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Synthesis and SAR of heteroaryl-phenyl-substituted pyrazole derivatives as highly selective and potent canine COX-2 inhibitors

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Abstract—The discovery of heteroaryl-phenyl-substituted pyrazole derivatives as canine selective COX-2 inhibitors is described. Structure–activity relationship (SAR) studies of this class of compounds led to the identification of compound **1** which demonstrated a canine whole blood COX-2 inhibitory IC_{50} of 12 nM and selectivity ratio of COX-1/COX-2 greater than 4000-fold.

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin exhibit their anti-inflammatory effect by inhibiting cyclooxygenase (COX)[1–3] which catalyzes the first step in arachidonic acid metabolism.[4] It was realized in the late 1980s that the isozyme COX-1 is constitutive and responsible for the physiological production of prostaglandins, while COX-2 is inducible and responsible for the elevated production of prostaglandins during inflammation.[5] COX-3, another isozyme of COX, was also reported recently.[6] The chronic use of NSAIDs to treat pain and inflammation is often accompanied by side effects such as gastric ulceration, bleeding, and renal function suppression. It is believed that a selective COX-2 inhibitor will greatly reduce these side effects.[7–10] Several COX-2 selective inhibitors, including celecoxib (Celebrex®),[11] valdecoxib (Bextra®),[12] rofecoxib (Vioxx®),[13] and etoricoxib (Arroxin®), have shown excellent efficacy in humans with few side effects.

Progressive degenerative joint disease, or osteoarthritis, is the most common cause of chronic pain in dogs.[14] It is estimated that one out of every five adult dogs, or approximately 8 million animals, has osteoarthritis, yet nearly half (48%) of these patients are untreated.[15] As with humans, chronic use of NSAIDs in dogs is often associated with GI side effects.[16] Carprofen (Rimadyl®) and deracoxib (Deramaxx®), with moderate COX-2 selectivity, are marketed agents for the treatment of pain and inflammation for dogs.[17a,17b] Another canine COX-2 selective inhibitor under clinical evaluation is firocoxib.[18] In the search for the next generation canine COX-2 selective inhibitor, in addition to the pyrazole derivatives reported recently,[19] we discovered that heteroaryl-phenyl-substituted pyrazole derivatives such as compound **1** are highly selective and potent canine COX-2 inhibitors. The synthesis and SAR of this class of compounds will be discussed.

The synthesis of 2′-pyridine-4-methylsulfone pyrazole derivatives[20] is outlined in Scheme 1. The pyrazoles were prepared via condensation of the hydrazine with the respective diketone. The commercially available 2,5-dibromopyridine **2** was converted to 2-bromo-5-methylsulfanylpyridine **3** by a lithium–halogen exchange

Keywords: Cox-2 inhibitor; Anti-inflammation; Pain; Dog.
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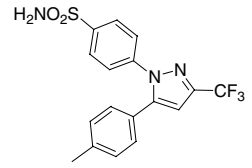
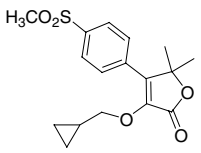
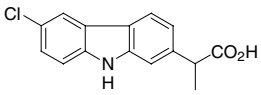


