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5-Heteroatom substituted pyrazoles as canine COX-2 inhibitors. Part III: Molecular modeling studies on binding contribution of 1-(5-methylsulfonyl)pyrid-2-yl and 4-nitrile

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Abstract—The structure–activity relationship toward canine COX-1 and COX-2 in vitro whole blood activity of 4-hydrogen versus 4-cyano substituted 5-aryl or 5-heteroatom substituted *N*-phenyl versus *N*-2-pyridyl sulfone pyrazoles is discussed. The differences between the pairs of compounds with the 4-nitrile pyrazole derivatives having substantially improved in vitro activity are highlighted for both COX-2 and COX-1. This difference in activity may be due to the contribution of the hydrogen bond of the 4-cyano group with Ser 530 as shown by our molecular modeling studies. In addition, our model suggests a potential contribution from hydrogen bonding of the pyridyl nitrogen to Tyr 355 for the increased activity over the phenyl sulfone analogs.

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 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 maintenance of the GI tract, NSAIDs that 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 the 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 decreased 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 8 million animals, has 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. Firocoxib, with increased selectivity for the canine COX-2 enzyme,[16b] has also been recently approved for treatment in dogs. None of these are approved in the US for use in cats

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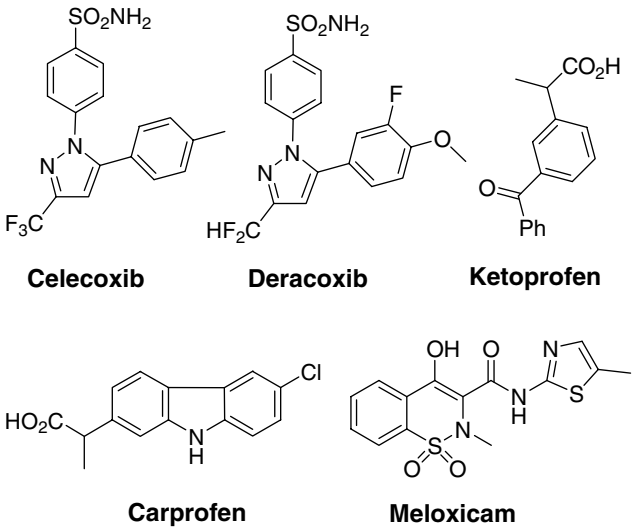


Figure 1. Structures of marketed COX-2 inhibitors.

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 to identify superior agents in this area led to the identification of 5-aryl pyrazole **3c** (Fig. 2) which had enhanced canine COX-2 selectivity and in vivo efficacy relative to carprofen.[19] In addition, we have disclosed the synthesis and SAR related to the alkyl ether[20] and amino pyrazoles[21] that led to the identification of the lead compound, **5c,** which showed in vivo efficacy in both canine and feline synovitis models. During our studies on the nitrile substituted pyrazoles, we have consistently noticed the differences in activity between the 4-nitrile and 4-hydrogen (non-substituted) as well as other substitutions at that position. Several literature disclosures have indicated the potential contribution of the nitrile group as a strong hydrogen-bond acceptor in contributing to activity of compounds in various drug discovery efforts.[22] In addition, a recent study was done to look at the specific contribution of the 4-substituted pyrazoles in the COX-2 activity of 1,4,5-substituted pyrazoles.[23] This study demonstrated a potential contribution of the hydrogen bond acceptor groups based on some modeling studies, but no in vitro whole cell or enzyme activity was reported. We would like to highlight the contribution of the 4-cyano and 2-pyridyl nitrogen as hydrogen bond acceptors leading to improvements in activity in our in vitro assay.

