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In vitro and in vivo profile of 2-(3-di-fluoromethyl-5-phenylpyrazol-1-yl)-5-methanesulfonylpyridine, a potent, selective, and orally active canine COX-2 inhibitor

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Abstract—The synthesis of a novel canine COX-2 selective inhibitor, 2-(3-difluoromethyl-5-phenylpyrazol-1-yl)-5-methanesulfonylpyridine, and its in vitro and in vivo profile are described. Pyrazole **8** demonstrated excellent potency and selectivity for canine COX-2 in both in vitro and ex vivo whole blood assays. This novel COX-2 inhibitor also showed a good pharmacokinetic profile (pk) following oral (po), intravenous (iv), and subcutaneous (sc) dosing and demonstrated excellent in vivo efficacy in a canine synovitis model.

1. Introduction

A major trend in veterinary medicine is the increasing recognition of the need for pain management and the associated benefits to pet health and quality of life. Progressive degenerative joint disease (osteoarthritis) is the most common cause of chronic pain in dogs.[1] It is estimated that one out of every five dogs, or approximately eight million dogs, have osteoarthritis, yet nearly half (48%) of the patients go untreated.[2] Nonsteroidal anti-inflammatory drugs (NSAIDs), whose mechanism of action is the inhibition of cyclooxygenase enzymes (both COX-1 and COX-2) involved in the conversion of arachidonic acid to the prostaglandins,[3–5] have been used most commonly to treat pain and inflammation in dogs.[6] The chronic use of such non-selective NSAIDs has been associated with side effects such as gastric ulceration, bleeding and renal function suppression and is attributed to the inhibition of the constitutively expressed COX-1 enzyme necessary for normal platelet activity and homeostatic maintenance of the gastric mucosa and renal function. The inducible COX-2 enzyme leads to excess prostaglandin production during injury resulting in pain and inflammation.[7,8] Thus, the inhibition of COX-2 alone is thought to be enough to provide the anti-inflammatory activity.[9–12] A moderately COX-2 selective agent, carprofen[13] (**1**), is the most prescribed drug currently used for the treatment of inflammation and pain in dogs and has been shown to have reduced or no side effects associated with gastric ulceration.[14,15] Deracoxib[16] (**2**), another COX-2 selective NSAID, has been recently marketed by Novartis for dogs. Here we disclose the synthesis and the in vitro and in vivo profile of a highly potent and selective novel canine COX-2 inhibitor, 2-(3-difluoromethyl-5-phenylpyrazol-1-yl)-5-methanesulfonylpyridine[17] (Fig. 1).

2. Chemistry

2-(3-Difluoromethyl-5-phenylpyrazol-1-yl)-5-methanesulfonylpyridine (**8**) was synthesized as outlined in Scheme 1. The commercially available 2,5-dibromopyridine (**3**) was converted to 2-bromo-5-methylsulfanylpyridine (**4**) by a lithium–halogen exchange reaction followed by the addition of methyldisulfanylmethane. The methylsulfanyl functional group was then transformed into methanesulfonyl moiety in **5** by MCPBA-mediated oxidation. Displacement of the bromine

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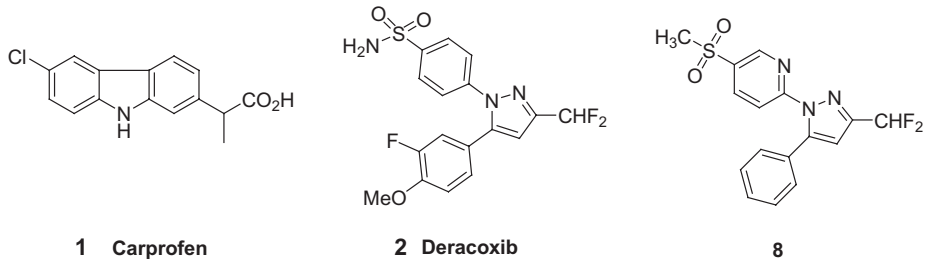
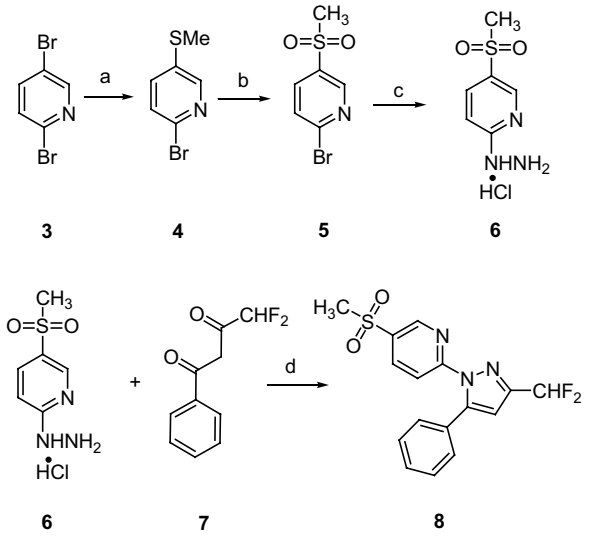


