaluation and nitric oxide release studies

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ABSTRACT

A new group of hybrid nitric oxide (NO) releasing anti-inflammatory (AI) coxib prodrugs (NO-coxibs) wherein the *para*-tolyl moiety present in celecoxib was replaced by a *N*-(4-nitrooxybutyl)piperidyl **15a–b**, or *N*-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridyl **17a–b**, NO-donor moiety was synthesized. All compounds released a low amount of NO upon incubation with phosphate buffered saline (PBS) at pH 7.4 (2.4–5.8% range). In comparison, the percentage NO released was higher (3.1–8.4% range) when these nitrate prodrugs were incubated in the presence of L-cysteine. In vitro COX-1/COX-2 isozyme inhibition studies showed this group of compounds are moderately more potent, and hence selective, inhibitors of the COX-2 relative to the COX-1 enzyme. AI structure–activity relationship data acquired showed that compounds having a $MeSO_2$ COX-2 pharmacophore exhibited superior AI activity compared to analogs having a H_2NSO_2 substituent. Compounds having a $MeSO_2$ COX-2 pharmacophore in conjunction with a *N*-(4-nitrooxybutyl)piperidyl (ED_{50} = 132.4 mg/kg po), or a *N*-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridyl (ED_{50} = 118.4 mg/kg po), moiety exhibited an AI potency profile that is similar to aspirin (ED_{50} = 128.7 mg/kg po) but lower than ibuprofen (ED_{50} = 67.4 mg/kg po).

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever, and inflammation. The use of NSAIDs that selectively inhibit the inducible cyclooxygenase-2 (COX-2) isozyme in the periphery provided a useful drug design concept. This discovery resulted in the development of effective anti-inflammatory (AI) drugs that were devoid of adverse cardiovascular effects and gastrointestinal ulcerogenicity believed to be associated with inhibition of the constitutive cyclooxygenase isoform (COX-1).[1] Therefore, COX-2 selective inhibitors (coxibs) such as celecoxib (Celebrex®, **1**), rofecoxib (Vioxx®, **2**), and valdecoxib (Bextra®, **3a**) were 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 **2** and valdecoxib **3a** alter the natural balance in the COX biochemical 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).[3–5] 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®).[6,7]

Nitric oxide (NO) exhibits a number of useful pharmacological actions that include vascular relaxation (vasodilation), and inhibition of platelet aggregation and adhesion.[8] Hybrid 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. In this regard, NO-coxibs having a nitrate ester NO-donor moiety such as the oxazole (**3b**) exhibit anti-inflammatory activity similar to valdecoxib with antithrombotic action at higher doses,[9] and the pyrazole **4** that exhibits anti-inflammatory activity.[10] Accordingly, we showed that a novel group of hybrid NO-releasing NONO-NSAID ester prodrugs possessing a 1,3-dinitrooxy-2-propyl moiety attached directly to the carboxylic group of aspirin (**6b**), indomethacin (**7b**) or ibuprofen (**9b**) showed approximately equipotent anti-inflammatory activity to the parent NSAID, without significant gastric toxicity when administered orally.[11] (see structures in Fig. 1). Studies carried out by others have shown that nitrate-based NO-NSAIDs such as NO-aspirin (**5**, **6a**),[12,13] NO-indomethacin (**7a**, **8**),[14,15] and NO-ibuprofen (**9a**),[15] are gastrointestinal sparing while simultaneously suppressing prostaglandin synthesis similar to the parent drugs. As part of our ongoing research program to develop

