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Article

Design, synthesis, biological evaluation, and nitric-oxide release studies of a novel series of celecoxib prodrugs possessing a nitric-oxide donor moiety

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A new group of hybrid nitric oxide-releasing anti-inflammatory drugs (NONO-coxibs), in which an *O*^{2}-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate NO-donor moiety is attached directly to the carboxylic acid group of 1-(4-aminosulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylic acids (**6a–c**), were synthesized. A low amount of NO was released from the diazen-1-ium-1,2-diolate compounds **6a–c** upon incubation with phosphate buffer saline (PBS) at pH 7.4 (range: pH 7.97–8.51), whereas, the percentage of NO released was significantly higher (84.5%–85.05% of the theoretical maximal release of two molecules of NO/molecule of the parent hybrid ester prodrug) when the diazen-1-ium-1,2-diolate ester prodrugs were incubated in the presence of rat serum. These incubation studies demonstrated that both NO and the anti-inflammatory 1-(4-aminosulfonylphenyl)-5-(4-H, 4-F or 4-Me-phenyl)-1*H*-pyrazol-3-carboxylic acid (**4a–c**) would be released from the parent NONO-coxib upon in vivo cleavage by non-specific serum esterases. The parent compounds **4a-c** displayed good anti-inflammatory effects (ID_{50}=81.4–112.4 mg/kg p.o.) between those exhibited by the reference drugs, aspirin (ID_{50}=114.3 mg/kg p.o.) and celecoxib (ID_{50}=12.6 mg/kg p.o.). Hybrid ester anti-inflammatory/NO-donor prodrugs (NONO-coxibs) offer a potential drug-design concept directed toward the development of anti-inflammatory drugs that are lacking adverse ulcerogenic and/or cardiovascular effects.

Keywords: Anti-inflammatory/development/drugs. NONO-coxib esters. 1,5-dihydropyrazoles.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) represent a miscellaneous group of compounds that are essentially used to combat fever, pain, and inflammation. NSAIDs remain by far among the most commonly used classes of medications, accounting for 2.5% of all prescription dollars in the world (Trelle *et al.*, 2011). NSAIDs exert their pharmacological action by inhibiting the synthesis of prostaglandins (PGs) by non-selectively blocking cyclooxygenases (COX)-1 and COX-2, or by selectively blocking COX-2. (Laine, 2002; Gunter *et al.*, 2017). COX-1 is constitutively expressed in many tissues, and it physiologically functions in the maintenance of renal function, the protection of gastric mucosa, and the regulation of platelet aggregation, while COX-2 is inducible by pro-inflammatory mediators (Gunter *et al.*, 2017). The use of NSAIDs that selectively inhibit the inducible 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) (Thomsen *et al.*, 2006). Therefore, COX-2 selective inhibitors (coxibs), such as celecoxib, **I**, rofecoxib, **II**, and valdecoxib, **III**, (Figure 1) were developed for the long-term treatment of patients suffering from chronic pain and inflammation (Turini, DuBois,

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2002). Unfortunately, some selective COX-2 inhibitory drugs that include rofecoxib **II** and valdecoxib **III** alter the natural balance in the COX biochemical pathway. In this regard, the amount of desirable vasodilatory and anti-aggregatory prostacyclin (PGI2) produced is decreased, and this is accompanied by a simultaneous increase in the level of the undesirable prothrombotic thromboxane A2 (TxA2) (Hinz, Brune, 2002; Patel, Gross, 2002). 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, **II** and valdecoxib, III (Scheen, 2004; Dogné, Supuran, Pratico, 2005).

Nitric oxide (NO) displays a number of favorable pharmacological actions that include vascular relaxation (vasodilation) and inhibition of platelet aggregation and adhesion (Serafim *et al.*, 2012). Brueggeman *et al.*(2009) found that celecoxib, but not rofecoxib, dilated preconstricted small mesenteric arteries. The authors attributed the vasodilatory activity of celecoxib to the enhancement of KCNQ potassium currents and the suppression of L-type voltage-sensitive calcium currents. NO was shown to exhibit some of the general properties of PGs within gastric mucosa, and thus it should augment the local mucosal defense mechanism, thereby compensating the reduced gastric PGs produced by NSAIDs (Vannini *et al.*, 2015).