1 Carprofen **2** Deracoxib **8**

Figure 1. Structures of selected canine COX-2 inhibitors.



Scheme 1. Reagents and conditions: (a) *n*-BuLi, Et_2O, −78 °C, $(MeS)_2$; (b) MCPBA, DCM, rt; (C) i. NH_2NH_2, EtOH, reflux, ii. HCl/EtOH; (d) CF_3CH_2OH, reflux.

moiety with hydrazine furnished 5-methanesulfonylpyridin-2-ylhydrazine, which was converted to its hydrochloride salt **6** in situ. The condensation of hydrazine **6** with the 1,3-dicarbonyl **7** in trifluoroethanol then provided the 1,5-diarylpyrazole **8** in 70–80% yield.[18] The structure of pyrazole **8** was verified by NMR, MS, CHN elemental analysis, and X-ray crystallography.

3. Results and discussion

Carprofen (**1**), deracoxib (**2**), and pyrazole (**8**) were all tested in the in vitro canine whole blood COX inhibition assays[17] as shown in Table 1. The pyrazole **8** is about 20-fold more potent toward the COX-2 enzyme compared to carprofen (**1**), slightly more potent than deracoxib (**2**). Both pyrazole **8** and deracoxib (**2**) demonstrated excellent selectivity compared to carprofen (**1**). Pyrazole **8** would be expected to exhibit an excellent therapeutic index and safety profile based on its selectivity.

Table 1. In vitro activity of carprofen (**1**), deracoxib (**2**), and pyrazole (**8**)

Compound	Ratio, COX-1/2	IC_{50} (μg/mL)	
		COX-1	COX-2
1	5	13.5	2.70
2	36.5	9.13	0.25
8	153	16.8	0.11

The pharmacokinetics of pyrazole **8** were assessed in female beagle dogs.[19] Table 2 summarizes the pk data for a 2 mg/kg dose in female beagle dogs following single po (0.5% methylcellulose suspension), sc (80% PEG400/20% ethanol), and iv administration (80% PEG400/20% water). The pyrazole **8** exhibited multi-exponential plasma kinetics following iv dosing with slow plasma clearance. Following po and sc administration, pyrazole **8** was detected in the plasma within 30 min. Peak plasma concentrations were achieved more rapidly following po dosing (T_{max} = 1.75 h) compared to sc dosing (T_{max} = 13.25 h) and maximal plasma concentrations were approximately twofold greater following po dosing than sc dosing. Due to the prolonged absorption phase, elimination of the compound following sc dosing seemed to be absorption rate dependent (flip-flop kinetics). The prolonged absorption phase following sc dosing helped maintain plasma concentrations of **8** above 163 ± 69 ng/mL for at least 24 h. This may be advantageous in maintaining efficacy for 24 h or longer once desired plasma concentrations (2–3X COX-2 IC_{50}) are achieved with an appropriate dose. Unlike sc dosing, po administration provided mean plasma concentrations at 24 h (83 ± 106 ng/mL) that were lower than the COX-2 IC_{50}. The bioavailability following sc dosing was approximately twofold greater than with po dosing (Fig. 2).

The excellent selectivity of pyrazole **8** for the COX-2 enzyme was also demonstrated by ex vivo studies.[20]

Table 2. Mean (±SD) pharmacokinetic parameters of **8** in female beagles following a single intravenous (iv), oral (po), or subcutaneous (sc) dose at 2 mg/kg

	T_{max} (h)	C_{max} (ng/mL)	C_{24} (ng/mL)	AUC (ng h/mL)	$T_{1/2}$ (h)	CL (mL/kg h)	Vss (mL/kg)	K_{el}	F (%)
iv	NA	1929 (369)	181 (62)	13993 (4467)	13	155 (49)	2500 (693)	0.0544 (0.0226)	NA
sc	13.25 (12.5)	183 (54)	163 (69)	9483 (4529)	21	NA	NA	0.0331 (0.0176)	66 (13)
po	1.75 (1.5)	339 (114)	83 (106)	5788 (5682)	8	NA	NA	0.0864 (0.0504)	38 (29)