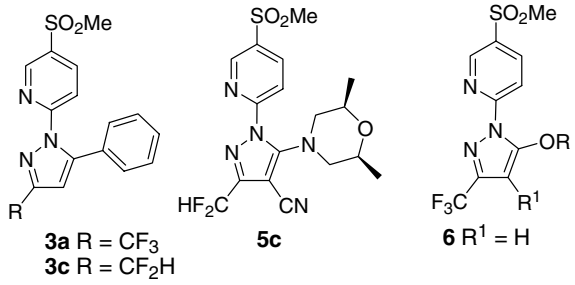
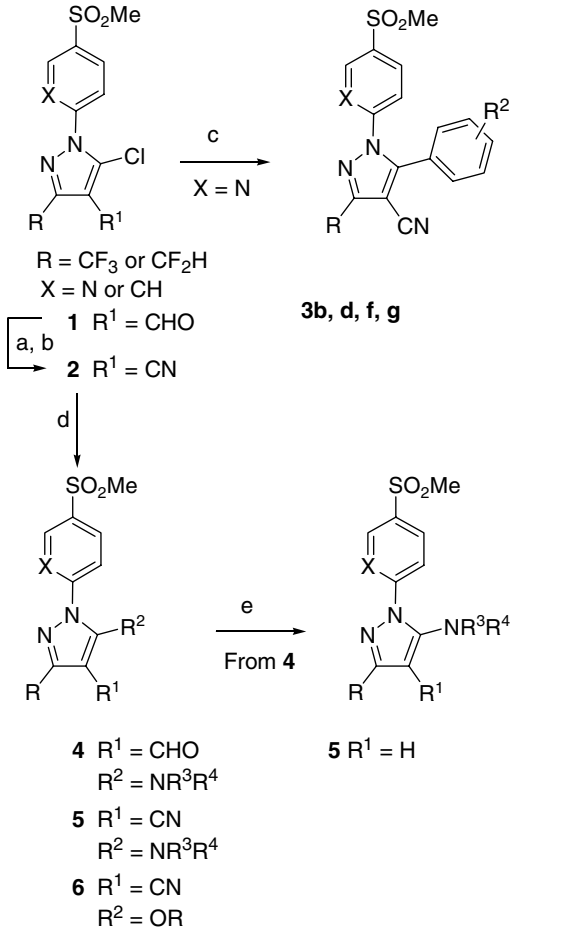


Figure 2. Canine COX-2, selective leads.

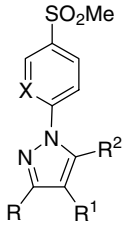
The synthesis of 5-phenyl pyrazoles **3a**, **3c** and 5-ethers **6** with 4-H has been disclosed in previous reports.[19,20] Preparation of 5-aryl-4-cyano pyrazoles (**3b**, **3d**, **3f**, **3g**) was accomplished using Suzuki–Miyauri coupling conditions (Scheme 1). The preparation of the 5-aminoalkyl-4-cyano substituted pyrazoles was done as shown in Scheme 1. The substitution of the 5-chloro-4-cyano pyrazoles **2** under mild base-mediated conditions gave the 4-cyano-5-aminoalkyl and 5-ether pyrazoles (**5** and **6**).[24,25] Reactions of the 4-formyl-5-amino-pyrazoles **4**, obtained in an analogous manner to compounds **5** and **6**, with hydroxyl amine hydrochloride in methanol, in an attempt to obtain the oximes, resulted in decarbonylation by-products **5**. The reactions were general enough to obtain up to 60% of the 4-H by-products **5**.

Table 1 shows the activity comparison of the analogs tested in the canine whole blood (CWB) COX-1 and COX-2 assay.[26] The compounds are grouped according to the 5-substitution on the pyrazole with comparisons



Scheme 1. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, TFE, reflux, 2 h, >80% (b) Cl_3CCOCl, Et_3N, DCM, 0 °C, 4–6 h, >90%; (c) $ArB(OH_2)$, $Pd(OAc)_2$, tris[2-(2-methoxyethoxy)ethyl]amine, 50–80%; (d) For **4**: Method A: R^3R^4NH, Et_3N, DCE, 80 °C; For **6**: Method B: ROH, KF, DMSO, RT to 80 °C; For **5**: Method A or Method B (at RT); (e) **4**, 1.2 equiv $NH_2OH{\cdot}HCl$, MeOH, reflux, 8–60%.