The last decade has seen an excessive development of NO-based hybrid drugs in which an appropriate NO-releasing chemical moiety was linked to the parent – already marketed – drugs to refine the overall pharmacotherapeutic efficacy, as well as to reduce any related noxious effects (Serafim *et al.*, 2012; Consalvi, Biava, Poce, 2015; Abdellatif *et al.*, 2017). *In vivo* and *in vitro* studies on NO-aspirin displayed more potent antithrombic properties when compared to aspirin. Furthermore, in an animal model of chronic neurodegenerative disease, NO-flurbiprofen and NO-aspirin incapacitated the brain's inflammatory response (Shinde, Modi, Kulkarni, 2017). In 2011, Abdellatif and co-workers (Abdellatif *et al.*, 2011) disclosed the anti-inflammatory and vascular-relaxing activity of a series of diazen-1-ium-1,2-diolated NO donor ester prodrugs of 3-(4-hydroxymethylphenyl)-4-(4-methanesulfonylphenyl)-5*H*-furan-2-one. Other new perspectives of NO donor molecules, including cardioprotectives, have been discussed in a recent study in which the researchers revealed the molecular mechanism of NO action on blood vessels (Kruzliak, Kovacova, Pechanova, 2013; Martelli *et al.*, 2013; Kruzliak, Novák, Novák, 2014). In 2014, Martinez *et al*. patented a series of NO-releasing guanidine coxibs capable of releasing NO through an enzymatic pathway involving the guanidine moiety while retaining the scaffold of celecoxib (Martinez *et al.*, 2014). Furthermore, Kontrek *et al.* have revealed that the conjugation of celecoxib with an NO donor moiety retains the anti-inflammatory properties of the parent drug while improving cardiovascular safety and antitumor efficacy, which is in line with other drugs of the NO-NSAIDS class (Konturek *et al.*, 2006). In another study by Knaus *et al.*, the non-ulcerogenic NONO-NSAID ester prodrugs of aspirin (**IV**) and indomethacin (V) (Figure 1) that have an NO-donor diazen-1-ium-1,2-diolate (NONOate) moiety that are effectively cleaved by esterases to release the AI drug and NO, were reported (Velázquez *et al.*, 2008). Therefore, the attachment of a NO-donor moiety to highly selective COX-2 inhibitors (NONO-coxibs) offers a potential drug-design concept that can be leveraged to outwit adverse cardiovascular events (Martelli *et al.*, 2013).

Diazen-1-ium-1,2-diolate ions, after their cleavage from the parent hybrid NONO-coxib, can release up to two equivalents of NO without further metabolic activation. These ions are structurally diverse and they possess a rich derivatization chemistry that facilitates delivery of NO to specific organ and/or tissue sites (Keefer, 2003). These features distinguish the NONO-coxib **IV** and **V** (Figure 1) from nitrate-based NO-coxibs (NMI-1093, Figure 1), which require redox activation before NO is released (Dhawan *et al.*, 2005).

Accordingly, the attachment of a *N*-diazen-1-ium-1,2-diolate moiety offers a potential drug-design concept to circumvent the adverse cardiovascular events associated with the chronic clinical use of highly selective COX-2 inhibitors.

RESULTS AND DISCUSSION

A COX-2 pharmacophore, such as methanesulfonyl, sulfonamide, methanesulfonamide, azido, or tetrazole, is required at the R^1 position on the N^1-phenyl ring for potent and selective COX-2 inhibitory activity (Zarghi *et al.*, 2011; Zarghi *et al.*, 2012). Studying the CYP2C9 structure–metabolism relationships to optimize the metabolic stability of COX-2 inhibitors, Ahlstrom *et al.* revealed that the pyrazole ring C-3 position (R^2 substituent) imposes very few steric restrictions pertaining to COX-2 inhibitors, implying that the inhibitory properties of the COX-2 isozyme should be retained (Ahlström *et al.*, 2007). It was also disclosed that the R^2 carboxyl substituent at the C-3 position containing compounds may undergo an electrostatic interaction with Arg120 in the binding pocket of the COX-2 enzyme. The

