Bioorganic & Medicinal Chemistry Letters 20 (2010) 4544–4549

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Celecoxib prodrugs possessing a diazen-1-ium-1,2-diolate nitric oxide donor moiety: Synthesis, biological evaluation and nitric oxide release studies

Khaled R. A. Abdellatif, Morshed A. Chowdhury, Carlos A. Velázquez, Zhangjian Huang, Ying Dong, Dipankar Das, Gang Yu, Mavanur R. Suresh, Edward E. Knaus *

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8

ARTICLE INFO

Article history:
Received 17 May 2010
Revised 2 June 2010
Accepted 3 June 2010
Available online 8 June 2010

Keywords:
Nitric oxide donors
Cyclooxygenase inhibition
Anti-inflammatory activity

ABSTRACT

A new class of anti-inflammatory (AI) cupferron prodrugs was synthesized wherein a diazen-1-ium-1,2-diolato ammonium salt, and its O^2-methyl and O^2-acetoxyethyl derivatives, nitric oxide (NO) donor moieties were attached directly to an aryl carbon on a celecoxib template. The percentage of NO released from the O^2-methyl and O^2-acetoxyethyl compounds was higher (18.0–37.8% of the theoretical maximal release of one molecule of NO/molecule of the parent compound) upon incubation in the presence of rat serum, relative to incubation with phosphate buffer saline (PBS) at pH 7.4 (3.8–11.6% range). All compounds exhibited weak inhibition of the COX-1 isozyme (IC_{50} = 5.8–17.0 μM range) in conjunction with weak or modest inhibition of the COX-2 isozyme (IC_{50} = 1.6–14.4 μM range). The most potent AI agent 5-[4-(O^2-ammonium diazen-1-ium-1,2-diolato)phenyl]-1-(4-sulfamoylphenyl)-3-trifluoromethyl-1*H*-pyrazole exhibited a potency that was about fourfold and twofold greater than that observed for the respective reference drugs aspirin and ibuprofen. These studies indicate that use of a cupferron template constitutes a plausible drug design approach targeted toward the development of AI drugs that do not cause gastric irritation, or elevate blood pressure and induce platelet aggregation that have been associated with the use of some selective COX-2 inhibitors.

The development of celecoxib (**1**),[1] rofecoxib (**2**),[2] and valdecoxib (**3**)[3] validated the original concept that selective cyclooxygenase-2 (COX-2) inhibitors would be effective anti-inflammatory agents with a diminished gastrointestinal (GI) and renal toxicity (see structures in Fig. 1).[4–7] Unfortunately, some selective COX-2 inhibitory drugs including rofecoxib and valdecoxib alter the natural biochemical balance in the COX pathway. In this regard, the amount of the desirable vasodilatory and anti-aggregtory prostacyclin (PGI_2) produced is decreased together with a simultaneous increase in the level of the undesirable vasoconstrictory and prothrombotic thromboxane A_2 (TxA_2).[8–10] These two adverse biochemical changes in the COX pathway are believed to be responsible for the increased incidences of high blood pressure and myocardial infarction that ultimately prompted the withdrawal of rofecoxib and valdecoxib.[11,12] Nitric oxide (NO) exhibits a number of useful pharmacological actions that include vascular relaxation (vasodilation), and inhibition of platelet aggregation and adhesion.[13] Accordingly, attachment of a NO-donor moiety to highly selective COX-2 inhibitors (NONO-coxibs) offers a potential drug design concept to circumvent adverse cardiovascular events. In previous studies, we reported NONO-coxib ester prodrugs (**4–6**), having a NO-donor diazen-1-ium-1,2-diolate moiety, that are effectively cleaved by esterases to release NO. The spontaneous decomposition reaction of *N*-diazeniumdiolates **4–6**, upon esterase-mediated hydrolysis, would release the active components (coxib and NO) in conjunction with 1 molecule of a secondary amine, 1 molecule of formaldehyde, and one molecule of acetic acid for **5** and **6**.[14–16] In developing drug design strategies, it is important to minimize or prevent the potential risk of exposure to contraindicated products that may be formed in vivo.[17] This concern can be addressed by preparing NO-coxib prodrugs that release only the active coxib and NO. This objective was not achieved in the case of the hybrid NO-donor diazen-1-ium-1,2-diolate derivatives (**7, 8**) which could not be isolated since these salts undergo spontaneous decomposition to release NO and a *N*-nitroso product.[18,19] It has been reported (i) that the direct attachment of a NO donor group to an aromatic carbon atom provides a class of compounds called cupferrons that are stable under protonating conditions,[20,21] and (ii) this type of cupferrons release one molecule of NO together with a nitroso product that is stated to be non-carcinogenic even though no biological data was provided to support this claim.[22–24] As part of our ongoing program, we now report the synthesis, in vitro COX-1/COX-2 inhibitory activity, in vivo anti-inflammatory (AI) activity and NO release data for a new class of nitric oxide-releasing AI com-

* Corresponding author. Tel.: +1 780 492 5993; fax: +1 780 492 1217.
E-mail address: eknaus@pharmacy.ualberta.ca (E.E. Knaus).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.06.022