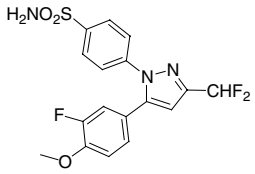
Celecoxib



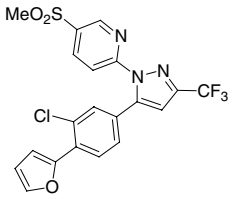
Firocoxib



Carprofen



Deracoxib



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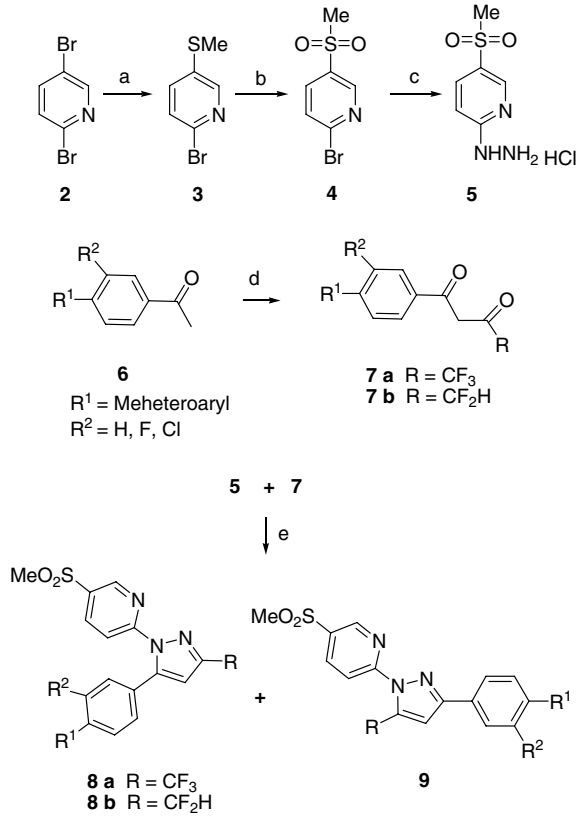
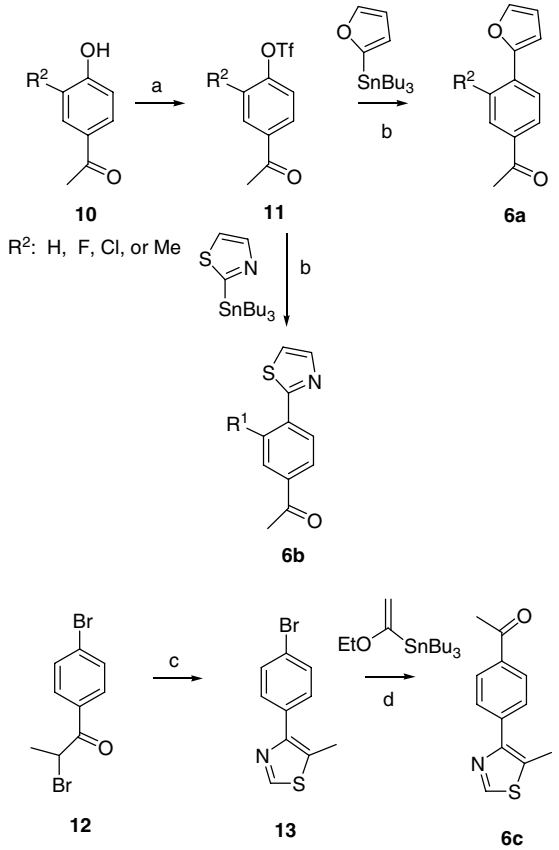
reaction followed by addition of methyldisulfanylmethane. The methylsulfanyl functional group was transformed to a methanesulfonyl moiety in **4** by MCPBA-mediated oxidation. Displacement of bromine with hydrazine furnished 5-methanesulfonylpyridin-2-yl-hydrazine, which was converted to its HCl salt **5** in situ. The 1,3-diketones **7a** and **7b** were synthesized using Claisen condensation of the heteroaryl-substituted acetophenones **6** with either ethyl trifluoroacetate or ethyl difluoroacetate in DME. The condensation of hydrazine **5** with the 1,3-dicarbonyl **7** in ethanol then provided the 1,5-diarylpyrazole **8** as the major product, which was readily separated from its 1,4-diarylpyrazole isomer **9** by flash chromatography.

The preparation of the heteroaryl-substituted acetophenones **6** is illustrated in Scheme 2. The hydroxy group in acetophenone **10** was activated by treatment with triflic anhydride. The resultant triflate **11** was reacted with 2-furyl-tributyltin by palladium-mediated coupling to afford acetophenone derivative **6a**. When compound **11** was reacted with 2-thiazolyl-tributyltin, acetophenone derivative **6b** was obtained. To synthesize acetophenone derivative **6c**, the α-bromoketone **12** was condensed with thioformamide to give **13**, which was then converted to acetophenone **6c** by palladium mediated coupling with tributyl-(1-ethoxy-vinyl)-stannane.

The chemistry that was employed to prepare **6a** was followed to synthesize the acetophenone precursors for pyrazole analogs **18**, **19**, **20**, **21**, and **29**.