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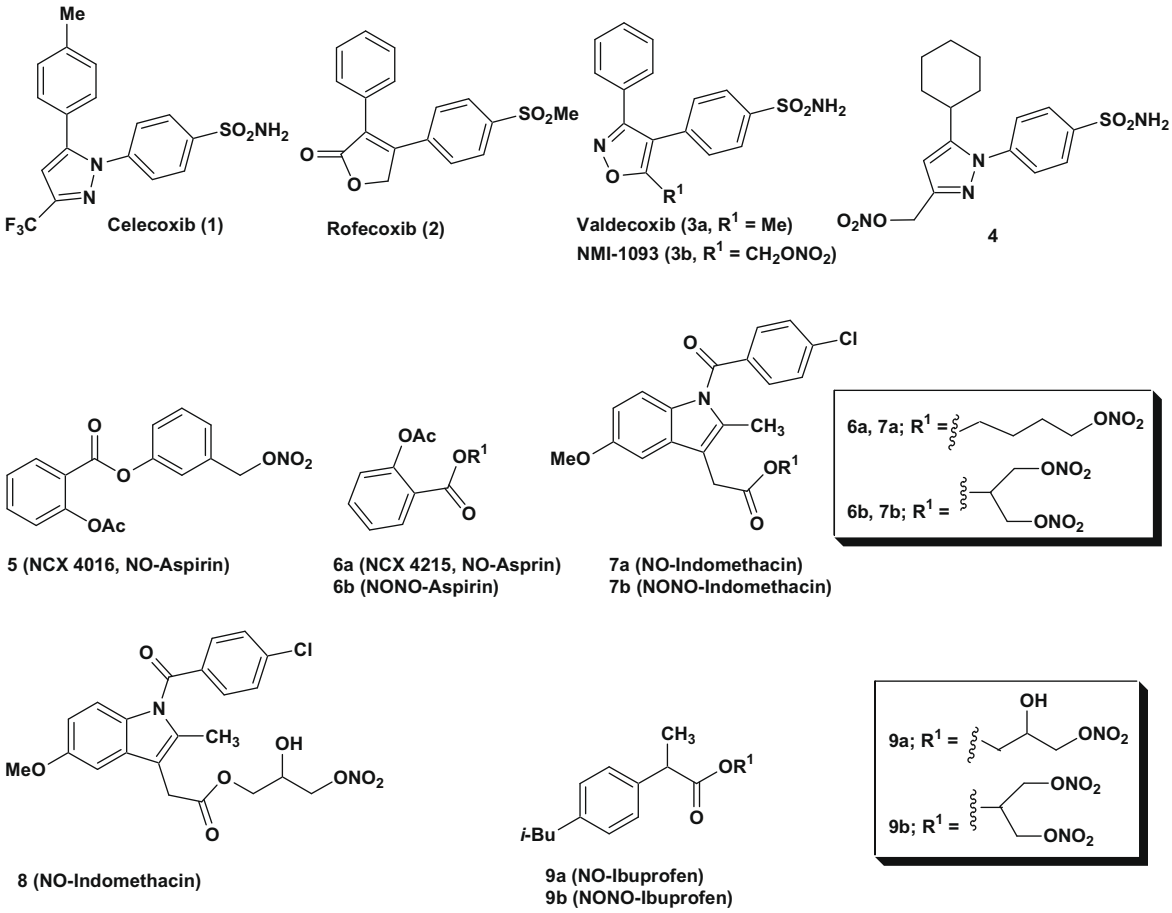


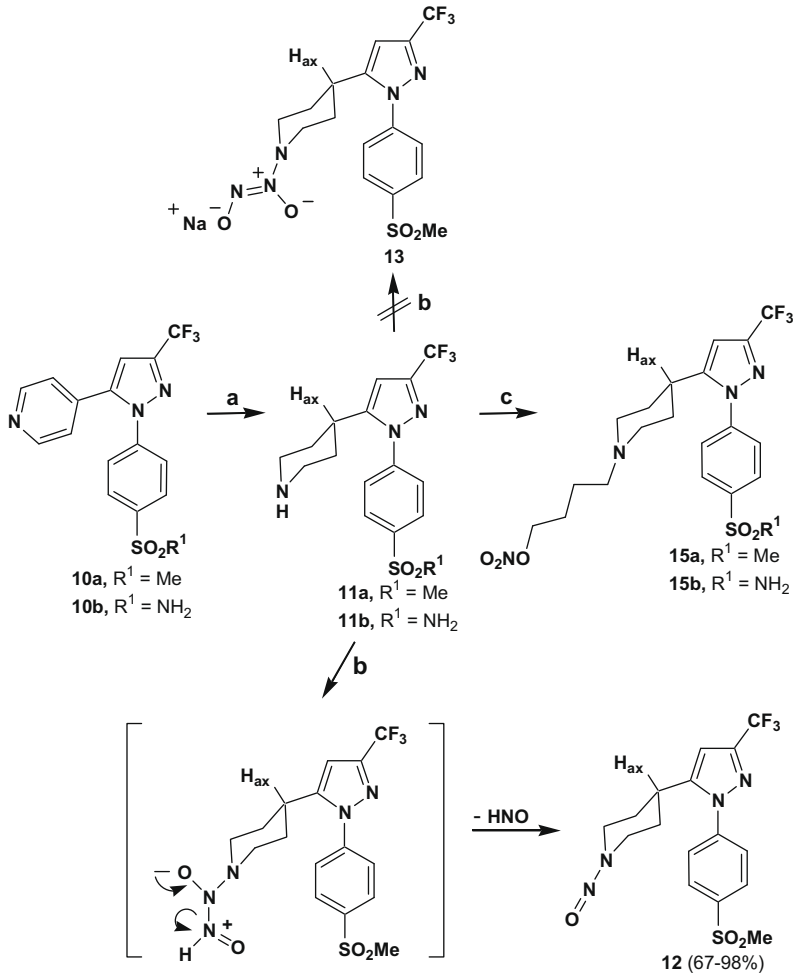
Figure 1. Chemical structures of the selective cyclooxygenase-2 (COX-2) inhibitors celecoxib (**1**), rofecoxib (**2**), and valdecoxib (**3a**), and the nitric oxide donor nitrate esters NMI-1093 (**3b**) and **4**, the 3-(nitrooxymethylphenyl) ester of aspirin (**5**), some nitrooxybutyl ester prodrugs of aspirin (**6a**) and indomethacin (**7a**), and the nitroglyceryl esters of indomethacin (**8**) and ibuprofen (**9a**), and 1,3-dinitrooxy-2-propyl esters of aspirin (**6b**), indomethacin (**7b**), and ibuprofen (**9b**).

anti-inflammatory agents devoid of side effects, we now report the synthesis, in vitro COX-1/COX-2 inhibitory activity, in vivo anti-inflammatory activity and nitric oxide release data for a group of coxib prodrugs that possess a *N*-(4-nitrooxybutyl)piperidyl **15a–b**, or a *N*-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridyl **17a–b**, NO-donor moiety in place of the tolyl substituent present in celecoxib.