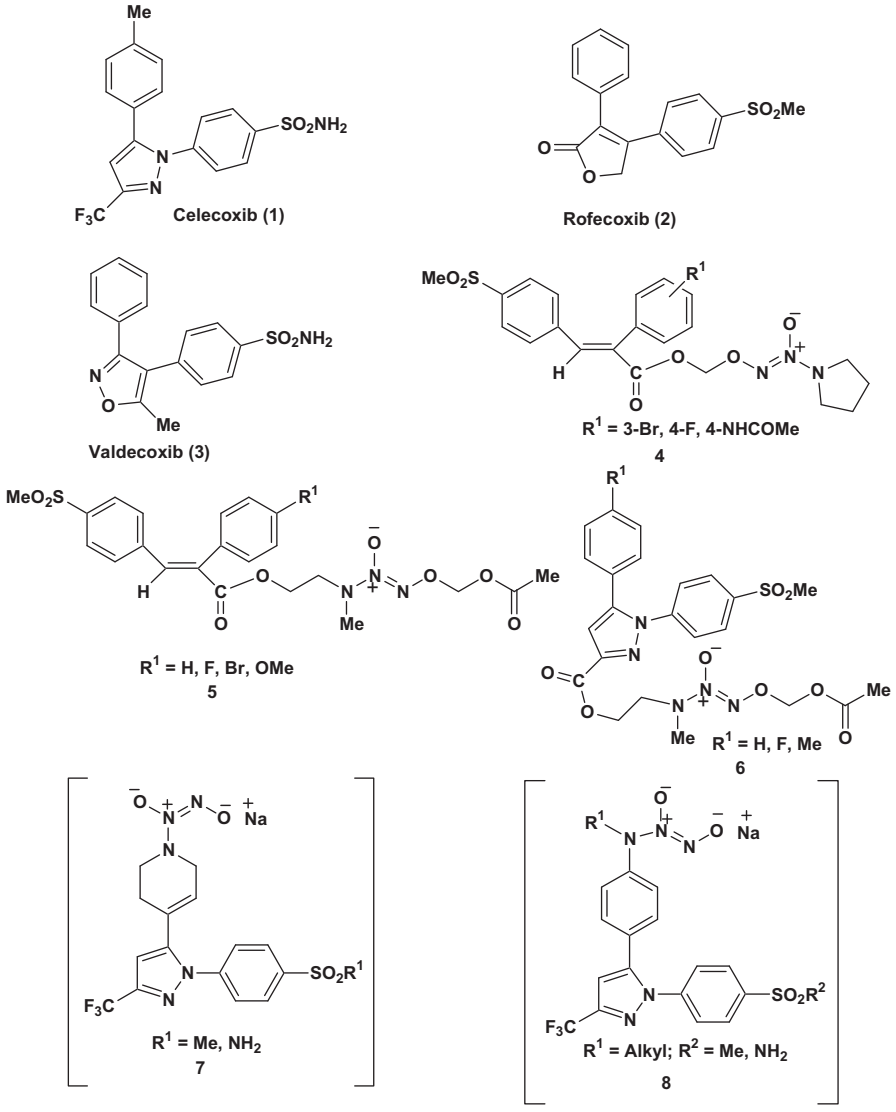


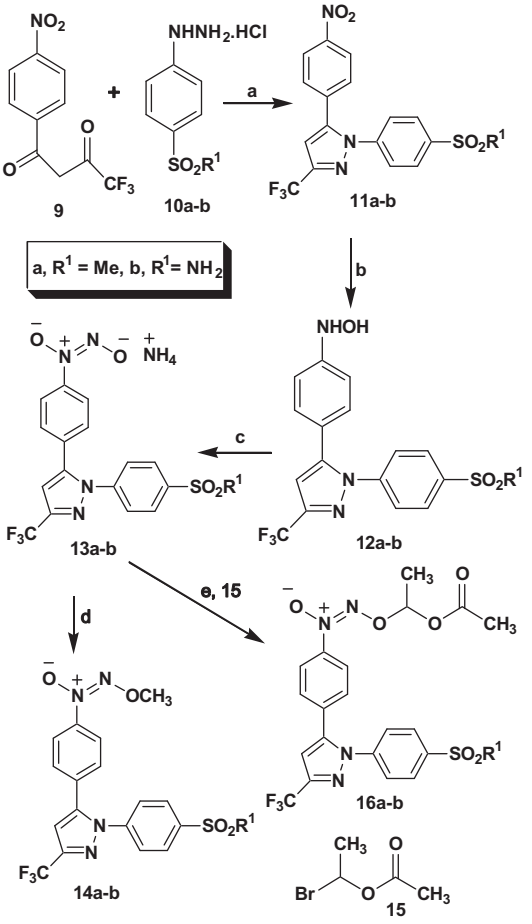
Figure 1. Chemical structures of the selective cyclooxygenase-2 (COX-2) inhibitors celecoxib (**1**), rofecoxib (**2**), and valdecoxib (**3**), diazen-1-ium-1,2-diolated ester prodrugs (**4**, **5**, **6**), and the unisolabile model hybrid NO-donor diazen-1-ium-1,2-diolate compounds (**7, 8**)[18,19] that spontaneously release one molecule of NO and a *N*-nitroso product.

pounds (**13a–b**, **14a–b**, **16a–b**) in which a NO donor moiety is attached directly to an aromatic carbon (cupferron template) rather than a traditional secondary amino nitrogen atom as in the case of compounds **4–8** (Fig. 1). It is expected that the cupferron compounds **13**, **14** and **16** will be devoid of adverse ulcerogenic and cardiovascular effects.

The target diazen-1-ium-1,2-diolated cupferrons **13a–b**, **14a–b** and **16a–b** were synthesized using the reaction sequence illustrated in Scheme 1. Accordingly, reaction of the dione **9** with either 4-methylsulfonylphenylhydrazine hydrochloride (**10a**), or 4-sulfamoylphenylhydrazine hydrochloride (**10b**), afforded the respective pyrazole product **11a** (56%) or **11b** (72%). Reduction of the nitro compounds **11a–b** afforded the *N*-hydroxylamino derivatives 12a–**b** which were subsequently reacted with ammonia gas and butyl nitrite to afford ammonium salts **13a–b** in moderate yields (32–68%). O^2-methylation of the ammonium salts **13a–b** using dimethyl sulfate furnished the respective O^2-methyldiazen-1-ium-1,2-diolate products (**14a–b**) in good yield (58–68%). A similar reaction of the ammonium salts **12a–b** with 1-bromoethyl acetate (**15**) afforded the O^2-(1-acetoxyethyl) protected compounds (**16a–b**) in 23–37% yield.