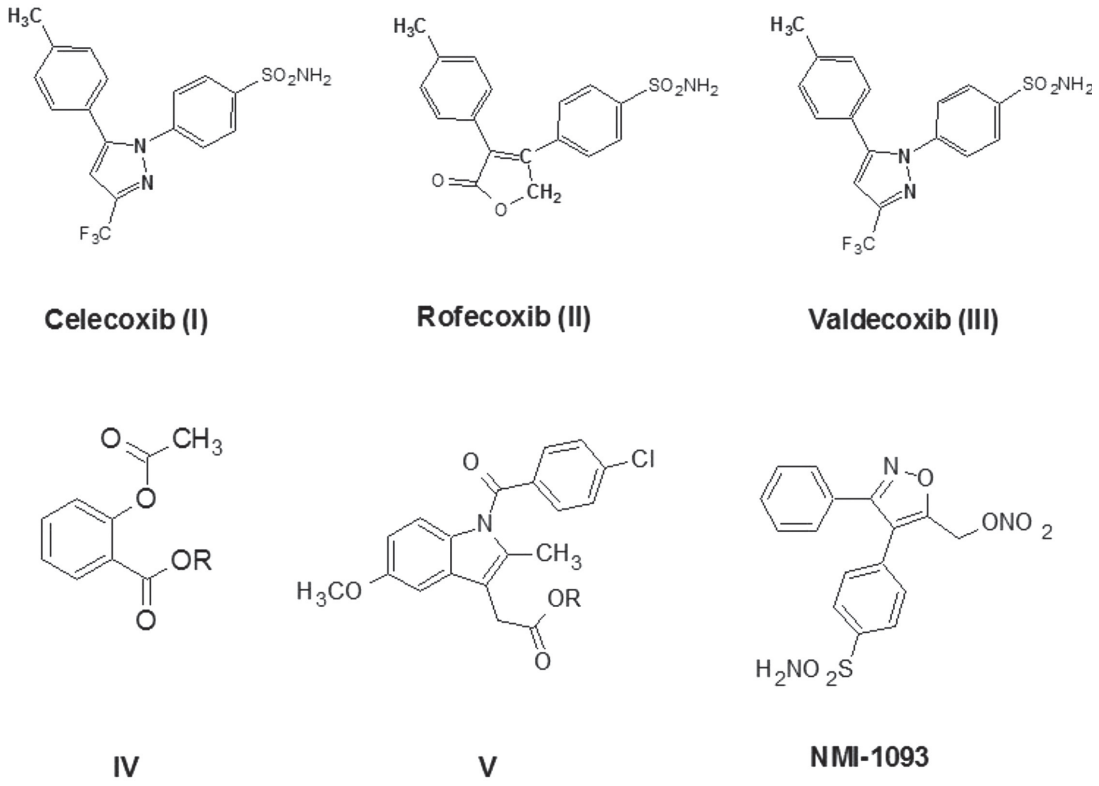


FIGURE 1 - Chemical structure of the selective COX-2 inhibitors celecoxib (I), rofecoxib (II), and valdecoxib (III), diazen-1-ium-diolate ester prodrugs of Aspirin (IV) and indomethacin (V), and nitrate-based prodrug (NMI-1093).

R^3 methyl substituent (benzylic carbon) in celecoxib (see the structure in Figure 2) undergoes sequential metabolic biotransformation to inactive metabolites;
Me → CH_2OH → CO_2H → CO_2 glucuronide conjugate) thus, it was concluded that the R^3 carboxyl substituent on the pyrazole C-5 phenyl ring was not tolerable (Abdellatif *et al.*, 2009). Based on this structural information, it was decided that the *O*^{2}-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino) diazen-1-ium-1,2-diolate NO-donor moiety be coupled to a pyrazole ring from the C-3 CO_2H group via an ester moiety to prepare the target NONO–coxib hybrid ester prodrugs (**6a**–**c**).

The percentage of NO released from the hybrid ester prodrugs (**6a**–**c**) upon incubation in phosphate buffered saline (PBS; pH 7.4), and in the presence of rat serum, was calculated (see data in Table I). One type of chemical modification was used to control the rate of NO release from diazen-1-ium-1,2-diolate; this is the attachment of alkyl substituents to the O^2-position (Huang *et al.*, 2012). *O*^{2}-substituted-diazen-1-ium-1,2-diolates are stable compounds that hydrolyze slowly, even in an acidic solution (Hrabie *et al.*, 1993). Consistent with these observations, when compounds **6a**–**c** were incubated for 1.5 hours in PBS at pH 7.4, the percentage of NO released varied from 7.97% to 8.51%, suggesting slow NO release. Conversely, the effect of non-specific esterases present in rat serum was higher (range: 65.9%–74.0%). These data indicate that the non-specific serum esterases present in rat serum cleave these hybrid prodrug esters more effectively than PBS at pH 7.4. The hybrid ester prodrugs **6a**–**c** cannot release NO prior to cleavage of the acetoxy moiety present in the terminal *O*^{2}-acetoxymethyl-1-(*N*-methylamino)diazen-1-ium-1,2-diolate NO-donor moiety. This requirement is consistent with the observation that the *O*^{2}-sodium diazen-1-ium-1,2-diolate, which does not possess an ester group that requires prior ester cleavage, releases 84.5% and 85.0% of the theoretical maximal release of two molecules of NO and the molecule of the parent NO donor, respectively, as disclosed earlier (Abdellatif *et al.*, 2008). An earlier study revealed that there are two possible pathways for the ester hydrolysis of hybrid ester prodrugs containing an *O*^{2}-acetoxymethyl-1-[*N*-(2-ethoxy)-*N*-methylamino] diazen-1-ium-1,2-diolate moiety. Moreover, the study described the subsequent release of acetic acid, formaldehyde, two molecules of NO, and *N*-methylethanolamine (Velázquez *et al.*, 2007). The hybrid ester NO-donor prodrugs **6a**–**c** were designed with a one-carbon methylene spacer between the terminal acetoxy group and the diazen-1-ium-1,2-diolate O^2-atom, such that the *O*^{2}-(hydroxymethyl)diazen-1-