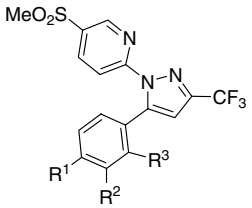


Scheme 1. Reagents and conditions: (a) *n*-BuLi, Et_2O, −78 °C; $(MeS)_2$, 98%; (b) MCPBA, DCM, 95%; (c) NH_2NH_2, EtOH, reflux, then conversion to HCl salt, 100%; (d) RCOOEt, NaOMe, DME, rt, 60–95%. (e) EtOH, reflux, 50–95%.



Scheme 2. Reagents and conditions: (a) $(CF_3SO_2)_2O$, Et_3N, −40 °C, 15 min, 100%; (b) $Pd(PPh_3)_4$, LiCl, dioxane, 110 °C, 1 h, 70%; (c) $HCSNH_2$, dioxane, reflux, 95%; (d) (1) $Pd(PPh_3)_4$, LiCl, dioxane, reflux; (2) 2 M HCl, THF, reflux, 78%.

Table 1. In vitro whole blood COX-1 and COX-2 inhibition data for CF_3 substituted pyrazole derivatives



Compound	R^1	R^2	R^3	WB IC_{50} (μM)		Ratio
				COX-1	COX-2	COX 1/2
1		Cl	H	>50	0.012	>4167
14		H	H	18.2	0.020	759
15		CN	H	2	0.013	154
16		F	H	27.9	0.050	558
17		Me	H	3.4	0.01	340
18		$C(O)NH_2$	H	2	0.41	4.9
19		CO_2Me	H	—	>0.500	—
20		CN	H	23.4	0.11	212
21		H	H	>50	0.16	>313
22		Me	H	15.5	0.09	172
23		Cl	H	16	<0.05	>320
24		H	F	22.7	0.29	78
25		Me	H	12.7	0.03	487
26		Me	H	11.1	0.065	171
27		H	H	—	>0.5	—
28		H	H	50	0.21	238
29		H	H	2.7	0.18	15
30	H		H	—	>0.5	—

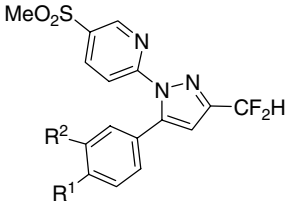
Compounds were tested in canine whole blood in vitro assays for inhibition against COX-1 and COX-2 enzymes.[21] The biological activities of the trifluoromethyl substituted pyrazoles are summarized in Table 1. Compounds with furyl, oxazole or thiazole at the para position of the phenyl ring demonstrated good COX-2 inhibitory activity and selectivity. Interestingly, compound **29** only exhibited moderate selectivity when the thiazole is attached to the phenyl ring at position 5′ compared to position 2′ in the case of **28** or position 4′ in the case of **21**. When the heteroaryl ring is attached to the phenyl ring at meta position as shown in **30**, or there is a methyl group on the heteroaryl ring as shown in **27**, no COX-2 inhibitory activity was observed.

Next, we examined the effects of other substituents (R^2, R^3) on the biological activity of these compounds. When the substituents are F, Cl, Me or CN, the resultant pyrazoles demonstrated very good potency and high selectivity. However, when the substituents are formamide, or methoxcarbonyl, the resultant pyrazole derivatives showed little activity. These results indicate the SAR is very sensitive for this class of pyrazoles in the canine whole blood in vitro assay.

Finally, we investigated the effects of changing CF_3 to CF_2H on the pyrazole ring; the latter analogs being more polar than the CF_3 analogs. As illustrated in Table 2, when the CF_3 is replaced by CF_2H, the potency of the resultant compounds remained about the same, however, the selectivity was slightly negatively impacted.

Pharmacokinetic studies with compound **1** demonstrated 34% oral bioavailability and 16.4 h half-life. It was then progressed to a canine in vivo synovitis model.