A group of new nitric oxide-releasing AI compounds (**13a–b**, **14a–b**, **16a–b**) in which a diazen-1-ium-1,2-diolate moiety is attached directly to an aryl carbon providing a class of cupferron compounds, was synthesized. The O^2-methyl protected cupferron compounds **14a–b** were designed to release one molecule of NO and a nitroso product after metabolic O^2-demethylation.[25–27] In comparison, the O^2-(1-acetoxyethyl) protected compounds **16a–b** were designed to release one molecule of NO, a nitroso coxib, one molecule of acetic acid formed after esterase cleavage of the terminal acetoxy group, and one molecule of acetaldehyde which in turn should be metabolized to non-toxic acetic acid. It is anticipated that acetaldehyde is a less toxic by-product compared to formaldehyde that would be produced upon decomposition of the previously reported *N*-diazeniumdiolates **4–6** (Fig. 1) as illustrated in the metabolic pathway (Fig. 2).

In vitro COX-1/COX-2 enzyme inhibition studies (Table 1) showed that these cupferron compounds are weak inhibitors of the COX-1 isozyme (IC_{50} = 5.8–17.0 μM range). In comparison, compounds **14a**, **14b** and **16b** exhibited moderate COX-2 isozyme inhibitory activities (IC_{50} = 1.6, 3.9 and 3.3 μM, respectively) that are relatively similar to the reference drugs aspirin and ibuprofen,



Scheme 1. Reagents and conditions: (a) EtOH, reflux, 20 h; (b) Zn, NH_4Cl, MeOH/H_2O (4:1, v/v), reflux, 1.5 h; (c) NH_3 gas, butyl nitrite, THF, Et_2O, 0 °C, 1.5 h; (d) $(CH_3)_2SO_4$, MeOH, 0 °C for 1.0 h and then 25 °C for 24 h; (e) CH_3CN, 25 °C, 18 h.

but much less potent than celecoxib. This group of cupferron compounds showed low COX-2 selectivity indices in the 1.0–6.6 range.

The percentage NO released from the cupferron compounds (**14a–b, 16a–b**), upon incubation in phosphate-buffered-saline (PBS) at pH 7.4, varied over a narrow range (3.8–11.6%) of the theoretical maximal release of one molecule of NO/molecule of the parent NO donor, which is indicative of slow NO release. In comparison, the percentage NO released was higher (18–37.8% range) in the presence of rat serum. These data indicate the incubations performed in the presence of rat serum cleave these O^2-methyl **14a–b**, and O^2-acetoxyethyl **16a–b** cupferron compounds more effectively than incubations in PBS at pH 7.4. The release of NO from the ammonium salts **13a–b**, which do not require enzymatic activation to release NO, is similar in the presence and the absence of serum. A putative metabolic activation pathway for the release of NO, from O^2-(1-acetoxyethyl) protected cupferron compounds **16a–b** is illustrated in Figure 2.

The AI activities exhibited by the cupferron compounds (**13b**, **14a–b**, **16a–b)** were determined using a carrageenan-induced rat foot paw edema model (see data in Table 1). Compounds **13b**, **14b** and **16b** having a sulfamoyl (SO_2NH_2) substituent exhibited higher AI activities (65.7%, 47.7% and 18.6% inhibition of inflammation, respectively) than the corresponding methylsulfonyl (SO_2CH_3) **14a** (9.8%) and **16a** (5.1%) compounds for the same oral dose (100 mg/kg). The two most potent AI compounds possess a sulfamoyl substituent (**13b**, **16b)**. The ammonium salt **13b** exhibited AI activity (ED_{50} = 168.1 μmol/kg po) that was about fourfold more potent than aspirin (ED_{50} = 710 μmol/kg po), twofold more potent than ibuprofen (ED_{50} = 327 μmol/kg po), but less active than celecoxib (ED_{50} = 30.9 μmol/kg po). The O^2-methyl diazen-1-ium-1,2-diolate compound **14b** exhibited an AI activity (ED_{50} = 240.6 μmol/kg po) between that observed for the reference drugs ibuprofen and celecoxib. It is highly probable that the in vivo AI activity exhibited by this group of cupferron compounds (**13**, **14**, **16**) is due, at least in part, to formation of a nitroso metabolite as illustrated in Figure 2.

In conclusion, a new class of nitric oxide-releasing AI cupferron compounds (**13a–b**, **14a–b**, **16a–b**) in which a NO donor moiety is

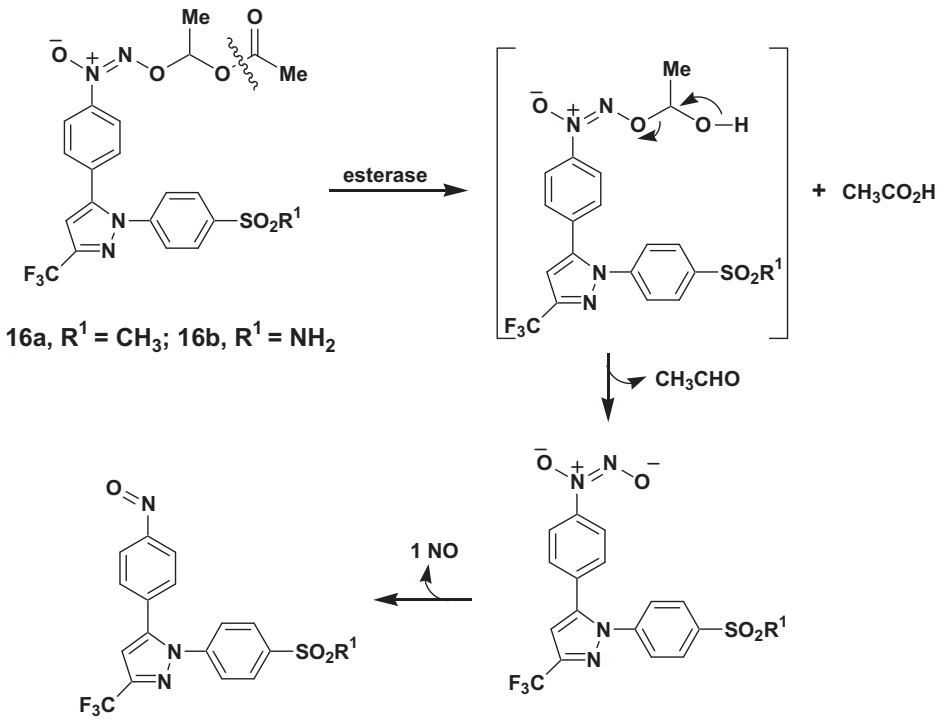
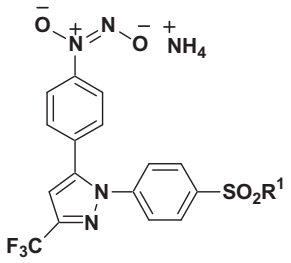
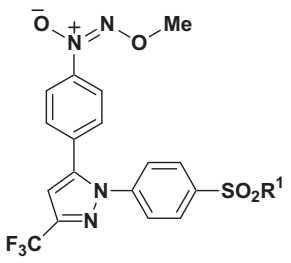


