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Comparative structure–activity relationship studies of 1-(5-methylsulfonylpyrid-2-yl)-5-alkyl and (hetero)aryl triazoles and pyrazoles in canine COX inhibition

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Abstract—Structure–activity relationship (SAR) studies of novel 5-alkyl and 5-aryl/heteroaryl substituted 1,2,4-triazoles are described. The in vitro activity is compared to the pyrazole class of compounds with analogous side-chains to delineate the contribution of the triazole ring nitrogen in binding to the active site. Both series are quite potent and selective in the canine whole blood (CWB) COX-2 assay, suggesting the increased binding contribution of the hydrophobic side-chains.

The cyclooxygenase (COX) enzymes, which catalyze the first step in arachidonic acid metabolism,[1] were identified as the molecular targets of all non steroidal anti-inflammatory drugs (NSAID).[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 primarily expressed in the brain and the heart is thought to be the target for acetaminophen.[6] The COX-2 enzyme, after being over expressed at the site of injury, is a catalyst for the production of the prostaglandins that illicit an immune response to the site causing inflammation and pain.

Because 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

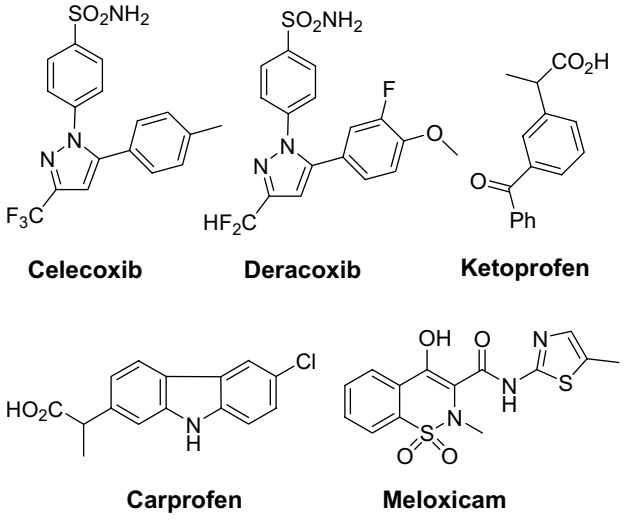

Figure 1. Structures of marketed COX-2 inhibitors.

Keywords: Pyrazole; Triazole; Cyclooxygenase; COX; Canine; Anti-inflammatory.
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selective drugs, including celecoxib (Celebrex®),[11] (Fig. 1) for treating pain and inflammation associated with arthritis have been shown to be well tolerated and reduced 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 increased selectivity for canine COX-2 enzyme,[16b] has also been recently approved for treatment in dogs. Neither carprofen, deracoxib, nor firocoxib are 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 to identify superior agents led to the identification of 5-aryl pyrazole **1** (Fig. 2) which had enhanced canine COX-2 selectivity and in vivo efficacy compared to carprofen.[19]

In addition, we have disclosed the synthesis[20,21] and SAR related to the amino pyrazoles with 4-CN group that led to the identification of the lead compound, **2**, which showed in vivo efficacy in both canine and feline synovitis models.[22] The increased activity was explained by subsequent molecular modeling studies, in which the 4-nitrile substituted pyrazoles had contributions from hydrogen bonding of the nitrile with the active site side chain serine 530 hydroxyl group.[23] Similarly, we surmised that the 1,2,4-triazoles with the ring 4-nitrogen might also contribute to this potential hydrogen bonding and thus provide enhanced activity compared to the pyrazoles with 4-H group. Thus, in this paper, we disclose the synthesis and in vitro activity of a novel class of canine COX-2 selective 5-alkyl and 5-(hetero) aryl triazoles compared to some of the 4-unsubstituted pyrazoles with similar 5-sidechains.

The general synthesis of the analogs for SAR is shown in Scheme 1. Reaction of the pyridyl hydrazine with trifluoromethyl amidine **3** in methanol at room temperature gave the hydrazine substituted trifluoromethylacetamidine **5**. Coupling of the amidine with the acid followed by thermal cyclization gave the desired triazoles **7** in good yields.[24]

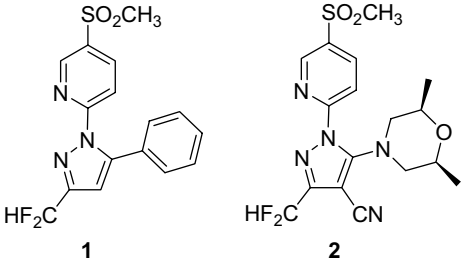
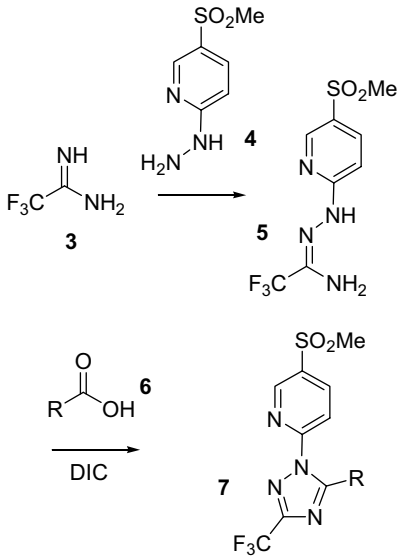


Figure 2. Canine COX-2 selective leads.



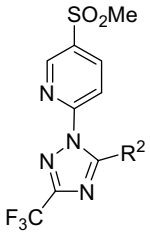
Scheme 1. Reagents and condtions: (a) THF, RT, 4 d, 96%; (b) R-CO_2H, DMAP, DIC, 90 °C, 48 h.

