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5-Heteroatom-substituted pyrazoles as canine COX-2 inhibitors: Part 2. Structure–activity relationship studies of 5-alkylethers and 5-thioethers

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Abstract—Structure–activity relationship (SAR) studies of novel 2-[3-trifluoromethyl-5-alkyl(thio)ether pyrazo-1-yl]-5-methanesulfonyl pyridine derivatives for canine COX enzymes are described. The 4-cyano-5-alkyl ethers were found to have excellent potency and selectivity, whereas the 5-thioethers were potent but less selective than the ether analogs in a canine whole blood (CWB) COX-2 assay.

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 in the gastrointestinal tract, and is believed to be responsible for the homeostatic maintenance 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 primary 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 elicit an immune response to the site causing inflammation and pain.

Because the COX-1 is involved in the maintenance 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 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]

As in humans, 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 8 million animals, has osteoarthritis, yet nearly half (48%) of these patients are untreated.[14] 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. Firocoxib, with

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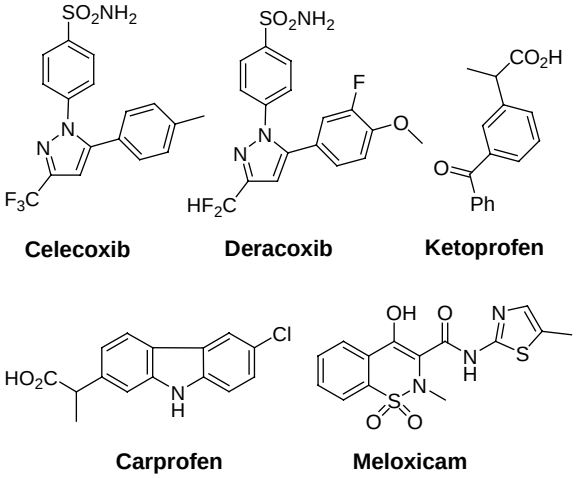


Figure 1. Structures of marketed COX-2 inhibitors.

increased selectivity for canine COX-2 enzyme,[16b] has also been recently approved for treatment in dogs. Neither carprofen, deracoxib, nor firocoxib is approved in the US for use in cats for pain and inflammation. Meloxicam, a marginally selective NSAID for canine COX-2, was recently approved in the US for use in cats.[18]

Our initial efforts in this area led to the identification of 5-aryl pyrazole **1** (Fig. 2) which had enhanced canine COX-2 selectivity and in vivo efficacy compared to those of carprofen.[19]

In addition, we have disclosed the synthesis and SAR of the 5-alkylamino pyrazoles that led to the identification of the lead compound, **2**, which showed in vivo efficacy in both canine and feline synovitis models.[20] In this paper, we disclose the synthesis and in vitro SAR of a novel class of canine COX-2 selective 4-unsubstituted and substituted 5-alkylether and 5-alkylthioether pyrazoles.

The general synthesis of the analogs for SAR is shown in Schemes 1 and 2. Reaction of the ketoesters **3** with the hydrazine hydrochloride **4** in refluxing ethanol followed by addition of sodium hydroxide and stirring for 30 min provided the desired pyrazolone **5**. The alkyl ethers were prepared by reacting the pyrazolone salt, generated with potassium carbonate, with alkyl halides at room temperature or at 50–60 °C. In the case of alkyl bromides and chlorides, 2 equiv of potassium iodide were added to the reaction mixture.[21] The chloride **8** was prepared by