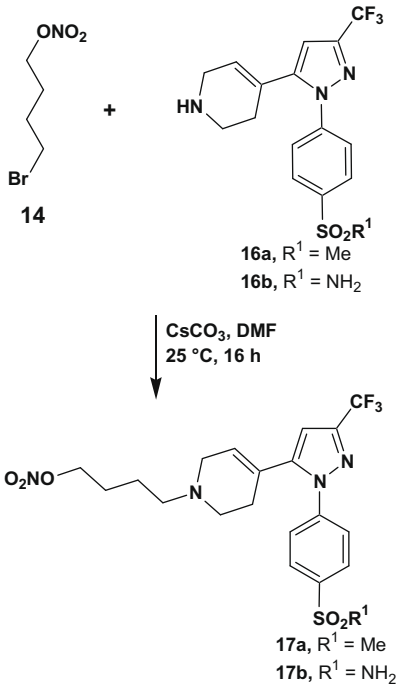
A group of 1-(4-methane(amino)sulfonylphenyl)-5-[1-(4-nitrooxybutyl)piperidin-4-yl]-3-trifluoromethyl-1*H*-pyrazoles (**15a–b**), and 1-(4-methane(amino)sulfonylphenyl)-5-[1-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridin-4-yl]-3-trifluoromethyl-1*H*-pyrazoles (**17a–b**), hybrid nitric oxide donor prodrugs were synthesized using the reaction sequence illustrated in Schemes 1 and 2, respectively. Hydrogenation of 1-(4-methane(amino)sulfonylphenyl)-5-(pyridin-4-yl)-3-trifluoromethyl-1*H*-pyrazole (**10a–b**) in glacial acetic acid in the presence of Pd/C catalyst[16] reduced the pyridine ring to a piperidine ring to furnish the respective piperidyl products **11a–b** in 70–77% yields. The observation that the C-4 piperidine proton possessed two large vicinal coupling constants ($J_{3ax,4ax}$ and $J_{4ax,5ax}$ = 12.2 Hz) indicates that the pyrazole ring in **11a–b** assumes an equatorial orientation. An initial study was directed toward synthesis of the target hybrid nitric oxide donor *N*-diazen-1-ium-1,2-diolate derivative **13** of 1-(4-methanesulfonylphenyl)-5-(piperidin-4-yl)-3-trifluoromethyl-1*H*-pyrazole (**11a**). However, reaction of the piperidine compound **11a** with nitric oxide gas (40 psi) under a variety of experimental conditions (aprotic and protic solvent systems, various temperatures) afforded the *N*-nitrosopiperidine **12** as the sole isolable product rather than the desired product **13** (see Scheme 1). For example, reaction in the aprotic THF solvent system in the presence of NaOMe (95%) at −65 °C afforded the *N*-nitrosopiperidyl product **12** in 67% yield. Alternatively, reaction of **11a** in a protic solvent system consisting of NaOMe in MeOH (25% w/v) and MeCN at 25 °C furnished **12** (98%). The formation of the *N*-nitroso product **12** upon reaction of **11a** with nitric oxide indicates that the unstable intermediate *N*-amino-*N*-diazen-1-ium-1,2-diolate product **13** must undergo protonation of the diazen-1-ium-1,2-diolate N^2-nitrogen which subsequently eliminates a HNO species (see mechanism shown in Scheme 1).[17] On the other hand, reaction of the piperidyl compounds **11a** or **11b** with 4-nitrooxybutyl bromide (**14**) in the presence of cesium carbonate in dry DMF afforded the target *N*-(4-nitrooxybutyl)piperidyl products **15a–b** in 11–23% yield (Scheme 1).

Alternatively, reaction of a 1,2,3,6-tetrahydropyridinyl compound **16a** or **16b** with **14** furnished the respective *N*-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridyl product **17a** or **17b** in 27–47% yields as illustrated in Scheme 2.

Hybrid nitric oxide donor prodrugs such as the *N*-(4-nitrooxybutyl)piperidines **15a–b** and the corresponding 1,2,3,6-tetrahydropyridine analogs **17a–b** constitute a potential class of selective COX-2 inhibitor agents that are devoid of adverse cardiovascular properties. Compounds **15a–b** and **17a–b** were designed based on structure–activity data showing that (i) a COX-2 pharmacophore such as $MeSO_2$ or H_2NSO_2 at the *para*-position of a N^1-phenyl ring on a pyrazole ring template confers potent and selective COX-2 inhibitory activity,[18] (ii) attachment of a 1,2,3,6-tetrahydropyridine ring substituent in compounds **17a–b** via their C-4 sp^2



Scheme 1. Reagents and conditions: (a) AcOH, 10% Pd/C, H_2 (60 psi), 25 °C, 20 h; (b) THF, NaOMe (95%), NO (40 psi), −65 °C, 1 h or MeCN, NaOMe (25% in MeOH), NO (40 psi) 25 °C, 24 h; (c) 4-nitrooxybutyl bromide (**14**), $CsCO_3$, DMF, 25 °C, 16 h.