Figure 2. Theoretical metabolic activation (esterase hydrolysis) and nitric oxide release from the 5-[4-(O^2-(1-acetoxyethyl) diazen-1-ium-1,2-diolato)phenyl]-1-(4-methylsulfonyl(or sulfamoyl) phenyl)-3-trifluoromethyl-1*H*-pyrazoles (**16a–b**).

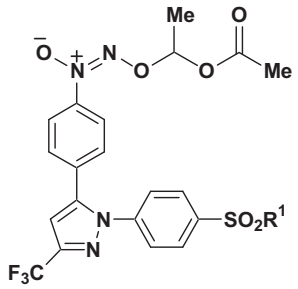
Table 1
In vitro COX-1 and COX-2 inhibition, percent (%) nitric oxide release and anti-inflammatory (AI) data for 5-[4-(O^2-ammonium diazen-1-ium-1,2-diolato)phenyl]-1-(4-methylsulfonyl(sulfamoyl)phenyl)-3-trifluoromethyl-1*H*-pyrazoles (**13a–b**), 5-[4-(O^2-methyl diazen-1-ium-1,2-diolato)phenyl]-1-(4-methylsulfonyl(sulfamoyl)phenyl)-3-trifluoromethyl-1*H*-pyrazoles (**14a–b**), O^2-(1-acetoxyethyl) protected compounds (**16a–b**), and the reference drugs celecoxib, aspirin and ibuprofen



13a, $R^1 = CH_3$; **13b**, $R^1 = NH_2$



14a, $R^1 = CH_3$; **14b**, $R^1 = NH_2$



16a, $R^1 = CH_3$; **16b**, $R^1 = NH_2$

Compound	IC_{50}[a] (μM)		COX-2 S.I.[b]	% NO released[c]		AI activity[f]	
	COX-1	COX-2		PBS[d]	Serum[e]	ED_{50} (μmol/kg)	% Inhibition (100 mg/kg)
13a	—	—	—	30.2	33.4	—	—
13b	5.8	6.1	0.95	37.2	36.0	168.1	65.7
14a	10.5	1.6	6.6	7.8	18.0	—	9.8
14b	12.1	3.9	3.1	3.8	20.6	240.6	47.7
16a	17.0	14.4	1.2	9.0	36.0	—	5.1
16b	11.3	3.3	3.4	11.6	37.8	—	18.6
Celecoxib	115.9	0.065	1783	—	—	30.9	—
Aspirin	0.3	2.4[g]	0.13	—	—	714	—
Ibuprofen	2.9	1.1[g]	2.64	—	—	327	—

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

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

[c] Percent of nitric oxide released based on a theoretical maximum release of 1 mol of NO/mol of the test compounds (**13a–b**, **14a–b** and **16a–b**). The result is the mean value of three measurements ($n = 3$) where variation from the mean % value was $\leqslant$0.2%.

[d] A solution of the test compound (2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer at pH 7.4 was incubated at 37 °C for 1.5 h.

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

[f] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as the % inhibition of inflammation at 3 h after oral administration of the test compound at the specified dose (μmol/kg).

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

attached directly to an aromatic carbon, rather than a traditional secondary amine nitrogen atom, was synthesized[28] for evaluation as COX-1/COX-2 isozyme inhibitors,[29] NO donors,[30] and as AI agents.[31] Structure–activity and biological stability studies showed that (i) these cupferron compounds exhibit weak-to-moderate COX-2, and weak COX-1, inhibitory activities, (ii) the O^2-methyl (**14a–b**) and O^2-(1-acetoxyethyl) **16a–b** compounds are relatively stable in phosphate-buffered saline at pH 7.4 where NO release is in the 3.8–11.6% range in comparison to incubations carried out in the presence of serum where the percentage of NO released was much higher (18–37.8% range), (iii) the ammonium salt **13b** does not require enzymatic activation, and the O^2-methyl derivative **14b** which requires enzymatic activation, to release NO exhibited the most potent AI activities, and (iv) cupferron compounds that possess a suitable NO donor moiety offers a drug design concept to circumvent adverse ulcerogenic effects associated with the use of non-steroidial anti-inflammatory drugs (NSAIDs) that cause gastrointestional irritation, and contraindicated cardiovascular effects such as an increase in blood pressure and platelet aggregation associated with the use of highly selective COX-2 inhibitors.[11,12]

Acknowledgement

We are grateful to the Canadian Institutes of Health Research (CIHR) (MOP-14712) for financial support of this research.