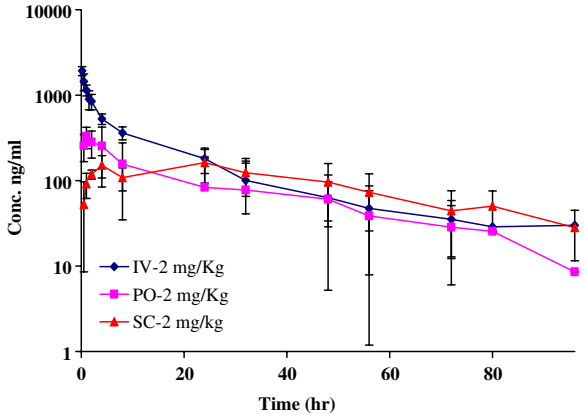


Figure 2. Pharmacokinetics of pyrazole **8** in female beagles following a single intravenous (iv), oral (po), or subcutaneous (sc) dose at 2 mg/kg.

Four dogs per group were dosed at 10 and 15 mg/kg via oral administration for 5 days. The whole blood ex vivo assessment was conducted at 0, 2, 8, and 24 h on days 1 and 5. On day 1, COX-2 inhibition reached a high of 76% at 10 mg/kg and 93% at 15 mg/kg at the 2 h time-point (Fig. 3). Inhibition levels remained at 55% and 85% for 10 and 15 mg/kg through 24 h, respectively. On day 5, inhibition was at 88% and 97% at the '0' h time-point and remained at 92% and 98% for 10 and 15 mg/kg 24 h after the last dose (Fig. 4). No COX-1 inhibitory activity was detected throughout the study.

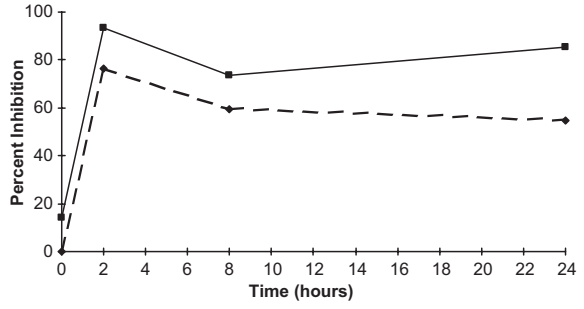


Figure 3. COX-2 inhibition (time-point control) of **8** at 10 mg/kg (dotted line) and 15 mg/kg (solid line) po day 1.

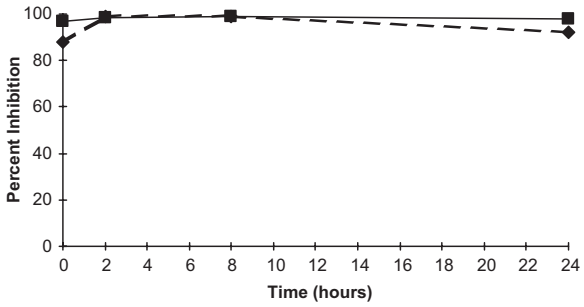


Figure 4. COX-2 inhibition (time-point control) of **8** at 10 mg/kg (dotted line) and 15 mg/kg (solid line) po day 5.

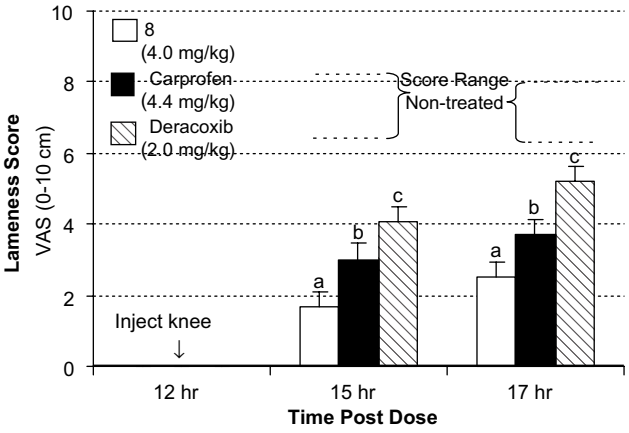


Figure 5. Comparison of lameness scores (bars show mean + standard error of the mean) for dogs given **8**, carprofen (**1**), and deracoxib (**2**) prior to induction of acute knee joint synovitis in one hind leg.