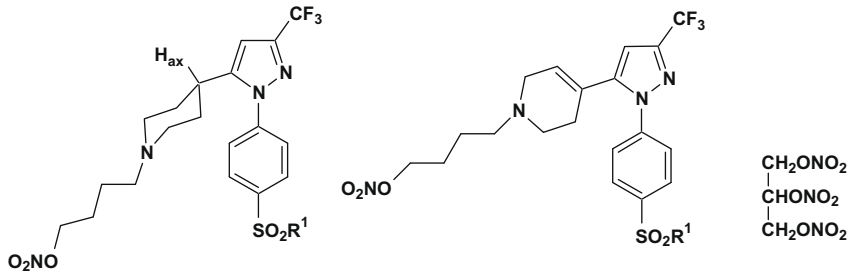


Scheme 2.

hybridized carbon atom is consistent with the observation that two aryl rings on adjacent positions of a five-membered heterocyclic ring template (scaffold) generally provide optimum COX-2 inhibitory activity,[19] (iii) the piperidyl and 1,2,3,6-tetrahydropyridyl secondary amino group, such as that present in compounds **11** and **16**, provides a logical synthon for elaboration to the corresponding nitric oxide donor *N*-diazen-1-ium-1,2-diolates or *N*-nitrates,[20–25] and (iv) the nitrooxybutyl group present in compounds **15a–b** and **17a–b** could release a COX-2 inhibitory compound and nitric oxide (NO).[10] Unfortunately, reaction of the piperidyl compound **11a** with nitric oxide (see Scheme 1) afforded the undesired *N*-nitroso product **12** rather than the desired unisolable *N*-diazen-1-ium-1,2-diolate product **13**.

In vitro COX-1/COX-2 enzyme inhibition studies (Table 1) showed that the piperidyl compound **15a** did not inhibit the COX-1 isozyme at the highest test compound concentration used (100 μM). Like the reference drug celecoxib (COX-1 IC_{50} = 7.7 μM), compounds **15b** and **17a–b** were weak inhibitors of COX-1 (IC_{50} = 16.2–34.7 μM range). Compounds **15a–b** and **17a–b** were moderately more potent inhibitors of COX-2 (IC_{50} = 7.5–16.8 μM range) than COX-1 that resulted in a modest selectivity for the COX-2 isozyme with COX-2 selectivity indexes in the 1.2 to >6 range. The COX-2 selectivity indexes shown by compounds **15a–b** and **17a–b** are higher than reference drug aspirin (selectivity index = 0.13) and comparable with ibuprofen (selectivity index = 2.64). The $MeSO_2$ and H_2NSO_2 pharmacophores are not a

Table 1
In vitro COX-1 and COX-2 inhibition, percent (%) nitric oxide release and anti-inflammatory (AI) data for 1-(4-methane(amino)sulfonylphenyl)-5-[1-(4-nitrooxybutyl)piperidin-4-yl]-3-trifluoromethyl-1*H*-pyrazoles (**15a–b**) and 1-(4-methane(amino)sulfonylphenyl)-5-[1-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridin-4-yl]-3-trifluoromethyl-1*H*-pyrazoles (**17a–b**), glycerol trinitrate (GTN, **18**), and the reference drugs celecoxib, aspirin, and ibuprofen



15a, R^1 = Me; **15b,** R^1 = NH_2 **17a,** R^1 = Me; **17b,** R^1 = NH_2 **18** (GTN)

Compound	IC_{50}[a] (μM)		COX-2 S.I.[b]	% NO released[c]		AI activity[f]: ED_{50} (mg/kg)
	COX-1	COX-2		PBS[d]	L-Cysteine[e]	
15a	>100	15.6	>6	3.8	8.4	132.4 ± 4.7
15b	19.8	16.8	1.2	2.4	3.1	Weak activity[g]
17a	34.7	12.8	2.7	3.1	3.8	118.4 ± 4.1
17b	16.2	7.5	2.2	5.8	7.1	Inactive[h]
18	—	—	—	2.8	10.1	—
Celecoxib	7.7	0.12	64.2	—	—	10.8
Aspirin	0.3	2.4[i]	0.13	—	—	128.7
Ibuprofen	2.9	1.1[i]	2.64	—	—	67.4