References and notes

1. Penning, T. D.; Tally, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Doctor, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.
2. Prasit, P.; Wang, Z.; Brideau, C.; Chan, C. C.; Charleson, S.; Cromlish, W.; Ethier, D.; Evans, J. F.; Ford-Hutchinson, A. W.; Gauthier, J. Y.; Gordon, R.; Guay, J.; Gresser, M.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Leger, S.; Mancini, J.; O'Neill, G. P.; Quellet, M.; Percival, M. D.; Perrier, H.; Riendeau, D.; Rodger, I.; Tagari, P.; Therien, M.; Vickers, P.; Wong, E.; Xu, L. J.; Young, R. N.; Zamboni, R.; Boyce, S.; Rupniak, N.; Forrest, M.; Visco, D.; Patrick, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1773.
3. Talley, J. A.; Brown, D. L.; Carter, J. S.; Masferrer, M. J.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. *J. Med. Chem.* **2000**, *43*, 775.
4. Turini, M. E.; DuBois, R. N. *Ann. Rev. Med.* **2002**, *53*, 35.
5. Rodrigues, C. R.; Veloso, M. P.; Verli, H.; Fraga, C. A.; Miranda, A. L.; Barreiro, E. J. *Curr. Med. Chem.* **2002**, *9*, 849.
6. Ferraz, M. B.; Hawkey, C. J.; Hochberg, M. C.; Kvien, T. K.; Schnitzer, T. J. *N. Eng. J. Med.* **2000**, *343*, 1520.
7. Silverstein, F. E.; Faich, G.; Goldstein, J. L.; Simon, L. S.; Pincus, T.; Whelton, A.; Makuch, R.; Eisen, G.; Agrawa, N. M.; Stenson, F. W.; Burr, A. M.; Zhao, W. W.; Kent, J. D.; Lefkwith, J. B.; Verburg, K. M.; Geis, G. S. *JAMA* **2000**, *284*, 1247.
8. Hinz, B.; Brune, K. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 367.
9. Patel, H. H.; Gross, G. J. *J. Mol. Cell. Cardiol.* **2002**, *34*, 1.
10. Mukherjee, D. *Biochem. Pharmacol.* **2002**, *63*, 817.
11. Scheen, A. J. *Rev. Med. Liege* **2004**, *59*, 565.
12. Dogné, J.-M.; Supuran, C. T.; Pratico, D. *J. Med. Chem.* **2005**, *48*, 2251.
13. Butler, A. R.; Williams, D. L. H. *Chem. Soc. Rev.* **1993**, *22*, 233.

14. Abdellatif, K. R. A.; Dong, Y.; Chen, Q.-H.; Chowdhury, M. A.; Knaus, E. E. *Bioorg. Med. Chem.* **2007**, *15*, 6796.
15. Abdellatif, K. R. A.; Chowdhury, M. A.; Dong, Y.; Chen, Q.-H.; Knaus, E. E. *Bioorg. Med. Chem.* **2008**, *16*, 3302.
16. Abdellatif, K. R. A.; Chowdhury, M. A.; Dong, Y.; Knaus, E. E. *Bioorg. Med. Chem.* **2008**, *16*, 6528.
17. Preussmann, R.; Stewart, B. W. *Chemical Carcinogens*; American Chemical Society: Washington DC, 1984. pp 643–868.
18. Chowdhury, M. A.; Abdellatif, K. R. A.; Dong, Y.; Knaus, E. E. *Bioorg. Med. Chem.* **2008**, *16*, 8882.
19. Abdellatif, K. R. A.; Chowdhury, M. A.; Knaus, E. E. *J. Heterocycl. Chem.* **2008**, *45*, 1707.
20. Balaban, A. T.; Garfield, R. E.; Lesko, M. J.; Seitz, W. A. *Org. Prep. Proced. Int.* **1998**, *30*, 439.
21. Keefer, L. K.; Christodolou, D.; Dunams, T. M.; Hrabie, J. A.; Maragos, C. M.; Saavedra, J. E.; Wink, D. A.; Nitrosamines, related N-nitroso compounds Chemistry and Biochemistry. In *A.C.S. Symp. Series No. 553*; Loeppky, R. N., Michedja, C. J., Eds.; Amer. Chem. Soc: Washington, D.C., 1994; p 136.
22. Hou, Y.; Xie, W.; Janczuk, A. J.; Wang, P. G. *J. Org. Chem.* **2000**, *65*, 4333.
23. Garfield, R. E.; Balaban, A. T.; Seitz, W. A.; Klien, D. J.; Lesko, M. J. U.S. Patent 5,698,738 December 16, 1997.
24. Taylor, D. K.; Bytheway, I.; Barton, D. H. R.; Bayse, C. A.; Hall, M. B. *J. Org. Chem.* **1995**, *60*, 443.
25. Guengerich, F. P.; MacDonald, T. L. *FASEB J.* **1990**, *4*, 2453.
26. Ortiz de Montellano, P. R. *Trends Pharmacol. Sci.* **1989**, *10*, 354.
27. Guengerich, F. P. *MacDonald, Acc. Chem. Res.* **1984**, *17*, 9.

28. *Experimental procedures and spectral data for compounds* **11a–b**, **12a–b**, **13a–b**, **14a–b**, **16a–b**. *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. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AM-300 spectrometer in $CDCl_3$, or $CDCl_3$ + DMSO-d_6, with TMS as the internal standard. Mass spectra (MS) were recorded on a Water's Micromass ZQ 4000 mass spectrometer using the ESI ionization mode. Microanalyses were performed for C, H, N (Micro Analytical Service Laboratory, Department of Chemistry, University of Alberta). Compounds **11a–b**, **12a–b**, **13a–b**, **14a–b** and **16a–b** showed a single spot on Macherey–Nagel Polygram Sil G/UV254 silica gel plates (0.2 mm) using a low, medium, and highly polar solvent system, and no residue remained after combustion, indicating a purity >95%. Silica gel column chromatography was performed using Merck Silica Gel 60 ASTM (70–230 mesh). All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification. 1-(4-Nitrophenyl)-4,4,4-trifluorobutan-1,3-dione (**9**),[1] 4-methylsulfonylphenylhydrazine hydrochloride (**10a**),[32] 4-sulfamoylphenylhydrazine hydrochloride (**10b**),[33] and 1-bromoethyl acetate (**15**)[34] were prepared according to literature procedures. The in vivo AI assay was carried out using a protocol approved by the Health Sciences Animal Welfare Committee at the University of Alberta.

