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5-Heteroatom substituted pyrazoles as canine COX-2 inhibitors. Part 1: Structure–activity relationship studies of 5-alkylamino pyrazoles and discovery of a potent, selective, and orally active analog

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Abstract—Structure–activity relationship (SAR) studies of the novel 2-[3-di and trifluoromethyl-5-alkylamino pyrazo-1-yl]-5-methanesulfonyl (SO_2Me)/sulfamoyl (SO_2NH_2)-pyridine derivatives for canine COX enzymes are described. The studies led to the identification of **2e** as lead with potent in vitro activity, selectivity, and in vivo activity in dogs and cats.

The cyclooxygenase (COX) enzymes, which catalyze the first step in arachidonic acid metabolism,[1] were identified as the molecular targets of all nonsteroidal anti-inflammatory drugs (NSAIDs).[2–4] COX-1, a constitutively expressed isoform, is found in platelets, kidneys, and the gastrointestinal tract and is believed to be responsible for the homeostatic maintainance of the kidneys and GI tract. The COX-2 enzyme is the inducible isoform that is produced by various cell types upon exposure to cytokines, mitogens, and endotoxins released during injury.[5] A recent discovery of the third COX isoform (COX-3) enzyme primarily expressed in the brain and the heart is thought to be the target for acetaminophen.[6] The COX-2 enzyme, after being overexpressed at the site of injury, is a catalyst for the production of the prostaglandins that result in inflammation and pain at the site. Because COX-1 is involved in the maintainance of the GI tract, NSAIDs which are inhibitors of both COX-2 and COX-1 have been found to cause side effects associated with gastrointestinal ulcers.[7–10] Thus, it was thought that a more selective COX-2 inhibitor would have reduced gastrointestinal side effects.[5]

Research efforts in the discovery of COX-2 selective agents have produced many classes of compounds having desired selectivity. Several marketed human COX-2 selective drugs, including celecoxib (Celebrex®),[11] (Fig. 1) for treating pain and inflammation associated with arthritis have been shown to be well tolerated with no gastrointestinal (GI) side effects.[12]

Progressive degenerative joint disease, or osteoarthritis, is the most common cause of chronic pain in dogs.[13] It is estimated that one out of every five adult dogs, or approximately eight million animals, have osteoarthritis, yet nearly half (48%) of these patients are untreated.[14] As in humans, chronic use of NSAIDs in dogs is often associated with GI side effects.[15] Carprofen (Rimadyl®),[16] and deracoxib (Deramaxx™),[11,17] two marketed agents for the treatment of inflammation and pain for dogs, have moderate COX-2 selectivity. Neither carprofen nor deracoxib is approved in the U.S. for use in cats