Table 2. In vitro whole blood COX-1 and COX-2 inhibition data for CF_2H-substituted pyrazole derivatives



Compound	R^1	R^2	WB IC_{50} (μM)		Ratio
			COX-1	COX-2	COX 1/2
31		H	12	0.130	92
32		Cl	5.5	0.010	550
33		F	5	0.060	83
34		Me	3.8	0.019	200

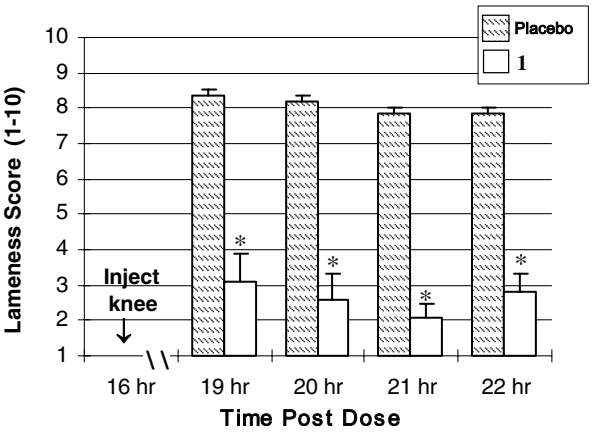


Figure 1. Comparison of lameness scores (bars show mean + standard error of the mean) for dogs given vehicle or 1 prior to induction of acute knee joint synovitis in one hind leg.[22] The asterisks over the error bars indicate statistical differences in the means at each time point ($p \leqslant 0.001$) as determined by an unpaired t-test.

The analgesic effect of **1** was evaluated in a beagle acute inflammatory model in which lameness develops following induction of synovitis in one knee joint. Dogs were dosed orally once a day with either vehicle or **1** at a dosage of 6.6 mg/kg for 3 consecutive days to achieve steady-state plasma concentrations and synovitis induced 16 h after the last drug dose. Compared to placebo dogs, lameness was improved at all time points from 19 to 22 h following the last dose as illustrated in Figure 1.

In summary, the heteroaryl-phenyl-substituted pyrazole derivatives demonstrated potent in vitro activity inhibiting canine COX-2 with very high selectivity in a canine whole blood assay. This resulted in the discovery of compound **1** which demonstrated canine whole blood COX-2 IC_{50} of 12 nM, COX-1/COX-2 selectivity greater than 4000, and an excellent efficacy profile for the treatment of pain and inflammation in dogs.

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21. Canine whole blood was collected by venal puncture into two tubes with and without heparin. All analogs were dissolved in DMSO. Microtubes containing 2 μl of various drugs at concentrations ranging from 500 to 0.005 μM were prepared ahead of time. Following collection, 500 μl of whole blood without heparin was immediately added to the drug tubes for COX-1 measurement. Samples were incubated for 1 h at 37 °C. Samples without drug were included as controls for maximum production of TXB2. COX-2 activity was measured by 500 μl of heparinized blood to tubes containing drug and 10 μg/ml of LPS to stimulate production of prostaglandin E2 (PGE2). LPS and vehicle-only controls were included for maximum PGE2 production and background values, respectively. Samples were incubated overnight at 37 °C. Following the respective COX-1 and COX-2 incubations, EDTA, 0.3% final concentration, was added to the samples to alleviate clotting upon sample thaw. Samples were centrifuged, and serum/plasma was collected into 96-well microtiter plates and stored at −20 °C until ready for analysis. Cayman EIA kits were used according to the manufacturer's instructions for measuring production of TBX2 and PGE2 for COX-1 and COX-2 activity, respectively. Samples were diluted to fall in the approximate range of the kit standards (1/10,000 for TXB2 and 1/1000 for PEG2).
22. Synovitis was induced by injection of media containing pro-inflammatory cytokines generated from a lipopolysaccharide-stimulated culture of canine histiocytes.[23] Animals were anesthetized and knee joints were injected 16 hours following the last administration of drug. Lameness was scored hourly from 3 to 6 h post-injection (19–22 h post-final dose) using a numerical rating scale scoring system where 1 represents no lameness and 10 the most severe lameness. Aqueous 80% PEG-400 vehicle was used to prepare a solution of **1**, which was administered by oral gavage to provide a dose of 6.6 mg/kg. Placebo animals received vehicle-only by oral gavage. An unpaired *t*-test was used to compare the mean lameness scores at each time point with significant differences ($p \leqslant 0.001$) indicated by an asterisk over the error bars (Fig. 1).
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