Table 1. In vitro COX-1 and COX-2 inhibition data of **3**, **5**, and **6**



Compound	X	R	R^1	R^2	Ratio COX 1/2	CWB* IC_{50} (μM)		CWB* COX-2 % inh at 0.5 μM	*C*log *P*	Kinetic aqueous sol (μg/ml)
						COX-1	COX-2			
3a	N	CF_3	H	Phenyl	>122	>50	0.41+	56.3+	2.95	50–200
3b	N	CF_3	CN	Phenyl	48.5	5.82	0.12	74.3	2.39	100–200
3c	N	CF_2H	H	Phenyl	155	48	0.31	62.7	2.25	> 200
3d	N	CF_2H	CN	Phenyl	>112	5.6	<0.05	98.3	1.84	ND
3e	CH	CF_2H	CN	Phenyl	>232	11.61	<0.05	97.4	2.89	ND
3f	N	CF_2H	CN	3,5-Difluorophenyl	131	22.34	0.17	96.8	2.13	50–100
3g	CH	CF_2H	CN	3,5-Difluorophenyl	>833	>50	0.06	86.4	3.18	ND
5a	N	CF_3	H	Cis-2,5-Dimethylmorpholine	—	>5	>5	−33.9	2.12	100–200
5b	N	CF_3	CN	Cis-2,5-Dimethylmorpholine	>208	>50	0.24	70.8	1.69	>200
5c	CH	CF_3	CN	*cis*-2,5-Dimethylmorpholine	—	>0.5	>0.5	38.0	2.15	ND
5d	N	CF_2H	CN	*cis*-2,5-Dimethylmorpholine	186	35.97	0.24	70.2	1.10	>200
5e	CH	CF_2H	CN	*cis*-2,5-Dimethylmorpholine	—	ND	>0.5	31.7	1.92	50–200
5f	N	CF_3	H	3-Methyl piperidine	—	>0.5	>0.5	6.2	2.62	ND
5g	N	CF_3	CN	3-Methyl piperidine	<1	<0.05	0.07	101.7	2.71	100–200
5h	CH	CF_3	CN	3-Methyl piperidine	>312	>50	0.16	87.1	3.01	ND
5i	N	CF_2H	CN	3-Methyl piperidine	100	3.02	0.03	102.8	1.60	ND
5j	CH	CF_2H	CN	3-Methyl piperidine	397	27.8	0.09	104.1	2.94	50–100
5k	N	CF_3	H	Cyclobutyl amine	—	>0.5	>0.5	−2.3	1.88	ND
5l	N	CF_3	CN	Cyclobutyl amine	5.39	1.24	0.23	78.7	1.56	ND
5m	N	CF_3	H	Cyclopropyl methyl amine	—	>0.5	>0.5	5.7	1.94	ND
5n	N	CF_3	CN	Cyclopropyl methyl amine	17	1.88	0.11	79.3	1.62	ND
5o	CH	CF_3	CN	Cyclopropyl methyl amine	—	>0.5	>0.5	1.1	2.54	ND
5p	CH	CF_2H	CN	Cyclopropyl methyl amine	—	>0.5	>0.5	−39.4	1.92	>200
5q	N	CF_3	H	Cyclopentylamine	—	ND	>0.5	21.3	2.44	25–100
5r	N	CF_3	CN	Cyclopentylamine	2.5	0.31	0.12	79.4	2.12	ND
5s	N	CF_2H	CN	Cyclopentylamine	151	12.08	0.08	100	1.50	25–50
5t	CH	CF_2H	CN	Cyclopentylamine	—	>0.5	>0.5	−38.2	2.41	100–200
5u	N	CF_3	H	Neopentylamine	—	ND	>0.5	−6.3	2.87	ND
5v	N	CF_3	CN	Neopentylamine	87	17.41	0.20	84.0	2.55	<25
5w	CH	CF_3	CN	Neopentylamine	—	ND	>0.5	13.8	3.46	ND
5x	CH	CF_2H	CN	Neopentylamine	—	>0.5	>0.5	−32.0	2.84	>200
6a	N	CF_3	H	Isobutyloxy	>12	>50	4.07	18.4	2.47	50–200
6b	N	CF_3	CN	Isobutyloxy	17	1.87	0.11	93.1	2.02	<25
6c	CH	CF_3	H	Isobutyloxy	—	ND	>0.5	−10.5	3.38	ND
6d	CH	CF_3	CN	Isobutyloxy	—	>0.5	>0.5	25.4	2.94	ND
6e	N	CF_3	H	Cyclopentyloxy	—	ND	>0.5	12.1	2.48	50–200
6f	N	CF_3	CN	Cyclopentyloxy	78	16.5	0.21	86.8	2.03	ND
6g	CH	CF_3	H	Cyclopentyloxy	—	ND	>0.5	−49.9	3.40	25–50
6h	N	CF_3	H	Cyclobutylmethyloxy	—	ND	>0.5	−77.4	2.54	100–200
6i	N	CF_3	CN	Cyclobutylmethyloxy	133	10.63	0.08	97.1	2.09	ND
6j	CH	CF_3	H	Cyclobutylmethyloxy	—	ND	>0.5	−34.7	3.46	25–50
6k	N	CF_3	H	Neopentyloxy	>2.5	>50	19.64	5.9	2.91	100–200
6l	N	CF_3	CN	Neopentyloxy	—	ND	<0.5	63.2	2.46	ND
6m	CH	CF_3	H	Neopentyloxy	—	ND	>0.5	−30.6	3.82	50–100
6n	CH	CF_3	CN	*n*-Butyl-2-oxy	—	>0.5	>0.5	14.7	2.85	50–100