1-(4-Methylsulfonylphenyl)-5-(4-nitrophenyl)-3-trifluoromethyl-1H-pyrazole (**11a**): 4-methylsulfonylphenylhydrazine hydrochloride (**10a**, 0.980 g, 4.4 mmol) was added to a stirred solution of the dione **9** (0.921 g, 4.0 mmol) in EtOH (50 mL), and the reaction was allowed to proceed at reflux for 20 h. After cooling to 25 °C, the solvent was removed in vacuo. The residue was dissolved in EtOAc (25 mL), washed with water and then brine, the EtOAc fraction was dried (Na_2SO_4), filtered, and the solvent was removed in vacuo to furnish **11a** (0.91 g, 56%) as a pale yellow powder; mp 203–204 °C; IR (film) 3014 (C–H aromatic), 2932 (C–H aliphatic), 1514, 1347 (NO_2), 1319, 1159 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.09 (s, 3H, SO_2CH_3), 6.93 (s, 1H, pyrazole H-4), 7.45 (dd, J = 1.8, 6.7 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.53 (d, J = 8.5 Hz, 2H, nitrophenyl H-2, H-6); 8.00 (dd, J = 1.8, 6.7 Hz, 2H, methanesulfonylphenyl H-3, H-5), 8.27 (d, J = 8.5 Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 44.4, 107.9, 120.8, 124.4, 125.8, 128.9, 129.7, 134.6, 140.8, 142.7, 144.5, 145.0, 148.2; MS m/z (ES^-) 410.9, $C_{17}H_{11}F_3N_3O_4S$ (M−H) requires 410.36.

5-(4-Nitrophenyl)-1-(4-sulfamoylphenyl)-3-trifluoromethyl-1H-pyrazole (**11b**): The title compound **11b** was synthesized, using a similar procedure to that described for the preparation of **11a**, using 4-sulfamoylphenylhydrazine hydrochloride (**10b)** in place of **10a**, in 72% yield as a pale brown powder; mp 169–172 °C; IR (film) 3235, 3183 (NH_2), 3017 (C–H aromatic), 2917 (C–H aliphatic), 1521, 1346 (NO_2), 1324, 1163 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.87 (br s, 2H, NH_2, exchangeable with D_2O), 6.92 (s, 1H, pyrazole H-4), 7.45 (d, J = 8.5 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.48 (d, J = 8.5 Hz, 2H, nitrophenyl H-2, H-6); 7.99 (d, J = 8.5 Hz, 2H, aminosulfonylphenyl H-3, H-5), 8.28 (d, J = 8.5 Hz, 2H, nitrophenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 107.7, 120.9, 125.6, 126.5, 127.9, 129.7, 134.6, 141.7, 142.2, 142.6, 144.3, 148.1; MS m/z (ES^-) 411.9, $C_{16}H_{10}F_3N_4O_4S$ (M−H) requires 411.34.

5-[4-(N-Hydroxylamino)phenyl]-1-(4-methylsulfonylphenyl)-3-trifluoromethyl-1H-pyrazole (**12a**): zinc powder (0.183 g, 2.8 mmol) and ammonium chloride (0.15 g, 2.8 mmol) were added to a solution of the nitro compound **11a** (0.582 g, 1.4 mmol) in methanol/water (4:1 v/v, 10 mL), and the reaction was allowed to proceed at reflux for 90 min with stirring. After cooling to 25 °C, the mixture was filtered using suction, the solvent from the filtrate was removed in vacuo, and the residue was extracted with EtOAc (3 × 20 mL). The combined extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography using EtOAc/hexane (1:1, v/v) as eluent to give **12a** as a yellow powder (0.30 g, 53%): mp 172–174 °C; IR (film) 3533–3350 (OH), 3335–3183 (NH), 3011 (C–H aromatic), 2925 (C–H aliphatic), 1319, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 3.00 (s, 3H, SO_2CH_3), 6.63 (s, 1H, pyrazole H-4), 6.92 (d, J = 8.5 Hz, 2H, hydroxylaminophenyl H-3, H-5), 7.01 (d, J = 8.5 Hz, 2H, hydroxylaminophenyl H-2, H-6); 7.28 (br m, 2H, NH, OH, exchangeable with D_2O), 7.47 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.84 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5); MS m/z (ES^+) 397.9, $C_{17}H_{15}F_3N_3O_3S$ (M+H) requires 398.37.

5-[4-(N-Hydroxylamino)phenyl]-1-(4-sulfamoylphenyl)-3-trifluoromethyl-1H-pyrazole (**12b**): the title compound **12b** was prepared, using a similar procedure to that described for the preparation of **12a**, using the nitro compound **11b** in place of **11a**, in 53% yield as a yellow powder; mp 103–105 °C; IR (film) 3530–3184 (OH, NH_2, NH), 3017 (C–H aromatic), 2923 (C–H aliphatic), 1338, 1161 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 4.78 (br s, 2H, NH_2, exchanges with D_2O), 6.62 (s, 1H, pyrazole H-4), 6.85 (d, J = 8.5 Hz, 2H, hydroxylaminophenyl H-3, H-5), 7.00 (d, J = 8.5 Hz, 2H, hydroxylaminophenyl H-2, H-6), 7.01 (br m, 2H, NH, OH, exchangeable with D_2O), 7.36 (d, J = 8.5 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.83 (d, J = 8.5 Hz, 2H, aminosulfonylphenyl H-3, H-5); MS m/z (ES^-) 397.1, $C_{16}H_{12}F_3N_4O_3S$ (M−H) requires 397.36.