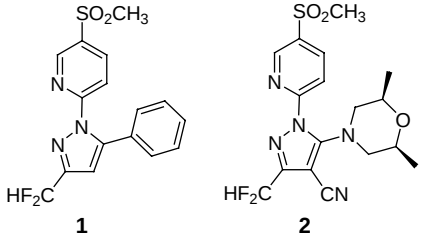
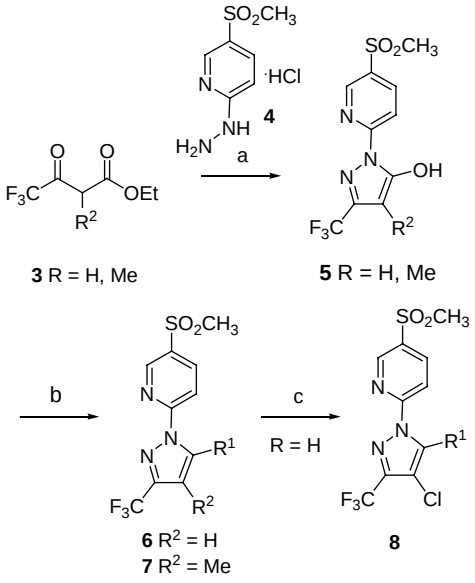
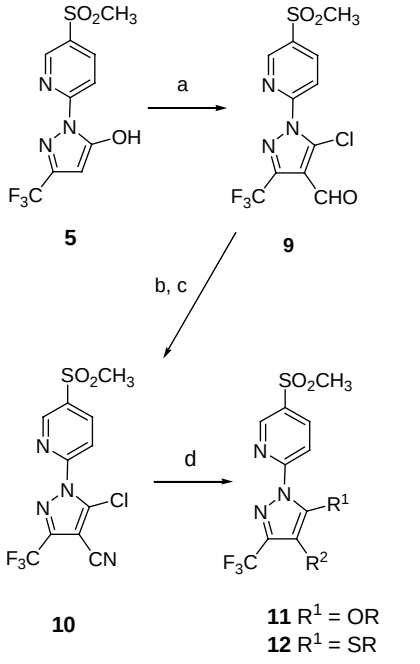


Figure 2. Canine COX-2 selective leads.



Scheme 1. Reagents and conditions: (a) **4**, EtOH, reflux, ~16 h; 2 equiv NaOH, EtOH, 30 min, 80–90%; (b) K_2CO_3, R^1-X, (for alkyl chloride and bromide, 2 equiv KI), DMF, 75 °C, 20–80%; (c) NCS, DMF, rt.



Scheme 2. Reagents and conditions: (a) $POCl_3$, 4 equiv DMF, 80 °C, 4 h, 70–80%; (b) $NH_2OH{\cdot}HCl$, TFE, reflux, 2 h, >80%; (c) Cl_3CCOCl, Et_3N, DCM, 0 °C, 4–6 h, >90%; (d) method A: ROH or RSH, KF or CSF, DMSO, rt–50 °C, 25–80%.

reacting the ether **6** with *N*-chlorosuccinimide in DMF at ambient temperature.[21]

For 4-cyano analogs, the key intermediate 4-formyl pyrazole was employed as shown in Scheme 2. The pyrazolone **5** was refluxed with $POCl_3$/DMF[22] mixture to provide the aldehyde **9** in good yields. Aldehyde **9** was

converted to the oxime followed by dehydration to the nitrile **10**, which proceeded in very good yields. Finally, the substitution of the 5-chloro pyrazoles **10** with various alcohols and thiols under potassium or cesium fluoride-mediated reaction, or under base promoted reaction, provided the ethers **11** and thioethers **13**. The details and scope of these mild substitution reactions have been highlighted in separate reports.[23,24]

More than 30 analogs of the 4-unsubstituted pyrazole ethers were prepared and tested in our single dose canine whole blood (CWB) COX-2 inhibition assay[25] and a subset of those analogs is shown in Table 1. Only the isobutyl (**6h**) and *n*-pentan-3-yl (**6i**) ethers showed greater than 50% COX-2 inhibition at 0.5 μM and gave IC_{50} values of 4.1 and 19.6 μM, respectively. Efforts to optimize the ether side chain by varying alkyl chain length, branched alkyl, cycloalkyl or arylmethyl derivatives did not lead to any fruitful increase in the inhibition of the COX-2 enzyme in our assay (Table 1).