ium-1,2-diolate compound formed following cleavage of the acetoxy group; this would spontaneously eliminate formaldehyde to produce the free diazen-1-ium-1,2-diolate compound that can subsequently fragment to release two molecules of NO. Contrariwise, cleavage of the second ester group attached directly to the C-3 position of the pyrazole ring, which releases the parent coxib **4a**–**c**, can occur either prior to or after NO release has occurred. The NO release properties of compounds **6a**–**c** were significant. Compounds **4a**–**c** exhibited AI activities (ID_{50} = 85.2-104.4 mg/kg p.o. range) between those exhibited by the reference drugs, aspirin (ID_{50} = 128.7 mg/kg p.o.) and celecoxib (ID_{50} = 10.8 mg/kg p.o.). The relative potency profile relative to the R-substituent was Me > F ≈ H. These AI data are aligned with the COX inhibition assay data, in which the prodrug with the R = Me displayed the highest COX-2 selectivity (SI) toward COX-enzyme, as compound 6c shares the same R^3 methyl substituent as celecoxib (c.f. Figure 2). In an earlier study by Kanus and co-workers, (Abdellatif *et al.*, 2008), the anti-inflammatory activity of a new series of diazen-1-ium-1,2-diolated NO donor ester prodrugs of 1-(4-methanesulfonylphenyl)-5-aryl-*1H*-pyrazol-3-carboxylic acids showed a similar potency profile with regard to the aryl substitution at the 5-position of the pyrazole ring. The AI activities exhibited by the hybrid ester prodrugs **6a**–**c** were not determined in this study, as it was previously reported that the same hybrid ester prodrug analogs of aspirin, ibuprofen, and indomethacin exhibited similar AI activities to aspirin, ibuprofen, and indomethacin for comparable ID_{50} μmol/kg oral dosage regimens (Velázquez *et al.*, 2007).

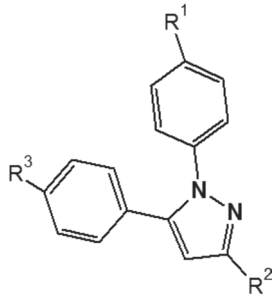


FIGURE 2 - Generic 1,5-diaryl-3-substituted-pyrazole selective COX-2 structure based on the structure of celecoxib (R^1 = SO_2NH_2, R^2 = CF_3, R^3 = CH_3).

Methodology

Chemistry

A group of 1-(4-aminosulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylate esters possessing an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate ester moiety (**6a**–**c**) were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, the reaction of 2,4-diketo ester 1a–c with (4-aminosulfonylphenyl)hydrazine hydrochloride (**2**) under reflux in ethanol will provide the corresponding pyrazole esters (**3a**–**c**). Alkaline hydrolysis of the esters (**3a**–**c**) with LiOH yielded the respective acids (**4a**–**c**). Nucleophilic displacement of the mesyloxy group present in the mesylate (**5**) by the respective sodium salt of the obtained acids **4a**–**c** in hexamethylphosphoramide (HMPA) furnished the target O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino) diazen-1-ium-1,2-diolate 1-(4-amino-sulfonylphenyl)-5-aryl-1*H*-pyrazol-3-carboxylate (**6a**–**c**).

The starting materials, methyl 2-hydroxy-4-oxo-4-aryl-2-butenoate (**1a**–**c**) (Abdellatif *et al.*, 2008), were prepared as follows: A solution of diethyloxalate and either acetophenone **1a**, 4-fluoroacetophenone **1b**, or 4-methylacetophenone **1c** in methanol was added dropwise to a solution of NaOH in MeOH, and the reaction was then refluxed for 2 hours. After cooling to room temperature, the mixture was poured into water acidified with HCl and extracted with diethylether. The combined extract was washed, dried over $MgSO_4$, and then the solvent was removed in a vacuum. Conversely, (4-aminosulfonylphenyl)hydrazine hydrochloride (2) (Soliman, 1979) and O^2-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino] diazen-1-ium-1,2-diolate (**5**) (Velázquez, Knaus, 2004) were prepared according to a literature procedure.