Keywords: Cyclooxygenase; COX-2 inhibitors; Canine; Feline; Pyrazoles.
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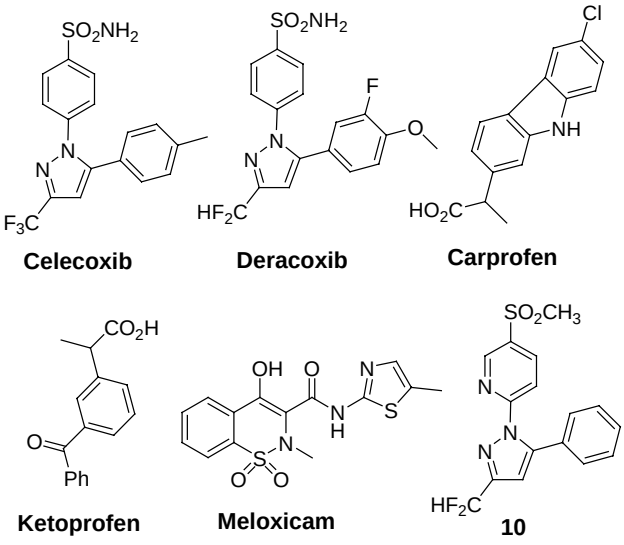
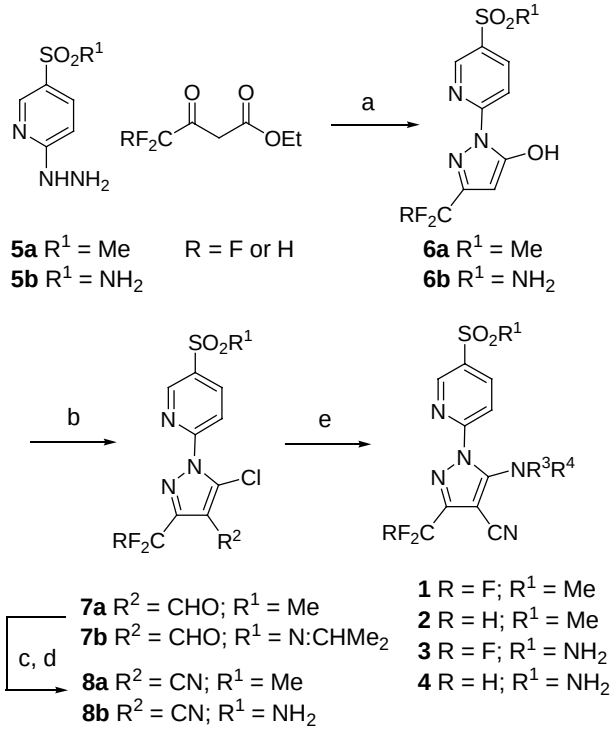


Figure 1. Structures of marketed COX-2 inhibitors and lead **10**.

for pain and inflammation. Meloxicam, a marginally selective (COX-1/COX-2, $IC_{50} = 3$) NSAID for canine COX-2, was recently approved in the U.S. for use in cats.[18]

Our initial efforts in this area led to the identification of 5-aryl pyrazole **10** (Fig. 1) which had enhanced canine COX-2 selectivity and in vivo efficacy than carprofen.[19] In this paper, we disclose the synthesis and structure–activity relationship (SAR) of a novel class of canine COX-2 selective inhibitors that culminated in the identification of 3-difluoromethyl-5-(*cis*-2,6-dimethylmorpholin-4-yl)-1-(5-methanesulfonyl-pyridin-2-yl)-1*H*-pyrazole-4-carbonitrile **2e** (Fig. 1) as a potent and selective agent for use in canine and feline inflammatory diseases.

The general synthesis of the analogs for SAR is shown in Scheme 1. The pyridyl hydrazines[19,20] were reacted with ethyl trifluoromethyl acetoacetate in ethanol at reflux to give the intermediate hydrazone, which was cyclized with sodium hydroxide to provide the pyrazolone **6** in very good yields. Reaction of ethyl difluoro acetoacetate under identical conditions gave very low yields of the desired pyrazolone. Thus, a modified procedure (*i*-PrOH and NH_4Cl) was used to obtain the desired pyrazolone in moderate yields (Scheme 1). Conversion to the chloroaldehyde ($POCl_3$ and DMF) was accomplished in good yields using a literature procedure.[21] During this step, the sulfonamide was converted to the dimethylformamide derivative **7b**. The aldehyde was then converted to the desired nitrile intermediate **8a** and **b** via the oxime intermediate in greater than 90% yield. For **8b**, the sulfonamide was recovered at this stage by acidic cleavage (2 N HCl) of the formamidine derivative. Final substitution of the chloronitrile **8a** and **b** with various primary and secondary amines in dichloroethane provided the desired analogs **1**–**4**. The details and scope of this reaction have been highlighted in a separate report.[22] Alternatively, the introduction of the amino substituent could also be effected with potassium fluoride in dimethylsulfoxide at room temperature.[23]