Next, we looked at some modification of the 4-position of the pyrazole to see if any increase in activity against the COX-2 enzyme could be gained (Table 2). Within the alkylether pyrazoles, only the nitrile substitution (**11a**, **11b**, and **11c**) showed greater than 50% inhibition of the COX-2 enzyme. Aromatic ethers or adding polar functionality to the side chains abrogated activity against the COX-2 enzyme (**11d** and **11f**). Extending the alkyl side chain also tended to lower activity (**11e**).

Table 3 shows the IC_{50} and selectivity data for some of the most potent ethers against the COX-2 and COX-1 enzymes in the whole blood assay. The compounds are subgrouped as alkylethers (**11h**–**11j**), cycloalkylmethylethers (**11k**–**11n**), cycloalkylethers (**11o**–**11r**), and aryl-ethers (**11s**–**11u**). Among the alkylethers, the branched secondary ethers tended to be very potent against the COX-2 enzyme and have great selectivity for the COX-2 enzyme (**11h**). Extensively branched alkyl group tended to have reduced activity against the COX-2 enzyme but maintained selectivity (**11j**). Among the cycloalkylmethylethers, the exocyclic olefin-containing 4-methylene cyclohexane methylether **11k** showed the most activity and selectivity. Smaller rings tended to have slightly reduced activity against the COX-2 enzyme while maintaining selectivity (**11m**). The directly connected cycloalkylethers showed increased potency with seven-membered ring ether (**11o**).

Whereas the six-membered and smaller rings (**11p**, **11q**, and **11r**) showed reduced potency for the COX-2 enzyme. While maintaining moderate selectivity. Interestingly, the arylethers had poor potency against the COX-2 and were surprisingly selective for the COX-1 enzyme (**11s**, **11t**, and **11u**).

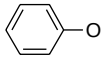
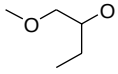
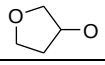
The thioether analogs also showed good potency for the COX-2 but only showed moderate selectivity (Table 4). Short linear and branched thioethers were the most potent compounds (**12e** and **12f**) followed by benzyl and arylthioethers (**12a**, **12b**, and **12c**). Long linear alkyl thioethers were only moderately active and selective for the COX-2 enzyme (**12g**, **12h**, and **12i**). Overall, the alkylethers were more selective for the COX-2 enzyme than the thioethers.

Table 1. In vitro CWB COX-2 inhibition at 0.5 μM and IC_{50} data of **6**

Compound	R_1	CWB COX-2 % inh at 0.5(μM)[a]	CWB COX-2 IC_{50} (μM)[a]
6a	$PhCH_2O$	12.5	>0.5
6b	3-F-$PhCH_2O$	10.7	>0.5
6c	4-Pyridyl-CH_2O	14.0	>0.5
6d	Naphthyl-1-CH_2O	11.3	>0.5
6e	Isopropyloxy	10.8	>0.5
6f	CH_3O	3.4	>0.5
6g	*n*-Butyloxy	−14.7	>0.5
6h	Isobutyloxy	106	4.1
6i	*n*-Pentan-3-yloxy	73.3	19.6
6j	Cyclopentyloxy	12.1	>0.5
6k	Cyclohexyloxy	−20.7	>0.5
6l	Cyclopropylmethyloxy	−67.2	>0.5
1	—	58–105	0.31

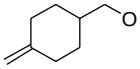
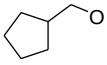
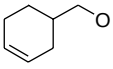
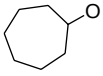
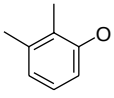
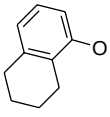
[a] Run in duplicate or triplicate.