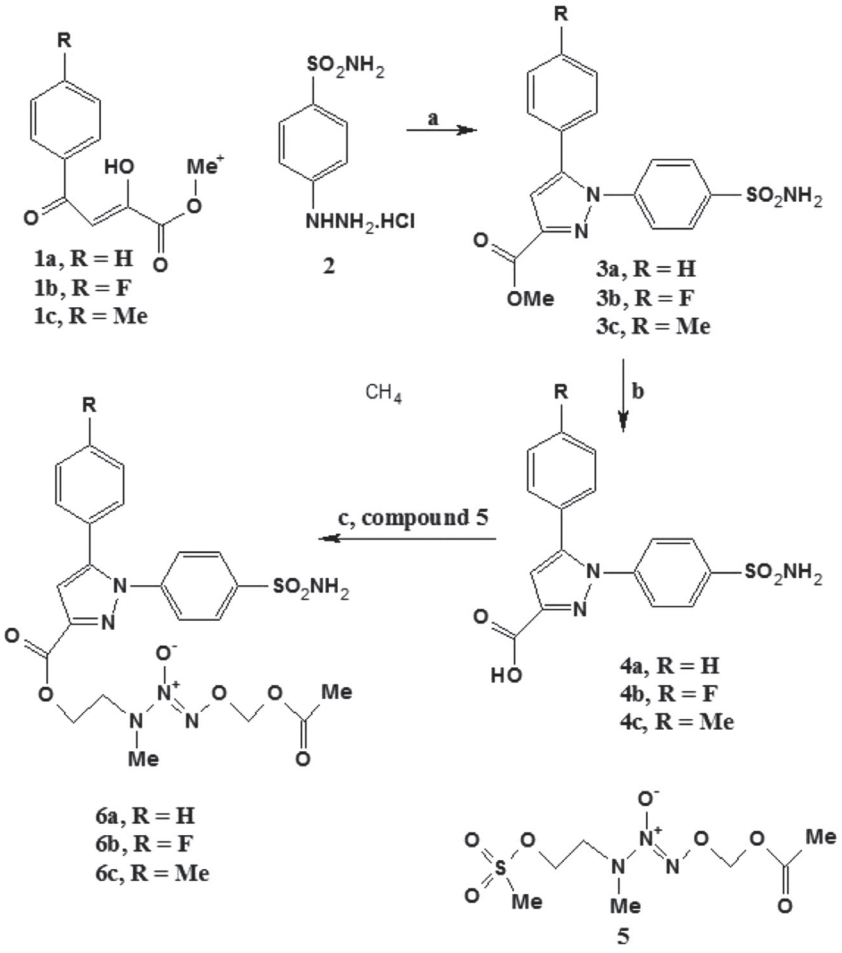
Characterization of the synthesized novel compounds was done using a specific melting point, Fourier-transform infrared (FT-IR), and 1*H* nuclear magnetic resonance (NMR), which were available at the College of Clinical Pharmacy, King Faisal University.

Statistical analysis

The results will be expressed as the mean ± standard error of the mean. The treated groups were compared with the controls to assess any statistically significant differences (P<0.05) using paired Student's t-test (IBM SPSS Statistics for Windows, Version 22.0; IBM Corporation, Armonk, NY, USA).

Cyclooxygenase inhibition assay

The ability of the test compounds to inhibit bovine COX-1 and human recombinant COX-2 was determined using an enzyme-immunoassay (EIA) kit following a previously reported procedure using a 96-well plate (Rao



SCHEME 1 - Reagents and conditions: (a) EtOH, reflux, 3 h; (b) THF/MeOH, LiOH (2 M), RT, 15 h; (c) Na_2CO_3, hexamethylphosphoramide (HMPA), 25 °C, 96 h.

et al., 2003). The procedure is described as follows: (a) 200 µL of Ellman's reagent was added to all 96 wells; (b) the plate was covered with a thin film; (c) the plate was incubated at room temperature for 90 minutes in the dark to develop the color; and (d) an ultraviolet (UV) plate reader was used at wavelengths ranging from 405–420 nm.

Nitric oxide release assay

The test compound was incubated at 37 °C for 1.5 hours 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 l L rat serum was added. It was then possible to determine the *in vitro* NO release via quantification of the nitrite produced by the reaction of NO with oxygen and water using the Griess reaction. NO release data were acquired for the test compounds (**6a–c**) using the reported procedures (Chowdhury *et al.*, 2010).

In vivo anti-inflammatory assay

The anti-inflammatory profiles of the test compounds and reference drugs, celecoxib and aspirin, were evaluated using an in vivo carrageenan-induced foot paw edema model, as reported previously (Winter, Risley, Nuss, 1962).

CONCLUSIONS

A new group of hybrid ester prodrugs (NONO-coxibs) in which an O^2-acetoxymethyl-1-(*N*-ethyl-*N*-methylamino)diazen-1-ium-1,2-diolate (**6a–c**) NO-donor moiety is attached directly to the carboxylic acid group of 1-(4-aminosulfonylphenyl)-5-(4-H, 4-F or 4-Me-phenyl)-1*H*-pyrazol-3-carboxylic acids (**4a–c**) were synthesized for a comparative biological evaluation. Compound 6c, where R= Me, displayed the highest AI activity and COX-2 selectivity, but it was still lower than that exhibited by celecoxib. In terms of biological stability, the NO-release studies (a) showed that the NONO-coxib prodrugs (**6a–c**) are relatively stable in PBS at pH 7, where the NO release is in the 7.97–8.51 range; (b) highlighted that the O^2-acetoxymethyl-1-(*N*-ethyl-*N*-

TABLE I - *In vivo* anti-inflammatory activities for 1-(4-methanesulfonylphenyl)-5-(4-substitutedphenyl)-1H-pyrazol-3-carboxylic acid (**4a-c**), and percent (%) nitric oxide release, data for diazeniumdiolate pyrazole esters (**6a-c**) and *in vitro* COX-1 and COX-2 inhibition.