The analgesic effect of pyrazole **8** was evaluated in an acute inflammatory model in beagles in which lameness occurs following induced synovitis of the stifle (knee) joint.[17] Dogs were dosed orally for five consecutive days with either **8**, carprofen (**1**) or deracoxib (**2**) and then synovitis was induced 12 h after the last dose. Compared to non-treated dogs, lameness was improved in all three groups of drug-treated animals. A once-a-day 4.0 mg/kg dose of **8** demonstrated efficacy superior to carprofen (**1**) dosed once daily at 4.4 mg/kg and deracoxib (**2**) at 2.0 mg/kg (Fig. 5).

In summary, a novel class of potent and selective canine COX-2 inhibitors were synthesized and evaluated. These efforts resulted in the discovery of pyrazole **8** with good canine COX-2 potency, selectivity and an excellent efficacy profile for the treatment of pain and inflammation in dogs.

4. Experimental

Proton and carbon magnetic spectra were recorded on Bruker DMX500, Varian XL-300, or Varian Unity 400 spectrometers. Chemical shifts are expressed in parts per million (δ) downfield from TMS. Low-resolution mass spectra were obtained on a Hewlett-Packard 5889A spectrometer using ammonia as the source of chemical ionization. All reagents, solvents, and drying agents were obtained from commercial sources and were used without further purification. Analytical thin-layer chromatography was carried out using silica plates (E. Merck Kieselgel 60 F254). Melting points were recorded on a Büchi 510 apparatus and are uncorrected. Combustion analyses were performed on Perkin–Elmer 2400 elemental analyzer.

4.1. Preparation of 5-methanesulfonylpyridin-2-ylhydrazine hydrochloride (6)

4.1.1. 5-Methylthio-2-bromopyridine (4). To a solution of 2,5-dibromopyridine (**3**) (23.4 g, 99 mmol) in ether

(500 mL), *n*-BuLi (1.52 M in *n*-hexane, 68 mL, 103 mmol) was added dropwise at −78 °C and the mixture was stirred for 1 h at that temperature. Dimethyldisulfide (9.8 mL, 110 mmol) was added slowly at −78 °C and the mixture was stirred for 1 h at that temperature and further 1 h at 0 °C. The mixture was quenched with aqueous 1 N HCl (200 mL) and extracted with ether, dried over $MgSO_4$, and concentrated gave compound **4** (18.9 g, 94%). An analytical sample can be obtained by crystallization of the product from hexane. ^{1}H NMR (400, $CDCl_3$): δ 2.48 (3H, s), 7.37 (1H, dd, J = 8.3, 0.83 Hz), 7.43 (1H, dd, J = 8.3, 2.49 Hz), 8.22 (1H, dd, J = 2.49, 0.83 Hz); ^{13}C NMR ($CDCl_3$): δ 16.08, 128.07, 135.44, 137.05, 138.56, 147.98; MS spectrum *m*/*z* 204 (M^++1).

4.1.2. 5-Methylsulfonyl-2-bromopyridine (5). To a solution of 5-methylthio-2-bromopyridine (**4**) from step 1 (18.9 g, 93 mmol) in DCM (600 mL), MCPBA (48 g, 190 mmol) was added portion wise at 0 °C and the mixture was stirred for 2 h at rt. Aqueous saturated Na_2SO_3 (200 mL) was added and stirred for 15 min and the organic phase was separated and washed with aqueous saturated $NaHCO_3$ (200 mL), dried ($MgSO_4$), and concentrated. The crude product was purified through flash chromatography on silica gel to give the title compound (20.9 g, 95%). ^{1}H NMR (400, $CDCl_3$): δ 3.11 (3H, s), 7.71 (1H, d, J = 8.3 Hz), 8.05 (1H, dd, J = 8.3, 2.49 Hz), 8.89 (1H, d, J = 2.49 Hz); ^{13}C NMR ($CDCl_3$): δ 45.14, 129.07, 136.55, 137.47, 148.20, 149.46; MS *m*/*z* 236 (M^++1).