5-[4-(O^2-Ammonium diazen-1-ium-1,2-diolato)phenyl]-1-(4-methylsulfonylphenyl)-3- trifluoromethyl-1H-pyrazole (**13a**): a vigorous stream of gaseous ammonia was bubbled into a cold solution of the *N*-hydroxylamino compound **12a** (1.91 g, 4.8 mmol) in THF (6 mL) and diethyl ether (50 mL) at 0 °C for 15 min. *n*-Butyl nitrite (0.744 g, 7.2 mmol) was added and ammonia gas was bubbled into the mixture for 1 h. The precipitated ammonium salt **13a** was separated by vacuum filtration and washed with diethyl ether (10 mL) to give **13a** as a pale yellow powder (0.68 g, 32%): mp 130–132 °C; IR (film) 3037 (C–H aromatic), 2927 (C–H aliphatic), 1317, 1153 (SO_2), 1239, 1097 (N=N–O) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 3.24 (s, 3H, SO_2CH_3), 4.34 (br s, 4H, NH_4, exchangeable with D_2O), 6.98 (s, 1H, pyrazole H-4), 7.24 (d, J = 8.6 Hz, 2H, phenyl H-2, H-6), 7.53 (d, J = 8.6 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.83 (d, J = 8.6 Hz, 2H, methanesulfonylphenyl H-3, H-5), 7.94 (d, J = 8.6 Hz, 2H, phenyl H-3, H-5); MS m/z (ES^+) 470.8, $C_{17}H_{12}F_3N_4O_4SNa_2$ (M+2Na) requires 471.36.

5-[4-(O^2-Ammonium diazen-1-ium-1,2-diolato)phenyl]-1-(4-sulfamoylphenyl)-3-trifluoromethyl-1H-pyrazole (**13b**): the title compound **13b** was prepared, using a similar procedure to that described for the preparation of **13a**, using the *N*-hydroxylamino compound **12b** in place of **12a**, in 68% yield as a pale yellow powder; mp 115–117 °C; IR (film) 3310, 3176 (NH_2), 3057 (C–H aromatic), 2928 (C–H aliphatic), 1338, 1163 (SO_2), 1237, 1097 (N=N–O) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 4.21 (br s, 2H, NH_2, exchangeable with D_2O), 6.88 (s, 1H, pyrazole H-4), 7.35 (dd, J = 1.8, 6.7 Hz, 2H, phenyl H-2, H-6), 7.45 (d, J = 8.5 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.81 (d, J = 8.5 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.89 (dd, J = 1.8, 6.7 Hz, 2H, phenyl H-3, H-5); MS m/z (ES^+) 427.1, $C_{16}H_{12}F_3N_5O_4S$ (M+H) requires 427.35.

5-[4-(O^2-Methyl diazen-1-ium-1,2-diolato)phenyl]-1-(4-methylsulfonylphenyl)-3-trifluoromethyl-1H-pyrazole (**14a**): a mixture of the ammonium salt **13a** (0.443 g, 1.0 mmol) and anhydrous Na_2CO_3 (0.16 g, 1.5 mmol) in anhydrous MeOH (10 mL) was cooled to 0 °C under a N_2 atmosphere. Dimethyl sulfate (0.19 g, 1.5 mmol) was added drop wise with stirring. The reaction mixture was maintained at 0 °C for 1 h prior to standing at 25 °C for an additional 24 h. The solvent was removed in vacuo, and the residue was extracted with CH_2Cl_2 (3 × 20 mL). The organic extract was dried (Na_2SO_4) over sodium sulfate, the solvent was removed in vacuo, and the residue obtained was purified by silica gel column chromatography using EtOAc/hexane (2:1, v/v) as eluent to give **14a** as a pale yellow powder (0.255 g, 58%): mp 171–173 °C; IR (film) 3012 (C–H aromatic), 2927 (C–H aliphatic), 1319, 1154 (SO_2), 1238, 1097 (N=N–O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.09 (s, 3H, SO_2CH_3), 4.27 (s, 3H, OCH_3), 6.89 (s, 1H, pyrazole H-4), 7.36 (d, J = 9.2 Hz, 2H, phenyl H-2, H-6), 7.53 (dd, J = 1.8, 6.7 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.99 (d, J = 9.2 Hz, 2H, phenyl H-3, H-5), 8.06 (dd, J = 1.8, 6.7 Hz, 2H, methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 44.4, 62.2, 107.4, 120.7, 122.0, 125.8 128.8, 129.4, 131.4, 140.5, 142.9, 143.3, 143.5, 144.3; MS m/z (ES^+) 441.1, $C_{18}H_{16}F_3N_4O_4S$ (M+H) requires 441.40. Anal. Calcd for $C_{18}H_{15}F_3N_4O_4S$: C, 49.09; H, 3.43. Found: C, 49.51; H, 3.52.

5-[4-(O^2-Methyl diazen-1-ium-1,2-diolato)phenyl]-1-(4-sulfamoylphenyl)-3-trifluoromethyl-1H-pyrazole (**14b**)*:* the title compound **14b** was prepared, using a similar procedure to that described for the preparation of **14a**, using the ammonium salt **13b** in place of **13a**, in 65% yield as a pale yellow powder; mp 88–90 °C; IR (film) 3346, 3250 (NH_2), 3014 (C–H aromatic), 2949 (C–H aliphatic), 1340, 1164 (SO_2), 1238, 1098 (N=N–O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.27 (s, 3H, OCH_3), 4.91 (br s, 2H, NH_2, exchangeable with D_2O), 6.88 (s, 1H, pyrazole H-4), 7.37 (d, J = 9.2 Hz, 2H, phenyl H-2, H-6), 7.49 (d, J = 9.2 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.96 (d, J = 9.2 Hz, 2H, aminosulfonylphenyl H-3, H-5), 8.05 (d, J = 9.2 Hz, 2H, phenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 62.2, 107.2, 120.6, 120.8, 125.6, 127.7, 129.5, 131.5, 141.9, 142.1, 143.2, 143.3, 144.1; MS m/z (ES^+) 442.1, $C_{17}H_{15}F_3N_5O_4S$ (M+H) requires 442.39.