Table 2. In vitro CWB COX-2 inhibition at 0.5 μM and IC_{50} data of **7**, **8**, and **11**

Compound	R^1	R^2	Max % inh CWB COX-2 at 0.5 (μM)[a]	CWB COX-2 IC_{50} (μM)[a]
8		—	5	>0.5
7a		Me	−2	>0.5
7b		Me	39	>0.5
7c		Me	−8	>0.5
11a		CN	63	<0.5
11b		CN	87	0.21
11c		CN	72	0.08
11d		CN	18	>0.5
11e		CN	47	>0.5
11f		CN	−18	>0.5
11g		CN	−39	>0.5

[a] Run in duplicate or triplicate.

Table 3. In vitro CWB COX-2 and CWB COX-1 IC_{50} data for **11**

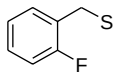
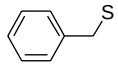
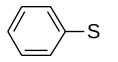
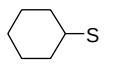
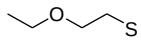
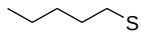
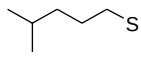
Compound	R^1	Ratio COX 1/2	CWB IC_{50} (μM)[a]	
			COX-1	COX-2
11h		127	11.8	0.09
11i		92	10	0.11
11j		287	>50	0.17
11k		340	29.7	<0.09
11l		6.8	0.4	0.06
11m		86	>50	0.13
11n		78	10.6	0.14
11o		117	8.0	0.07
11p		47	7.6	0.16
11q		35	7.1	0.20
11r		46.3	25.3	0.55
11s		0.3	0.1	0.17
11t		<0.01	<0.05	6.63
11u		0.1	0.1	0.90
1	—	155	48.0	0.31
2	—	>357	>50	0.14

[a] Run in duplicate or triplicate.

Having identified potent analogs in the ether and the thioether series, several of the lead compounds were profiled for their pharmacokinetic (PK) properties in beagle dogs at 2 mg/kg in 0.5% methylcellulose via oral gavage and compared to those of the leads **1** and **2**.

An initial PK study with 4-unsubstituted analog **6e** indicated the compound to have good exposure and a long half-life (Table 5). However, for the more active thioether and ether analogs with 4-nitrile substitution, the PK studies indicated the compounds to be poorly absorbed or rapidly eliminated with no drugs detected in vivo. Only compound **11o** was well characterized in the PK studies with C_{max} of 363 ng/ml and half-life of 2 h. Because the PK parameters fell far short of the leads **1** and **2**, both of which had excellent PK, these series were no longer pursued.[26]

Table 4. In vitro canine whole blood (CWB) COX-2 inhibition at 0.5 μM and IC_{50} data of **12**

Compound	R^1	Ratio COX 1/2	CWB IC_{50} (μM)[a]	
			COX-1	COX-2
12a		18.4	0.6	0.04
12b		12.1	0.9	0.08
12c		3.6	0.2	0.06
12d		31.2	3.2	0.10
12e		>29.5	1.5	<0.05
12f		>3.8	0.2	<0.05
12g		11.8	2.0	0.17
12h		11.7	3.5	0.30
12i		8.1	3.3	0.40
1	—	155	48.0	0.31
2	—	>357	>50	0.14

[a] Run in duplicate or triplicate.

In summary, several ether and thioether analogs with potent activity and selectivity for canine COX-2 enzyme

Table 5. Canine pharmacokinetic data of select analogs dosed PO

Compound	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	AUC (g h/mL)	$T_{1/2}$ (h)
1	5	1089	1.5	16,541	26
2	2	815	1.4	4830	11
6e	5	679	0.8	6985	34.8
11n	2	363	1	742	2

were identified. Unfortunately, PK was not improved over the lead amino pyrazole **2** or the aryl pyrazole **1**.

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25. 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 500–0.005 μM were prepared ahead of time. Five hundred microliters of whole blood without heparin 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. Five hundred microliters of heparinized blood was added to tubes containing drug and 10 μg/ml of 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 micro titer 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).
26. We did not have established canine microsomal stability assays while we were engaged in the COX-2 project. Although we had good rat and human microsomal stability data, it did not correlate well with the canine in vivo studies.