[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 (i) 1 mol of NO/mol of the oxynitrate test compounds (**15a–b**, **17a–b**) and (ii) 3 mol of NO/mol of glycerol trinitrate (**18**). The result is the mean value of three measurements ($n = 3$) where variation from the mean% value was ⩽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 which contained 5.0 mM L-cysteine), 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 ED_{50} value (mg/kg) at 3 h after oral administration of the test compound.
[g] A 9.0% inhibition of inflammation was observed using a 100 mg/kg oral dose.
[h] Inactive at a 100 mg/kg oral dose.
[i] Data acquired using ovine COX-2 (Catalog No. 56101, Cayman Chemical Inc.).

major determinant of COX-2 inhibitory potency since compounds **15a** and **15b**, and **17a** and **17b**, show similar potencies. Compounds 17a–**b** having a 1,2,3,6-tetrahydropyridyl ring are moderately more potent COX-2 inhibitors compared to the corresponding piperidyl compounds **15a–b**. This difference in potency may be due to the fact that the 1,2,3,6-tetrahydropyridyl ring is attached via a sp^2 hybridized carbon while the piperidyl ring is attached via a sp^3 hybridized carbon. The most potent COX-2 inhibitor 1,2,3,6-tetrahydropyridyl compound **17a** having a H_2NSO_2 moiety is about equipotent with aspirin but is less potent than celecoxib and ibuprofen.

The percent NO released from the hybrid prodrugs **15a–b** and **17a–b** upon incubation in phosphate-buffered-saline (PBS at pH 7.4), varied over a narrow range (2.4–5.8%) which is indicative of slow NO release[26] (see data in Table 1). The percentage of NO released from the *N*-nitrooxybutyl compounds **15a–b** and **17a–b** was higher in the presence of 5 mM L-cysteine (3.1–8.4% range) than in the absence of L-cysteine. This latter observation is consistent with reports that NO release from organic nitrates is facilitated by thiols.[27,28]

The oral AI activities exhibited by the piperidyl **15a**–**b**, and 1,2,3,6-tetrahydropyridyl **17a**–**b**, compounds were determined using a carrageenan-induced rat foot paw edema model (see data in Table 1). Structure–activity relationship data showed that the relative AI potency profile (i) with respect to the COX-2 pharmacophore moiety was $SO_2Me > SO_2NH_2$ irrespective of whether a *N*-(4-nitrooxybutyl)piperidyl (**15a** > **15b**) or *N*-(4-nitrooxybutyl)-1,2,3,6-tetrahydropiperidyl (**17a** > **17b**) moiety is present, (ii) within the *N*-(4-nitrooxybutyl)piperidyl group of compounds, **15a** (ED_{50} = 132.4 mg/kg po) is a more potent AI agent than **15b** which showed a weak 9.0% inhibition of inflammation for a 100 mg/kg oral dose, and (iii) within the *N*-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridyl group of compounds, **17a** exhibited AI activity (ED_{50} = 118.4 mg/kg po) between that of aspirin (ED_{50} = 128.7 mg/kg po), and ibuprofen (ED_{50} = 67.4 mg/kg po). In contrast, the sulfonamide compound **17b** showed no inhibition of inflammation at the maximal dose employed (100 mg/kg oral dose).

In conclusion, a new class of 1-(4-methane(amino)sulfonylphenyl)-5-[1-(4-nitrooxybutyl)piperidin-4-yl]-3-trifluoromethyl-1*H*-pyrazoles (**15a–b**) and 1-(4-methane(amino)sulfonylphenyl)-5-[1-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridin-4-yl]-3-trifluoromethyl-1*H*-pyrazoles (**17a–b**) was synthesized[29] for evaluation as COX-1/COX-2 isozyme inhibitors,[30] NO donors,[31] and as anti-inflammatory agents.[32] The structure–activity data acquired indicate that (i) compounds **15a–b** and **17a–b** exhibit weak in vitro COX-2 inhibitory activity, (ii) compounds having a $MeSO_2$ COX-2 pharmacophore show superior AI activity compared to the corresponding H_2NSO_2 compounds, (iii) both classes of prodrugs (**15a–b** and **17a–b**) are relatively stable in phosphate-buffered saline at pH 7.4 where NO release is in the 2.4–5.8% range, (iv) L-cysteine moderately enhances the release of NO (3.1–8.4% range), and (v) modification of celecoxib by replacement of the H_2NSO_2 by $MeSO_2$, and the *para*-tolyl moiety by either a *N*-(4-nitrooxybutyl)piperid-4yl (**15a**) or *N*-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyrid-4yl

(**17a**) moiety furnishes compounds that exhibit AI activity between that of the reference drugs aspirin and ibuprofen.

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29. *Experimental procedures and spectral data for compounds* **11**, **12**, **15** *and* **17**. *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 and ^{13}C NMR spectra were measured on a Bruker AM-300 spectrometer. Microanalyses (Micro Analytical Service Laboratory, Department of Chemistry, University of Alberta) were performed for C and H unless otherwise stated, and were within ±0.4% of theoretical values for all elements listed. Silica gel column chromatography was performed using Merck Silica Gel 60 ASTM (70–230 mesh). 1-(4-Methane(amino)sulfonylphenyl)-5-(pyridine-4-yl)-3-trifluoromethyl-1*H*-pyrazoles (**10a** and **10b**) and 1-(4-methane(amino)sulfonylphenyl)-5-(1,2,3,6-tetrahydropyridin-4-yl]-3-trifluoromethyl-1*H*-pyrazole (**16a** and **16b**) were prepared according to our previously reported procedure (Chowdhury, M. A.; Abdellatif, K. R. A.; Dong, Y.; Knaus, E. E. *Bioorg. Med. Chem.* **2008**, *16*, 8882). 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.