The details of the synthesis of the pyrazoles have been previously disclosed.[19]

All the analogs were tested in our single dose canine whole blood (CWB) COX inhibition assay.[25] Actives with inhibition greater than 50% were progressed to the COX-2 and COX-1 titration assay to determine their IC_{50}s, shown in Table 1.

The structure–activity relationship of these analogs follows very closely to that of the 5-aryl pyrazoles as discussed in previous papers.[19,23] Initial comparison of the phenyl side chain analogs—triazole **7a**, 4-nitrile pyrazole **8a** and 4-H pyrazole **8b**—suggest a slightly poorer binding based on slightly lower potency than the pyrazole class. However, only a slight modification of the ring with the 2-fluorophenyl side chain gives almost equivalent activity (0.12 uM) to the 4-nitrile pyrazole **8a**, whose improvement in activity is attributed to potential hydrogen bond interaction with the COX-2 binding pocket. In comparison, pyrazole **8c** has diminished activity with 2-fluorophenyl side-chain. On the other hand, 4-fluorophenyl side-chain gives comparable activity between the pyrazole **8d** and triazole **7c**. It is possible that with 2-fluorophenyl side chain, the 4-nitrogen of the triazole may be participating in binding whereas for the 4-fluorophenyl side-chain, the triazole ring 4-nitrogen may not be contributing to binding. However, among the 5-heteroaryl substituted pyrazoles and triazoles, the triazoles seem to have slightly improved activity over the pyrazole class (**7e** vs **8f**, **7f** vs **8g**, **7g** vs **8h**). Again, where the side-chains do not contribute significantly to binding, the ring nitrogen may be playing a role in contributing to improved activity for the triazoles. Only the 2-furanyl sidechains (**7d** vs **8e**) give similar activity between the two classes. Among alkyl side chains, the

Table 1. In vitro CWB COX-2 and COX-1 IC_{50} data for **7** and **8**



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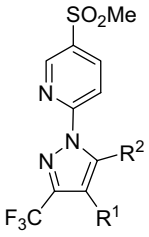

8

Compound	R^2	R^1	Ratio	CWB IC_{50}[a] (μM)	
			COX 1/2	COX-1	COX-2
7a	Ph		>122	>50	0.41
8a	Ph	CN	48.5	5.82	0.12
8b	Ph	H	>122	>50	0.41
7b	2-Fluorophenyl		>416	>50	0.12
8c	2-Fluorophenyl	H	—	>0.5	>0.5
7c	4-Fluorophenyl		71	17.71	0.25
8d	4-Fluorophenyl	H	69	19.2	0.28
7d	2-Furanyl		—	ND	>0.5
8e	2-Furanyl	H	—	>0.5	>0.5
7e	3-Pyridyl		138	33.08	0.24
8f	3-Pyridyl	H	—	>0.5	>0.5
7f	4-Pyridyl		—	>5	1.3
8g	4-Pyridyl	H	—	>0.5	>0.5
7g	2-Pyridyl		>104	>50	0.48
8h	2-Pyridyl	H		>0.5	>0.5
7h	Cyclobutyl		—	ND	>0.5
7i	Isobutyl		2.3	3.2	1.4
8i	Isobutyl	H	—	ND	>0.5
7j	Cyclohexyl		490	29.4	0.06
8j	Cyclohexyl	H	194	13.55	0.07
7k	3-Methyl-1-butyl		258	30.98	0.12
7l	2-Methyl-1-butyl		13	>50	3.88

[a] Run in duplicate or triplicate.

cyclohexyl substituent provided the most potent triazole **7j** and pyrazole **8j**, respectively, and had comparable activity between the two. With the exception of **7k**, which had good activity, other linear sidechains (**7i**, **7l**) or smaller cyclobutyl ring (**7h**), do not provide for improved activity. However, triazole **7i** does have improved activity over the pyrazole **8j**. Again the ring nitrogen could be contributing to the activity for compounds without side-chains with strong hydrophobic interactions.

We looked at the oral pharmacokinetic properties of these triazoles in beagles at 2 mg/kg in 0.5% methylcellulose and the results are listed in Table 2. For aryl and heteroaryl substituted triazoles (**7b**, **7e**), the C_{max} is better than that of the pyrazole **8b**, but the half-lives are long ($T_{1/2} > 20$ h). However, with the alkyl substituted triazoles **7k** and **7l**, the $T_{1/2}$ is much shorter (<3 h). Although these compounds had significant exposures in dogs, their half-lifes did not meet our guidelines for selection to move forward.

Table 2. 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)
8b[a]	5	611	0.8	4277	13.1
7b	2	745	1.5	14544	92
7e	2	566	24	19357	48.6
7k	2	751	0.8	4693	2.7
7l	2	321	0.8	898	1.8

[a] From Ref. 19.

In summary, this comparative study between the pyrazoles with 4-H substituent and the 1,2,4-triazoles of COX-2 inhibitors suggest that there may be a potential contribution from the ring 4-nitrogen towards the activity of the triazole with side-chains that do not have strong hydrophobic interactions but none or insignificant contributions for strong hydrophobic side chains. In conclusion, the majority of the activity in the triazole and the 4-H pyrazole class may be driven by the contribution of the strong hydrophobic 5-substituents. That is consistent with what we observed with our studies of the 4-nitrile substituted pyrazoles.[23] Although we were able to obtain potent and selective triazoles, none met our PK criteria.

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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 to 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 enyme immunoassay (EIA) kit. Samples without drug were included as controls for maximum production of TXB_2. 500 μl of heparinized blood was added to tubes containing drug and 10 l 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).