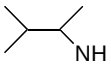
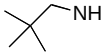
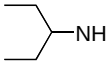
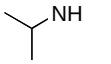
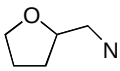
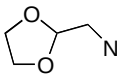
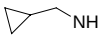
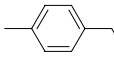
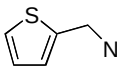
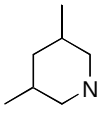
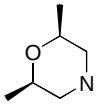
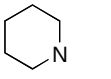
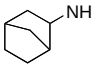
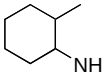


Scheme 1. Reagents and conditions: (a) for R = F: **5a** or **b**: EtOH, reflux, ~16 h, 2 equiv NaOH, EtOH, 30 min, 80–90%. For R = H: **5a** or **b**; *i*-PrOH, NH_4Cl, reflux, ~16 h, 50–60%; (b) $POCl_3$, 4 equiv DMF, 80 °C, 4 h, 70–80%; (c) $NH_2OH{\cdot}HCl$, TFE, reflux, 2 h, >80%; (d) Cl_3CCOCl, Et_3N, DCM, 0 °C, 4–6 h, >90%. For **8b**: 2 N aq HCl/CH_3CN (2:1), reflux, >95%; (e) method A: R^3R^4NH, Et_3N, DCE, 80 °C or method B: R^3R^4NH, KF, DMSO.

More than 200 5-alkylamino pyrazoles were prepared and tested in the in vitro canine whole blood (CWB) COX inhibition assays.[24] Data for a select group of compounds that met the requirements for further progression are listed in Tables 1–4. The compounds with desired activity and selectivity could be grouped into alkylamino (**1a**–**d**), cycloalkylmethyl or heterocycloalkylmethyl amino (**1e**–**g**), aryl or heteroarylmethylamino (**1h**,**i**), cycloalkylamino (**1m**,**n**), and heterocycloamino compounds (**1j**–**l**) (Table 1). Potent COX-2 activity and selectivity were found with all five classes of compounds. Some of the most potent and selective compounds were from the heterocycloalkylmethyl (**1e**) and heterocyclic amino (**1j**) group of compounds. In the alkyl amino series, the branched alkyl compounds showed better potency and selectivity than linear alkyl amino compounds (**1a**–**d**), with **1b** and **d** showing greater than 100-fold selectivity.

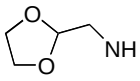
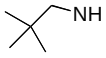
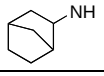
In the arylmethylamino and heteroarylmethylamino compounds, the arylmethyl compounds showed better selectivity (**1h** vs **i**). In the heterocyclic amino series, piperidine derivatives showed good potency and selectivity. The disubstituted morpholine compound **1k** and piperidine analogs **1j** and **l** turned out to be quite potent and highly selective in the in vitro assay. Other ring sizes (5- and 7-membered) were not tolerated (data not shown). Among the most active cycloalkylamino compounds, the norbornyl amino analog **1m** was slightly

Table 1. In vitro COX-1 and COX-2 inhibition data of **1a**–**n** (SO_2Me and CF_3 analogs)

Compound	R^3R^4N	Ratio COX 1/2	CWB IC_{50} (μM)[a]	
			COX-1	COX-2
1a		25.8	3.4	0.132
1b		>217	>50	0.23
1c		33.8	6.7	0.197
1d		113.6	40.8	0.36
1e		262.4	16.5	0.063
1f		>397	>50	0.126
1g		200	22.0	0.11
1h		186.9	21.5	0.115
1i		44.5	5.6	0.125
1j		526	>50	0.095
1k		>357	>50	0.14
1l		324.7	>50	0.15
1m		107.4	15.5	0.144
1n		36	10.8	0.297
10		16	48.0	0.31

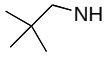
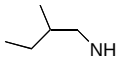
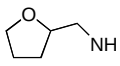
[a] Canine whole blood (CWB) assay: run in duplicate or triplicate.