5-[4-(O^2-(1-Acetoxyethy) diazen-1-ium-1,2-diolato)phenyl]-1-(4-methylsulfonylphenyl)-3-trifluoromethyl-1H-pyrazole (**16a**): freshly distilled 1-bromoethyl acetate (**15**, 0.184 g, 1.1 mmol) was added to a mixture of **13a** (0.443 g, 1 mmol) and potassium carbonate (0.138 g, 1 mmol) in acetonitrile (10 mL) at 25 °C with stirring. The reaction was allowed to proceed at 25 °C for 18 h with stirring, insoluble inorganic salts were removed by filtration, and the solvent was removed in vacuo. Dichloromethane (20 mL) was added to the residue and once again, the insoluble inorganic salts were removed by filtration. Removal of the solvent in vacuo gave a residue that was purified by silica gel column chromatography using EtOAc/hexane (1:1, v/v) as eluent to give **16a** as a yellow powder (0.19 g, 37%): mp 92–94 °C; IR (film) 3012 (C–H aromatic), 2929 (C–H aliphatic), 1760 (COO), 1320, 1155 (SO_2), 1243, 1098 (N=N–O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.72 (d, J = 5.5 Hz, 3H, $CHCH_3$), 2.13 (s, 3H, $COCH_3$), 3.10 (s, 3H,

SO_2CH_3), 6.82 (q, J = 5.5 Hz, 1H, $CHCH_3$), 6.88 (s, 1H, pyrazole H-4), 7.39 (d, J = 9.2 Hz, 2H, phenyl H-2, H-6), 7.55 (dd, J = 2.2, 8.9 Hz, 2H, methanesulfonylphenyl H-2, H-6), 8.00 (dd, J = 2.2, 8.9 Hz, 2H, methanesulfonylphenyl H-3, H-5), 8.03 (d, J = 9.2 Hz, 2H, phenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 18.8, 21.1, 44.4, 95.8, 107.5, 120.8, 122.2, 125.7 128.8, 129.4, 131.8, 140.5, 142.8, 143.2, 143.6, 144.3, 169.2; MS m/z (ES^+) 513.1, $C_{21}H_{20}F_3N_4O_6S$ (M+H) requires 513.46. Anal. Calcd for $C_{21}H_{19}F_3N_4O_6S$: C, 49.22; H, 3.74; N, 10.93. Found: C, 49.57; H, 3.72; N, 10.47.

5-[4-(O^2-(1-Acetoxyethy) diazen-1-ium-1,2-diolato)phenyl]-1-(4-sulfamoylphenyl)-3-trifluoromethyl-1H-pyrazole (**16b**): the title compound **16b** was prepared, using a similar procedure to that described for the preparation of **16a**, using **13b** in place of **13a**, in 23% yield after recrystallization from diethyl ether as yellow crystals; mp 128–130 °C; IR (film) 3353, 3258 (NH_2), 3016 (C–H aromatic), 2937 (C–H aliphatic), 1753 (COO), 1342, 1163 (SO_2), 1247, 1100 (N=N–O) cm^{-1}; ^{1}H NMR ($CDCl_3$ $_6$) δ 1.70 (d, J = 5.5 Hz, 3H, $CHCH_3$), 2.12 (s, 3H, $COCH_3$), 5.16 (br s, 2H, NH_2, exchangeable with D_2O), 6.80 (q, J = 5.5 Hz, 1H, $CHCH_3$), 6.87 (s, 1H, pyrazole H-4), 7.38 (d, J = 8.5 Hz, 2H, phenyl H-2, H-6), 7.46 (d, J = 8.5 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.94 (d, J = 8.5 Hz, 2H, aminosulfonylphenyl H-3, H-5), 8.02 (d, J = 8.5 Hz, 2H, phenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 18.8, 21.1, 95.8, 107.3, 120.8, 122.1, 125.6 127.8, 129.5, 131.9, 141.9, 142.1, 143.1, 143.5, 144.1, 169.3; MS m/z (ES^+) 514.1, $C_{20}H_{19}F_3N_5O_6S$ (M+H) requires 514.45. Anal. Calcd for $C_{20}H_{18}F_3N_5O_6S$: C, 46.78; H, 3.53; N, 13.64. Found: C, 47.22; H, 3.65; N, 13.20.

29. *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.).
30. *Nitric oxide release assays:* In vitro nitric oxide release, upon incubation of the test compound at 37 °C for 1.5 h with either 2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer at pH 7.4, or with 2.4 mL of a 1.0×10^{-2} mM solution in phosphate buffer at pH 7.4 to which 90 μL rat serum had been added, was determined by quantification of nitrite produced by the reaction of nitric oxide with oxygen and water using the Griess reaction. Nitric oxide release data were acquired for test compounds (**13a–b**, **14a–b**, **16a–b**) using the reported procedures (Velázquez, C.; Vo, D.; Knaus, E. E. *Drug Dev. Res.* **2003**, *60*, 204).
31. *In vivo anti-inflammatory assay*: The test compounds (**13b**, **14a–b**, **16a–b**), and the reference drugs aspirin, ibuprofen and celecoxib 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.
32. Pommery, N.; Taverne, T.; Telliez, A.; Goossens, L.; Charlier, C.; Pommery, J.; Goossens, J.-F.; Houssin, R.; Durant, F.; Henichart, J.-P. *J. Med. Chem.* **2004**, *47*, 6195.
33. Soliman, R. *J. Med. Chem.* **1979**, *22*, 321.
34. Himmel, E. S.; Young, B. G.; Fung, M. C. D.; Hollinshad, C. *Polyhydron* **1985**, *4*, 349.