Compound	R	AI activity[a] ID_{50} (mg/kg)	% •NO released[b]		IC_{50}[e] (μM)		COX-2 S.I.[f]
			PBS[c]	Serum[d]	COX-1	COX-2	
4a	H	112.4±7.6	—	—			
4b	F	110.1±6.8	—	—			
4c	Me	81.2±5.9	—	—			
6a	H	—	7.97±0.62	71.71±6.3	11.3±1.1	3.2±0.27	3.53
6b	F	—	9.04±0.81	65.91±5.8	3.1±0.25	0.7±0.06	4.42
6c	Me	—	8.51±0.72	73.91±6.7	5.6±0.49	1.1±0.98	5.1
Celecoxib		12.6±1.1			7.7±0.63	0.12±0.01	64.2
Aspirin		114.3±10.9			0.3±0.02	2.4±0.19	0.13

[a]Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as the ID_{50} value (mg/kg) at 3 h after oral administration of the test compound. [b]Percent of nitric oxide released based on a theoretical maximum release of 2 mol of NO/mol of the dia zen-1-ium-1,2-diolate test compounds (**6a-c**). The result is the mean value of 3 measurements (n = 3) where variation from the mean % value was ≤ 0.5%. [c]A solution of the test compound (2.4 mL of a 1.0 x 10^{-2} mM solution in phosphate buffer at pH 7.4), was incubated at 37 °C for 1.5 h. [d]A solution of the test compound (2.4 mL of a 1.0 x 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. [e]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 immune-assay kit (Catalog No. 560131, Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value. [f]*In vitro* COX-2 selectivity index (COX-1- IC_{50}/COX-2 IC_{50})

methylamino)diazen-1-ium-1,2-diolates (**6a–c**) undergo extensive cleavage of the terminal acetoxy group by rat serum esterase(s), which is followed by a significant NO release in the 60.51%–71.71% range; and (c) suggested that an alternative linker group to the ester moiety attached directly to the C-3 position of the pyrazole ring is vital to provide more potent AI activity.

EXPERIMENTAL SECTION

General. 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. ^1H NMR spectra were measured on a Bruker AM-300 spectrometer in D_2O, $CDCl_3$, or DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz (Hz). Microanalyses were performed for C, H, N (Micro Analytical Service Laboratory, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada) and were within ±0.4% of the theoretical values. Silica gel column chromatography was performed using a Merck silica gel 60 ASTM (70–230 mesh). All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI, USA), were used without further purification. Methyl 2-hydroxy-4-oxo-4-aryl-2-butenoate (1a–c) (Abdellatif *et al.*, 2010), (4-aminosulfonylphenyl) hydrazine hydrochloride (2), (Pommery *et al.*, 2004), and *O*^{2}-acetoxymethyl-1-[*N*-(2-methylsulfonyloxyethyl)-*N*-methylamino]diazen-1-ium-1,2-diolate (**5**) (Velázquez & Knaus, 2004) were prepared according to a literature procedure.

*General method for preparing methyl 1-(4-aminosulfonylphenyl)-5aryl-1H-pyrazole-3-carboxylates (**3a**–**c**).* (4-Aminosulfonylphenyl)hydrazine hydrochloride (**2**) (0.982 g, 4.4 mmol) was added to a stirred solution of the dione **1a**, **1b**, or **1c** (4.0 mmol) in 50 mL of EtOH. The mixture was heated to reflux and stirred for 3 hours. After cooling to room temperature, the reaction mixture was concentrated *in vacuo*. The residue was taken up in EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give **3a**–**c**. Physical and spectral data are listed below.

*Methyl 1-(4-aminosulfonylphenyl)-5-phenyl-1H-pyrazole-3-carboxylate (**3a**).* 78% yield; pale brown powder; IR (film) 3349, 3261 (NH_2), 2960 (C-H aromatic), 2913 (C-H aliphatic), 1723 (CO_2), 1341, 1161 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 3.72 (s, 3H, OCH_3), 6.79(s, 1H, pyrazole H-4), 6.94 (s, 2H, NH_2, D_2O exchangeable), 7.00-7.06 (m, 3H, phenyl H-3, H-4, H-5),

7.12-7.17 (m, 2H, phenyl H-2, H-6), 7.21 (d, *J* = 7.2 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.68 (d, *J* = 7.2 Hz, 2H, aminosulfonylphenyl H-3, H-5).