General procedure for the synthesis of 1-(4-methane(amino)sulfonylphenyl)-5-(piperidin-4-yl)-3-trifluoromethyl-1H-pyrazoles (**11a–b**)*:* Palladium-on-charcoal (1.13 g of 10% w/w) was added to a solution of one of 1-(4-methane(amino)sulfonylphenyl)-5-(pyridin-4-yl)-3-trifluoromethyl-1*H*-pyrazole (**10a** or **10b**, 4.08 mmol) in acetic acid (50 mL). The resulting suspension was then flushed with argon followed by three consecutive flushes with H_2 gas to remove any air or argon from the hydrogenation flask. The pressure in the hydrogenation flask was maintained at 60 psi with H_2 gas using a Parr apparatus. After shaking for 20 h the H_2 gas was released from the hydrogenation flask and the reaction mixture was filtered through a celite pad to remove any Pd/C catalyst. The filtrate was evaporated in vacuo to give a solid product as the acetate salt that was treated with a saturated Na_2CO_3 solution (100 mL) prior to extraction with ethyl acetate (3 × 100 mL). The combined organic fractions were washed successively with water and brine, and the organic fraction was dried ($MgSO_4$). Filtration and then removal of the solvent from the organic fraction in vacuo afforded the crude product which was recrystallized with acetone–hexanes to furnish the respective title compound **11a** or **11b**. Some physical and spectroscopic data for **11a–b** are listed below.
1-(4-Methanesulfonylphenyl)-5-(piperidin-4-yl)-3-trifluoromethyl-1H-pyrazole (**11a**): This compound was obtained as white crystals in 77% yield; mp 174–176 °C; IR (film) 3320 (br NH), 2950 (C–H aromatic), 2855 (C–H aliphatic), 1319, 1154 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.56 (br s, 1H, N*H* that exchanges with D_2O), 1.65 (dddd, J = 12.2, 12.2, 12.2, 3.7 Hz, 2H, piperidyl H-3ax, H-5ax), 1.81 (br d, J = 12.2 Hz, 2H, piperidyl H-3 equiv, H-5 equiv), 2.61 (ddd, J = 12.2, 12.2, 1.9 Hz, 2H, piperidyl H-2ax, H-6ax), 2.80 (dddd, $J_{3ax,4ax}$ = 12.2, $J_{4ax,5ax}$ = 12.2, $J_{3equiv,4ax}$ = 3.7, $J_{4ax,5equiv}$ = 3.7 Hz, 1H, piperidyl H-4ax), 3.12 (s, 3H, SO_2CH_3), 3.13 (br d, J = 12.2 Hz, 2H, piperidyl H-2 equiv, H-6 equiv), 6.55 (s, 1H, pyrazole H-4), 7.66 (d, J = 8.6 Hz, 2H, phenyl H-2, H-6), 8.11 (d, J = 8.6 Hz, 2H, phenyl H-3, H-5). Anal. Calcd for $C_{16}H_{18}F_3N_3O_2S$: C, 51.47; H, 4.86; N, 11.25; S, 8.59. Found: C, 51.24; H, 4.82; N, 10.89; S, 8.46.
1-(4-Aminosulfonylphenyl)-5-(piperidin-4-yl)-3-trifluoromethyl-1H-pyrazole (**11b**): This compound was obtained as white crystals in 70% yield; mp 205–206 °C; IR (film) 3320 (br NH), 2943 (C–H aromatic), 2858 (C–H aliphatic), 1350, 1166 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.45 (dddd, J = 12.2, 12.2, 12.2, 3.7 Hz, 2H, piperidyl H-3ax, H-5ax), 1.66 (br d, J = 12.2 Hz, 2H, piperidyl H-3 equiv, H-5 equiv), 2.39 (ddd, J = 12.2, 12.2, 1.9 Hz 2H, piperidyl H-2ax, H-6ax), 2.78 (dddd, $J_{3ax,4ax}$ = 12.2, $J_{4ax,5ax}$ = 12.2, $J_{3equiv,4ax}$ = 3.7, $J_{4ax,5equiv}$ = 3.7 Hz, 1H, piperidyl H-4ax), 2.90 (br d, J = 12.2 Hz, 2H, piperidyl H-2 equiv, H-6 equiv), 3.35 (br s, 1H, N*H* that exchanges with D_2O), 6.87 (s, 1H, pyrazole H-4), 7.40 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.77 (d, J = 8.6 Hz, 2H, phenyl H-2, H-6), 7.99 (d, J = 8.6 Hz, 2H, phenyl H-3, H-5). Anal. Calcd for $C_{15}H_{17}F_3N_4O_2S{\cdot}1.3H_2O$: C, 47.36; H, 4.68. Found: C, 47.57; H, 4.72.