Table 2. In vitro COX-1 and COX-2 inhibition data of **2a**–**f** (SO_2Me and CHF_2 analogs)

Compound	R^3R^4N	Ratio COX 1/2	CWB IC_{50} (μM)[a]	
			COX-1	COX-2
2a		75	2	0.03
2b		99.3	12.9	0.13
2c		>259	>50	0.19
2d		179.8	36.3	0.20
2e		186.2	43.8	0.24
2f		49.8	20.9	0.42

[a] Canine whole blood (CWB) assay: run in duplicate or triplicate.

Table 3. In vitro COX-1 and COX-2 inhibition data of **3a**–**f** (SO_2NH_2 and CF_3 analogs)

Compound	R^3R^4N	Ratio COX 1/2	CWB IC_{50} (μM)[a]	
			COX-1	COX-2
3a		107.4	15.5	0.14
3b		98.7	13.2	0.13
3c		23.1	4.5	0.19
3d		22.8	42.6	1.87
3e		11.7	14.6	1.25
3f		>6.3	>50	7.9

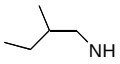
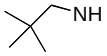
[a] Canine whole blood (CWB) assay: run in duplicate or triplicate.

more potent and selective than the cycloalkylamino compound **1n**.

Following the determination of the in vitro SAR with the 3-trifluoromethyl pyrazoles, some of the active side chains were incorporated into the sulfonamide and difluoromethyl compounds (**2**–**4**). The activities of the key methylsulfonyl difluoromethyl compounds are shown in Table 2.

With some exceptions, the methylsulfonyl 3-difluoromethyl analogs showed similar potency against COX-2 as the parent methanesulfonyl 3-trifluoromethyl analogs. The selectivity was somewhat decreased in all the series. Some of the most potent compounds were the heterocycloamines (**2a**,**b**). The most selective compound **2c** was the heterocyclicmethylamine. Among the cycloalkylamines, the norbornyl amine **2f** was the only active analog with good selectivity.

Table 4. In vitro COX-1 and COX-2 inhibition data of **4a–d** (SO_2NH_2 and CHF_2 analogs)

Compound	R^3R^4N	Ratio COX 1/2	CWB IC_{50} (μM)[a]	
			COX-1	COX-2
4a		21.9	4	0.20
4b		34.7	7	0.21
4c		48.2	18	0.36
4d		>10	>50	5.0

[a] Canine whole blood (CWB) assay: run in duplicate or triplicate.

The active sulfonamide 3-trifluoromethyl and difluoromethyl analogs are shown in Tables 3 and 4. In general, both trifluoromethyl and difluoromethyl pyrazoles in the sulfonamide series tended to show reduced potency and selectivity for the heterocyclic amino analogs. For the cycloalkylamino and the alkylamino analogs, the sulfonamide analogs showed similar potencies but reduced selectivity for COX-2 enzyme.

With many potent and selective compounds in hand, we next focused on identifying the optimum pharmacokinetic (PK) profile from this series. In vivo PK evaluations of the analogs were carried out in beagle dogs by oral gavage at 2 mg/kg. PK data for select analogs are summarized in Table 5. For the methylsulfonyl series with similar amino side chains, the $–CHF_2$ analogs were better absorbed compared to the $–CF_3$ analogs (i.e., **2e** vs **1k**). Similarly, for the trifluoromethyl series, the sulfonamide was better absorbed than the methylsulfonyl analogs (**1e** vs **3e**). The metabolism of the side chain was rapid for a majority of the analogs, thus producing short half-life compounds. The stable *cis*-dimethylmorpholine side chain was identified when **1k** showed a prolonged half-life (30 h) even though absorption was poor. The hindered side chain in **2e** proved to be stable enough to provide a 11 h half-life with very good exposure. Thus, **2e** was progressed into the feline PK studies and COX-2 inhibition assay.