*Methyl 1-(4-aminosulfonylphenyl)-5-(4-fluorophenyl)-1H-pyrazole-3-carboxylate (**3b**).* 75% yield; pale brown powder; IR (film) 3341, 3222 (NH_2), 2958 (C-H aromatic), 2922 (C-H aliphatic), 1733 (CO_2), 1339, 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 3.84 (s, 3H, OCH_3), 6.90 (s, 1H, pyrazole H-4), 6.93 (s, 2H, NH_2, D_2O exchangeable), 6.95-6.99 (m, 2H, fluorophenyl H-2, H-6), 7.13 (m, 2H, fluorophenyl H-3, H-5), 7.31 (dd, *J* = 8.7, 2.2 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.81 (dd, *J* = 8.7, 2.2 Hz, 2H, aminosulfonylphenyl H-3, H-5),

*Methyl 1-(4-aminosulfonylphenyl)-5-(4-methylphenyl)-1H-pyrazole-3-carboxylate (**3c**).* 89% yield; white powder; IR (film) 3348, 3256 (NH_2), 2957 (C-H aromatic), 2918 (C-H aliphatic), 1724 (CO_2), 1337, 1165 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 2.28 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 6.89 (s, 1H, pyrazole H-4), 6.90 (s, 2H, NH_2, D_2O exchangeable), 7.02 (d, *J* = 8.1, 2H, methylphenyl H-3, H-5), 7.08 (d, *J* = 8.1, 2H, methylphenyl H-2, H-6), 7.35 (d, *J* = 8.5 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.82 (d, *J* = 8.5 Hz, 2H, aminosulfonylphenyl H-3, H-5).

*General method for preparing 1-(4-aminosulfonylphenyl)-5-aryl-1H-pyrazol-3-carboxylic acids (**4a**–**c**).* The appropriate ester 3a, 3b, or 3c (1.40 mmol), was added to a stirred solution of THF (50 mL), MeOH (50 mL), and LiOH (2 M, 50 mL) and stirred for 15 hours. NaOH (1 M, 200 mL) was added and the mixture was extracted with EtOAc (200 mL). The aqueous phase was acidified with concentrated HCl (38 mL) to a pH level of 1.0, extracted with EtOAc (300 mL), dried over $MgSO_4$, and filtered and concentrated under vacuum to give the respective acids **4a**–**c**, for which physical and spectral data are listed below.

*1-(4-Aminosulfonylphenyl)-5-phenyl-1H-pyrazol-3-carboxylic acid (**4a**).* 83% yield; pale brown powder; mp 181-182 °C ; IR (film) 3628-3271 (OH, NH_2), 3070 (C-H aromatic), 3005 (C-H aliphatic), 1711 (CO_2), 1312, 1151 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 6.68 (s, 2H, NH_2, D_2O exchangeable), 6.75 (s, 1H, pyrazole H-4), 6.96-6.99 (m, 3H, phenyl H-3, H-4, H-5), 7.08-7.12 (m, 2H, phenyl H-2, H-6), 7.20 (d, *J* = 8.5 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.64 (d, *J* = 8.5 Hz, 2H, aminosulfonylphenyl H-3, H-5), 11.99 (br. s, 1H, COOH, D_2O exchangeable); MS 366.05 (M + Na).

*1-(4-Aminosulfonylphenyl)-5-(4-fluorophenyl)-1H-pyrazol-3-carboxylic acid (**4b**).* 65% yield; pale brown powder; mp 213-215 °C ; IR (film) 3637-3267 (OH, NH_2), 3069 (C-H aromatic), 3011 (C-H aliphatic), 1710 (CO_2), 1322, 1168 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 6.90 (s, 1H, pyrazole H-4), 6.96 (s, 2H, NH_2, D_2O exchangeable), 7.01-7.05 (m, 2H, fluorophenyl H-2, H-6), 7.13-7.18 (m, 2H, fluorophenyl H-3, H-5), 7.34 (d, *J* = 8.5 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.81 (d, *J* = 8.5 Hz, 2H, aminosulfonylphenyl H-3, H-5), 12.53 (br. s, 1H, COOH, D_2O exchangeable); MS 384.04 (M + Na).

*1-(4-Aminosulfonylphenyl)-5-(4-methylphenyl)-1H-pyrazol-3-carboxylic acid (**4c**).* 71% yield; white powder; mp 242-243 °C ; IR (film) 3574-3273 (OH, NH_2), 3071 (C-H aromatic), 3008 (C-H aliphatic), 1716 (CO_2), 1319, 1156 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 2.31 (s, 3H, CH_3), 6.79 (s, 2H, NH_2, D_2O exchangeable), 6.91 (s, 1H, pyrazole H-4), 7.05 (d, *J* = 8.1, 2H, methylphenyl H-3, H-5), 7.11 (d, *J* = 8.1, 2H, methylphenyl H-2, H-6), 7.40 (d, *J* = 8.5 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.84 (d, *J* = 8.5 Hz, 2H, aminosulfonylphenyl H-3, H-5); MS 380.08 (M + Na).