* Canine whole blood (CWB) assay: run in duplicate or triplicate; +, average of many runs.

made between 4-H (unsubstituted) and 4-CN substituted pyrazoles and between *N*-(4-methylsulfonyl) phenyl and/or *N*-(5-methylsulfonyl)pyrid-2-yl pyrazoles. The 3-position of the pyrazole is substituted with either CF_2H or CF_3, generally found to be optimal for activity.[11] Three types of 5-substituents; 5-aryls, 5-aminoalkyls, and 5-alkylethers, are highlighted in Table 1 with results from WBC COX-2 assay at 0.5 μM dose and titration data of the actives. (Actives: inhibition of COX-2 > 50% at 0.5 μM.)

Within the *N*-pyridyl 5-phenyl substituted pyrazoles **3**, the COX-2 activity does not vary that widely between compounds with 4-CN and 4-H pyrazoles, although there is a definite trend toward improved activity (3–6×) with the 4-CN substitution. However, there is a significant improvement in activity against the COX-1 enzyme (**3b**, **3d** vs **3a**, **3c**) as well. When comparing the contribution of the *N*-pyridyl versus *N*-phenyl sulfone to the activity within these 5-aryl pyrazoles, there does not seem to be any difference in activity against the COX-2 enzyme (**3d**, **3f** vs **3e**, **3g**). Against the COX-1 enzyme, however, the *N*-pyridylsulfone has improved activity compared to the *N*-phenyl sulfone analogs.

For both the 5-alkylamino, and 5-alkylether substituted *N*-(2-pyridyl methylsulfone) pyrazoles, **5** and **6,** the 4-CN substitution provides active compounds (**5b**, **5g** , **5l**, **5r**, **5v**, **6b**, **6f**, **6i**, **6l**) over 4-H compounds that are either inactive (**5a**, **5f**, **5k**, **5q**, **5u**, **6a**, **6e**, **6h**, **6k**) or weakly active (**6a**). As in the case of the 5-aryl pyrazoles, many of these 5-heteroatom substituted 4-CN pyrazoles show enhanced activity in the COX-1 assay as well (**5g**, **5l**, **5n**, **5r**, **5v**, **6b**) relative to its 4-H pyrazoles. The one exception is for **5b** where no COX-1 activity is seen. Among others that do show weak activity, comparison is difficult to make because no COX-1 titration data are available for the parent 4-H analogs (**6f**, **6i**). In the case of *N*-phenylmethylsulfonyl pyrazoles, the majority of the compounds with either 4-H or 4-CN substituted pyrazoles are inactive (**5e**, **5o**, **5p**, **5t**, **5w**, **5x**, **6c**, **6d**, **6g**, **6j**, **6n**, **6o**) or weakly active (5c (38% inh at 0.5 μM), 5e (31.7% inh at 0.5μM)) in the COX-2 assay. Only the 5-(3-methylpiperidine) substituted *N*-phenylsulfonyl and *N*-(2-pyridylmethylsulfonyl) pyrazoles with 4-CN group are active in the COX-2 assay. These 5-(3-methylpiperidine)-*N*-phenylmethylsulfonyl pyrazoles, however, are inactive (**5h**) or weakly active (**5j**) in the COX-1 assay, which provide for greater selectivity than the corresponding *N*-pyridyl analogs.