Table 5. Canine pharmacokinetic data of select analogs at 2 mg/kg (PO)

Compound	C_{max} (ng/ml)	T_{max} (h)	AUC (g h/ml)	$T_{1/2}$ (h)	C log *P*
10[a]	1089	1.5	16,541	26	2.5
2e	815	1.4	4830	11	1.4
3e	528	1.5	1362	1	1.4
4b	506	0.5	2102	1.5	3.1
3c	310	1.3	910	2.4	3.0
2a	151	0.5	450	4.6	3.3
1k	134	1	1954	30	2.0
2b	92	0.8	281	2.1	2.6
1e	86	0.5	118	2	1.3

[a] Dosed at 5 mg/kg.

In the feline in vitro whole blood COX-2 assay,[25] **2e** was shown to have a COX-2 IC_{50} of 0.028 μM and a 115-fold ratio ($n = 10$). The subcutaneous injectable PK of **2e** in cat was determined at 0.5 mg/kg. The half-life in cat was around 60 h due to a prolonged depot effect at the injection site. The C_{max} was 194 ng/ml (28 h T_{max}) and AUC was 26,809 ng h/ml.

The analgesic effect of **2e** was evaluated in a beagle acute inflammatory model in which lameness occurs following induced synovitis of the knee joint.[26] After three consecutive days of oral dosing with drug, synovitis was induced 12 h after the last dose. Compared to nontreated dogs, lameness was improved in both groups of drug-treated animals. A once a day 4.0 mg/kg dose of **2e** demonstrated efficacy similar to that of carprofen dosed once daily at 4.4 mg/kg. A similar efficacy study in a cat synovitis model at 2 mg/kg injection gave excellent pain relief compared to placebo.

In summary, a novel class of potent and selective canine COX-2 inhibitors were synthesized and evaluated. This resulted in the discovery of **2e** with high canine and feline COX-2 selectivity and an excellent efficacy profile for the treatment of pain and inflammation in dogs and cats.

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24. Whole blood was collected by venal puncture into two tubes with and without heparin. All analogs were dissolved in DMSO. Tubes containing 2 μl of various drugs at concentrations ranging from 500 to 0.005 μM were prepared ahead of time. Whole blood without heparin (500 μl) was immediately added to the tubes with drug. Following incubation for an hour at 37 °C, COX-1 activity was determined by measuring the thromboxane B_2 (TXB_2) synthesized from platelets, using an enzyme immunoassay (EIA) kit. Samples without drug were included as controls for maximum production of TXB_2. Heparinized blood (500 μl) was added to tubes containing drug and 10 μg/ml LPS (to stimulate production of PGE_2) for COX-2 activity. LPS and vehicle only samples, without drug, were included as controls for maximum PGE_2 production and background values respectively. Samples were incubated overnight at 37 °C. EDTA, 0.3% final concentration, was added to the samples to alleviate clotting of plasma after freeze–thaw. Samples were centrifuged, serum/plasma was collected in 96-well microtiter plates and stored at −20 °C for evaluation in the EIA kit. Cayman EIA kits were used according to manufacturer's instructions, to measure production of TBX_2 and PGE_2 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 TXB_2 and 1/1000 for PEG_2).
25. Although not validated extensively in-house, the assay was run analogous to the canine assay. See (a) Bridau, C.; Satden, C. V.; Chan, C. C. *Am. J. Vet. Res.* **2001**, *62*, 1755; (b) Giraudel, J. M.; Toutian, P.-L.; Lees, P. *Am. J. Vet. Res.* **2005**, *66*, 700.
26. Synovitis was induced by injection of media containing pro-inflammatory cytokines generated from a lipopolysaccharide-stimulated culture of canine histiocytes. Knee joints were injected 12 h following the last administration of drug and lameness was scored 3 and 5 h post-injection (15 and 17 h, respectively, post-final dose) using a 10 cm visual analog scale (VAS) scoring system where 0 cm represents no lameness and 10 cm the most severe lameness.