1-(4-Methanesulfonylphenyl)-5-(1-nitrosopiperidin-4-yl)-3-trifluoromethyl-1H-pyrazole (**12**): The piperidine compound **11a** (200 mg, 0.53 mmol) was added to a solution of NaOMe (65 mg of 95% purity, 0.53 mmol) in THF (10 mL) with stirring at −65 °C. This mixture was purged with argon for 5 min, and the reaction was allowed to proceed under an atmosphere of nitric oxide gas (40 psi internal pressure) with stirring at −65 °C for 1 h. The reaction mixture was allowed to warm to 25 °C and filtered to remove some inorganic materials. The solvent was removed from the filtrate to furnish product **12** in 67% yield. In a second reaction, nitric oxide gas was bubbled into a solution of **11a** using a protic solvent system consisting of NaOMe in MeOH (25% w/v) and MeCN at 25 °C which afforded **12** in 98% yield; mp 202–203 °C; IR (film) 3028 (C–H aromatic), 2931 (C–H aliphatic), 1317, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.55 and 1.90 (dddd, J = 12.2, 12.2, 12.2, 3.7 Hz, 1H each, piperidyl H-3ax and H-5ax), 1.94 and 2.10 (br d, J = 12.2 Hz, 1H each, piperidyl H-3 equiv and H-5 equiv), 2.51 and 3.68 (ddd, J = 12.8, 12.8, 3.1 Hz, 1H each, piperidyl H-2ax and H-6ax), 3.11 (dddd, J = 12.2, 12.2, 3.7, 3.7 Hz, 1H, piperidyl H-4ax), 3.12 (s, 3H, SO_2CH_3), 4.90 and 5.13 (dd, J = 12.1, 2.4 Hz, 1H each, piperidyl H-2 equiv and H-6 equiv), 6.54 (s, 1H, pyrazole H-4), 7.70 (dd, J = 8.6, 2.5 Hz, 2H, phenyl H-2, H-6), 8.14 (dd, J = 8.6, 2.5 Hz, 2H, phenyl H-3, H-5). Anal. Calcd for $C_{16}H_{17}F_3N_4O_3S$: C, 47.76; H, 4.26. Found: C, 47.92; H, 4.25.
General procedure for the synthesis of 1-(4-methane(amino)sulfonylphenyl)-5-[1-(4-nitrooxybutyl)piperidin-4-yl]-3-trifluoromethyl-1H-pyrazoles (**15a–b**) *and 1-(4-methane(amino)sulfonylphenyl)-5-[1-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridin-4-yl]-3-trifluoromethyl-1H-pyrazoles* (**17a–b**)*:* Cesium carbonate (0.22 g, 0.67 mmol) and 4-nitrooxybutyl bromide (**14**, 0.27 g, 1.34 mmol) were added to a solution of the piperidine **11a** or **11b**, or 1,2,3,6-tetrahydropyridine **16a** or **16b**, compound (1.34 mmol) in dry DMF (4 mL). The reaction mixture was stirred at 25 °C for 24 h, and water (10 mL) was added prior to extraction with EtOAc (3 × 20 mL). The combined organic fractions were washed successively with water and brine, and the organic fraction was dried ($MgSO_4$). Filtration and removal of the solvent from the organic fraction in vacuo afforded the crude product which was purified by silica gel column chromatography using hexanes/EtOAc (1:3, v/v) as eluent to furnish the respective title compound **15a–b** or **17a–b**. The low isolated yields of **15a–b** and **17b** is attributed to product decomposition during column purification. Some physical and spectroscopic data for **15a–b** and **17a–b** are listed below.
1-(4-Methanesulfonylphenyl)-5-[1-(4-nitrooxybutyl)piperidin-4-yl]-3-trifluoromethyl-1H-pyrazole (**15a**)*:* The product was obtained as a colorless gum in 23% yield; IR (film) 2957 (C–H aromatic), 2850 (C–H aliphatic), 1695, 1627, 1280 (ONO_2), 1319, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.52–1.70 (m, 2H, piperidyl H-3ax, H-5ax), 1.70–1.90 (m, 6H, piperidyl H-3 equiv, H-5 equiv, $CH_2CH_2CH_2ONO_2$), 2.65–2.83 (m, 2H, piperidyl H-2ax, H-6ax), 2.87 (dddd, J = 12.2, 12.2, 3.7, 3.7 Hz, 1H, piperidyl H-4ax), 3.14 (s, 3H, SO_2Me), 4.14 (t, J = 5.5 Hz, 2H, NCH_2), 4.07–4.35 (m, 2H, piperidyl H-2 equiv, H-6 equiv), 4.50 (t,