Thus, among all active compounds with 5-aryl or 5-heteroatom substituted-*N*-(2-pyridylmethylsulfone) pyrazoles, the substitution at the 4- position of the pyrazole ring with the cyano substituent confers substantial improvement in activity over its corresponding 4-H analogs. Depending on the binding contribution of the non-aryl 5-substituents, the contribution of the pyridyl nitrogen may not be required for activity since some of the *N*-phenyl methylsulfonyl compounds are active with a 4-cyano group present. Only in the case of 5-phenyl substituted pyrazoles, the 4-CN or 2-pyridyl group does not seem to be necessary for COX-2 activity.

Although our biological activities are based on canine whole blood assay, we strongly believe the contributions shown by the 4-cyano and *N*-2-pyridyl nitrogen for activity are based on COX-2 and COX-1 active site interactions and not just related to physical property differences in whole blood. There are no clear indications that the difference in activity is due to solubility or polarity of the molecules, based on their *C*log *P* or solubility data (Table 1). Thus, the in vitro biological results indicate a strong possibility of hydrogen bond interactions between 2-pyridyl nitrogen and 4-cyano nitrogen with appropriately situated hydrogen bond donors in the active site. Since the improvement in activity was seen for both the COX-2 and COX-1 activity, we presume the site of interaction to be present in both enzymes. Although there are many molecular modeling studies done with ligand interactions with COX-1 and COX-2 enzymes, we have been unable to find any modeling studies done with *N*-(5-methylsulfonylpyrid-2-yl) groups.[27] Studies with 4-cyano substituted pyrazoles have been mentioned in the literature.[23] Thus, we undertook molecular modeling studies to determine if there are some significant interactions with the 2-pyridyl and the 4-cyano nitrogen to afford such potent compounds. It is interesting to note that a recent molecular modeling study of many diaryl pyrazoles has indicated electrostatic and hydrogen bond interactions to be an important determinant for COX-2 selectivity along with the right conformation.[27d]

Canine and human COX-1 and COX-2 are highly similar (92.7% and 90.1% identical, respectively), and the active site residues of canine COX-1 and COX-2 are identical to human and rat enzymes.[28] Therefore, the available murine COX-2 X-ray co-crystal structure of SC-558[27a] was used as the starting point for molecular modeling. Manual docking followed by molecular mechanics minimization[29] of **3b** shows very similar interactions as in the case of SC-558 where the 5-phenyl is bound in the hydrophobic pocket formed by Phe 381, Leu 384, Tyr 385, Trp 387, Phe 513, and Ser 530. The backbone atoms of Gly 526 and Ala 527 are stacking against the phenyl ring and the *N*-2-pyridylmethylsulfonyl group is bound to the side pocket of COX-2, while the trifluoromethyl group is interacting with Arg 120. These interactions are similar in COX-1 except that the side pocket has Val 523 instead of Ile where the large Ile group potentially forms a smaller pocket in COX-1, thus providing for COX-2 selectivity.