4.1.3. 5-Methanesulfonylpyridin-2-ylhydrazine hydrochloride (6). A mixture of 5-methylsulfonyl-2-bromopyridine (**5**) (20.9 g, 89 mmol) and anhydrous hydrazine (5.6 mL, 180 mmol) in ethanol (200 mL) was refluxed for 4 h. After cooling to room temperature the mixture was concentrated. The residual solid was washed with aqueous saturated $NaHCO_3$ (100 mL) and H_2O and collected by filtration. The solid was then treated with 10% HCl/MeOH and the precipitate was collected by filtration to give the title compound **6** (9.8 g, 50%). ^{1}H NMR (400, DMSO-d_6): δ 3.16 (3H, s), 7.0 (1H, d, J = 9.1 Hz), 8.03 (1H, dd, J = 9.1, 2.49 Hz), 8.58 (1H, d, J = 2.49 Hz); ^{13}C NMR (DMSO-d_6): δ 44.82, 109.24, 129.22, 137.27, 147.79, 159.57; MS *m*/*z* 188 (M^++1).

4.2. Preparation of 2-(3-difluoromethyl-5-phenylpyrazol-1-yl)-5-methanesulfonylpyridine (8)

5-Methanesulfonylpyridin-2-ylhydrazine hydrochloride (**6**) (5.55 g, 21.2 mmol) and 4,4-difluoro-1-phenylbutane-1,3-dione (**7**) (4.21 g, 21.2 mmol) were dissolved in trifluoroethanol (250 mL). Concentrated H_2SO_4 (2 mL) was added to the reaction mixture. The mixture was refluxed overnight. The reaction mixture was cooled to rt and the solvent was removed under reduced pressure. The residue was then partitioned between EtOAc (200 mL) and $NaHCO_3$ (satd 200 mL). The aqueous layer was separated and extracted with EtOAc (3 × 50 mL). The organic extracts were combined and dried (Na_2SO_4). After removing the solvent the crude product was purified by flash chromatography on silica gel. The product obtained was then crystallized from isopropyl alcohol to furnish 5.29 g of 2-(3-difluoromethyl-5-phenylpyrazol-1-yl)-5-methanesulfonylpyridine **8** (71%), mp 124–125 °C; ^{1}H NMR (500, $CDCl_3$): δ 3.01 (3H, s), 6.73 (1H, s), 6.77 (1H, t, J = 54.8 Hz), 7.29 (2H, m), 7.38 (3H, m), 7.82 (1H, d, J = 8.7 Hz), 8.26 (1H, dd, J = 8.7, 2.1 Hz), 8.76 (1H, d, J = 2.1 Hz); ^{13}C NMR ($CDCl_3$): δ 45.31, 107.99, 109.39, 111.26, 113.12, 118.14, 128.86, 129.10, 129.42, 130.55, 135.77, 138.35, 146.82, 148.20, 155.61; MS *m*/*z* 350 (M^++1). Anal. Calcd for $C_{16}H_{13}F_2N_3O_2S$: C, 55.01; H, 3.75; N, 12.03. Found: C, 55.11; H, 3.67; N, 11.98.

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19. No gender effects on the pk profile of pyrazole **8** would be expected based on several earlier pk studies of pyrazole **8**.
20. The time-points evaluated in the whole blood ex vivo were 0, 2, 8 and 24 h on days 1 and 5. Four dogs per group were dosed at 10 and 15 mg/kg po, and blood from four untreated dogs was used as controls for each time-point. Drug was prepared in a 0.5% methylcellulose vehicle and dosed by oral gavage for 5 days. The best method for solubilizing the compound fully in a homogenous suspension was by the use of a tissue homogenizer. Drug solutions were made fresh each day. Dogs were not fed prior to dosing. Heparin tubes were used to collect blood for the LPS COX-2 assay. Microassay tubes were prepared with 2 μL of LPS (10 μg/mL final concn) as well as vehicle controls for background values. Blood was added (500 μL) and incubated at 37 °C overnight. EDTA (10 μL, 0.3% final concn) was added after incubation (prevents coagulation of plasma that sometimes occurs after thawing samples), samples were centrifuged at 4 °C, and plasma (~200 μL) was collected and stored at −20 °C in polypropylene 96-well plates. For the clotted blood COX-1 assay, vacutainer clot tubes were directly incubated at 37 °C for 1 h and centrifuged at approximately 4000 rpm for 15 min. Serum was removed and stored at −20 °C. To run the endpoints, Cayman EIA plates were used according to kit instructions to measure production of TXB_2 and PGE_2 for COX-1 and COX-2, respectively, utilizing competitive binding of a tracer to antibody and a colorimetric endpoint. Samples were diluted to approximate the range of the kit standards (1/10,000 for TXB_2, 1/1000 for PGE_2).