J = 6.1 Hz, 2H, CH_2ONO$_2$), 6.55 (s, 1H, pyrazole H-4), 7.68 (d, *J* = 8.6 Hz, 2H, phenyl H-2, H-6), 8.15 (d, *J* = 8.6 Hz, 2H, phenyl H-3, H-5). Anal. Calcd for $C_{20}H_{25}F_3N_4O_5S\cdot 1/6H_2O$: C, 48.67; H, 5.17. Found: C, 48.28; H, 4.98.

1-(4-Aminosulfonylphenyl)-5-[1-(4-nitrooxybutyl)piperidin-4-yl]-3-trifluoromethyl-1H-pyrazole (**15b**): The product was obtained as a colorless gum in 11% yield; IR (film) 3242 (br NH_2), 2928 (C–H aromatic), 2850 (C–H aliphatic), 1682, 1624, 1275 (ONO_2), 1320, 1160 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.55–1.71 (m, 2H, piperidyl H-3ax, H-5ax), 1.71-1.90 (m, 6H, piperidyl H-3 equiv, H-5 equiv, CH_2CH_2CH_2ONO$_2$), 2.65–2.87 (m, 3H, piperidyl H-2ax, H-4ax, H-6ax), 4.14 (t, *J* = 5.5 Hz, 2H, NCH_2), 4.05–4.35 (m, 2H, piperidyl H-2 equiv, H-6 equiv), 4.50 (t, *J* = 6.1 Hz, 2H, CH_2ONO$_2$), 5.04 (br s, 2H, SO$_2$NH_2 that exchanges with D_2O), 6.54 (s, 1H, pyrazole H-4), 7.62 (d, *J* = 8.6 Hz, 2H, phenyl H-2, H-6), 8.12 (d, *J* = 8.6 Hz, 2H, phenyl H-3, H-5). Anal. Calcd for $C_{19}H_{24}F_3N_5O_5S$: C, 46.43; H, 4.92. Found: C, 46.20; H, 4.76.

1-(4-Methanesulfonylphenyl)-5-[1-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridin-4-yl]-3-trifluoromethyl-1H-pyrazole (**17a**): The product was obtained as a colorless gum in 47% yield; IR (film) 2930 (C–H aromatic), 2850 (C–H aliphatic), 1699, 1627, 1280 (ONO_2), 1325, 1160 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.57–1.77 (m, 4H, CH_2CH_2CH_2ONO$_2$), 2.22–2.24 (m, 2H, tetrahydropyridyl H-3), 3.30 (s, 3H, SO$_2$*Me*), 3.40–3.55 (m, 2H, tetrahydropyridyl H-2), 3.91–3.93 (m, 2H, tetrahydropyridyl H-6), 4.04 (t, *J* = 6.1 Hz, 2H, NCH_2), 4.54 (t, *J* = 6.1 Hz, 2H, CH_2ONO$_2$), 5.84–5.86 (m, 1H, tetrahydropyridyl H-5), 7.07 (s, 1H, pyrazole H-4), 7.81 (d, *J* = 8.6 Hz, 2H, phenyl H-2, H-6), 8.08 (d, *J* = 8.6 Hz, 2H, phenyl H-3, H-5). Anal. Calcd for $C_{20}H_{23}F_3N_4O_5S\cdot 2H_2O$: C, 45.80; H, 5.19. Found: C, 46.09; H, 4.90.

1-(4-Aminosulfonylphenyl)-5-[1-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridin-4-yl]-3-trifluoromethyl-1H-pyrazole (**17b**): The product was obtained as a colorless gum in 27% yield; IR (film) 3232, 3104 (br NH_2), 2930 (C–H aromatic), 2855 (C–H aliphatic), 1684, 1627, 1281 (ONO_2), 1325, 1161 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.58–1.78 (m, 4H, CH_2CH_2CH_2ONO$_2$), 2.18–2.22 (m, 2H, tetrahydropyridyl H-3), 3.44-3.47 (m, 2H, tetrahydropyridyl H-2), 3.90-3.92 (m, 2H, tetrahydropyridyl H-6), 4.03 (t, *J* = 6.1 Hz, 2H, NCH_2), 4.53 (t, *J* = 6.1 Hz, 2H, CH_2ONO$_2$), 5.85 (br s, 1H, tetrahydropyridyl H-5), 7.05 (s, 1H, pyrazole H-4), 7.55 (s, 2H, SO$_2$NH_2 that exchanges with D_2O), 7.73 (d, *J* = 8.6 Hz, 2H, phenyl H-2, H-6), 7.96 (d, *J* = 8.6 Hz, 2H, phenyl H-3, H-5). Anal. Calcd for $C_{19}H_{22}F_3N_5O_5S\cdot 1/4H_2O$: C, 46.19; H, 4.59. Found: C, 46.02; H, 4.91.

30. *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).
31. *Nitric oxide release assays:* In vitro nitric oxide release, upon incubation of the test compound at 37 °C for 1.5 h with 2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer at pH 7.4 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 (**15a–b**, **17a–b**) using the reported procedures (Velázquez, C.; Vo, D.; Knaus, E. E. *Drug Dev. Res.* **2003**, *60*, 204).
32. *In vivo anti-inflammatory assay*: The test compounds **15a–b**, **17a–b**, and the reference drugs celecoxib, aspirin, 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).