*General method for preparing of the O^2-acetoxymethyl-1-(N-ethyl-N-methylamino) diazen-1-ium-1,2-diolate pyrazole esters (**6a-c**).* Sodium carboxylates of the respective acids **5a-c** (R = H, , F, Me) 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 (5, 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 to afford the respective product **6a**, **6b** or **6c** for which the physical and spectral data are listed below.

*O^2-Acetoxymethyl-1-(N-ethyl-N-methylamino) diazen-1-ium-1,2-diolate 1-(4-aminosulfonylphenyl)-5-phenyl-1H-pyrazol-3-carboxylate (**6a**).* Yield, 26%; white powder; mp 63-65 °C; IR (film) 3341, 3257 (NH_2), 2982 (C-H aromatic), 2934 (C-H aliphatic), 1738 (CO_2), 1339, 1165 (SO_2), 1226, 1065 (N=N-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.08 (s, 3H, $COCH_3$), 3.19 (s, 3H, NCH_3), 3.85, (t, *J* = 5.5 Hz, 2H, CH_2N), 4.59 (t, *J* = 5.5 Hz, 2H, CO_2CH_2), 5.09 (s, 2H, NH_2, D_2O exchangeable), 5.73 (s, 2H, OCH_2O), 7.03

(s, 1H, pyrazole H-4), 7.20-7.26 (m, 3H, phenyl H-3, H-4, H-5), 7.37-7.41 (m, 2H, phenyl H-2, H-6), 7.47 (d, *J* = 6.8 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.55 (d, *J* = 6.8 Hz, 2H, aminosulfonylphenyl H-3, H-5), MS 555.12 (M + Na); Anal. Calcd for $C_{22}H_{24}N_6O_8S$: C, 49.62; H, 4.54; N, 15.72. Found: C, 49.55; H, 4.62; N, 15.89.

*O^2-Acetoxymethyl-1-(N-ethyl-N-methylamino) diazen-1-ium-1,2-diolate 1-(4-aminosulfonylphenyl)-5-(4-fluorophenyl)-1H-pyrazol-3-carboxylate (**6b**).* Yield, 21%; white powder; mp 66-68 °C; IR (film) 3360, 3251 (NH_2), 2972 (C-H aromatic), 2935 (C-H aliphatic), 1729 (CO_2), 1344, 1167 (SO_2), 1221, 1062 (N=N-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.08 (s, 3H, $COCH_3$), 3.18 (s, 3H, NCH_3), 3.84 (t, *J* = 5.5 Hz, 2H, CH_2N), 4.59 (t, *J* = 5.5 Hz, 2H, CO_2CH_2), 5.05 (s, 2H, NH_2, D_2O exchangeable), 5.74 (s, 2H, OCH_2O), 7.02 (s, 1H, pyrazole H-4), 7.08-7.11 (m, 2H, fluorophenyl H-2, H-6), 7.20-7.24 (m, 2H, fluorophenyl H-3, H-5), 7.46 (d, *J* = 6.8 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.91 (d, *J* = 6.8 Hz, 2H, aminosulfonylphenyl H-3, H-5); MS 572.97 (M + Na). Anal. Calcd for $C_{22}H_{23}FN_6O_8S$: C, 48; H, 4.21; N, 15.27. Found: C, 48.22; H, 4.32; N, 15.38.

*O^2-Acetoxymethyl-1-(N-ethyl-N-methylamino) diazen-1-ium-1,2-diolate 1-(4-aminosulfonylphenyl)-5-(4-methylphenyl)-1H-pyrazol-3-carboxylate (**6c**).* Yield, 35%; white crystals; mp 142-144 °C; IR (film) 3347, 3257 (NH_2), 2965 (C-H aromatic), 2920 (C-H aliphatic), 1733 (CO_2), 1345, 1165 (SO_2), 1226, 1068 (N=N-O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.09 (s, 3H, $COCH_3$), 2.38 (s, 3H, CH_3), 3.19 (s, 3H, NCH_3), 3.85, (t, *J* = 5.5 Hz, 2H, CH_2N), 4.59 (t, *J* = 5.5 Hz, 2H, CO_2CH_2), 5.18 (s, 2H, NH_2, D_2O exchangeable), 5.74 (s, 2H, OCH_2O), 7.00 (s, 1H, pyrazole H-4), 7.10 (d, *J* = 7.9 Hz, 2H, methylphenyl H-2, H-6), 7.17 (d, *J* = 7.9 Hz, 2H, methylphenyl H-3, H-5), 7.47 (d, *J* = 6.7 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.88 (d, *J* = 6.7 Hz, 2H, aminosulfonylphenyl H-3, H-5),; MS 569.14 (M + Na). Anal. Calcd for $C_{23}H_{26}N_6O_8S.1/2H_2O$: C, 50.54; H, 4.79; N, 15.38. Found: C, 50.78; H, 4.90; N, 15.52.

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