Our molecular modeling revealed several unique interactions of *N*-2-pyridyl 5-alkylether/alkylamino 4-cyano pyrazoles with the COX-2 active site that would explain the observed structure–activity relationships. In contrast to a previous report[23], our models show that the cyano group is accepting a hydrogen bond from Ser 530 side-chain hydroxyl group. The observed distances of 3.0–3.5 Å were seen depending on substitution in 5-position, consistent with observation of nitrile hydrogen bonds.[31] In the case of **3b**, phenyl fits well into the hydrophobic pocket and Ser 530 sidechain rotated approximately 20° to form a weak hydrogen bond to the 4-cyano nitrogen. With smaller 5-alkylamino and 5-alkylether substitutions, like compound **6b**, the energy-minimized structure shows that the pyrazole ring shifts deeper by approximately 0.5 Å into the hydrophobic pocket. With a small rotation of Ser 530 hydroxyl (∼15°), a good hydrogen bond is formed with Ser 530 with a nitrogen to oxygen distance of 3.0 Å, as shown in Figure 3. We hypothesize that this is one of the factors in the contribution of 4-cyano versus 4-H substitution-related activity differences (37X for **6b** vs **6a** and **6h** vs **6i)**.

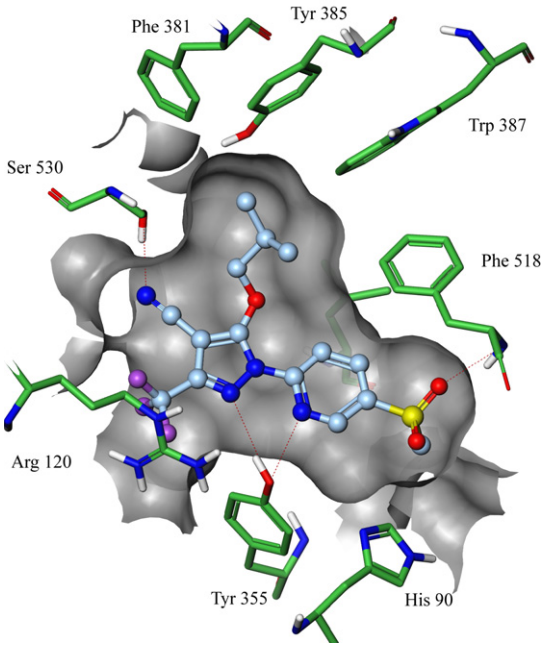


Figure 3. Energy-minimized model of **6b** in the COX-2 active site. Unique hydrogen bonds are 4-cyano nitrogen to Ser 530 and *N*-pyridyl nitrogen to Tyr-355.

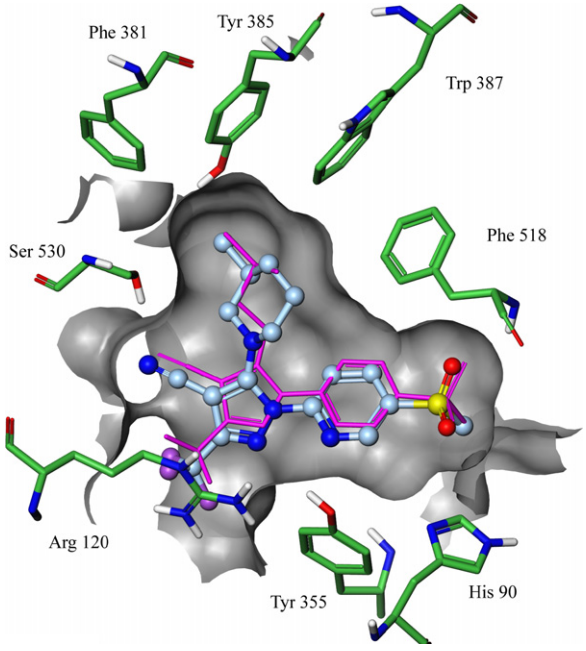


Figure 4. Model of **5g** (light blue ball and stick) shows interaction of methyl of 3-methyl piperidine that fits well into a small pocket near Phe 381, Tyr 385, and Ser 530. The model of **6b** is drawn in magenta to show slight shifting of **6b** model in the active site with respect to the binding model of **5g**.

The second factor that determines the relative 4-cyano contributions to potential electrostatic interactions/hydrogen bond is related to the electron-withdrawing capability of the 4-cyano group compared to other substituents. In the case of 5-aryl substitution, the aryl group is competing with the 4-cyano group to withdraw electrons from the pyrazole ring, as a result we expect to see weaker electrostatic interaction/hydrogen bond of the nitrile amine to Ser 530 hydrogen. Thus, contribution of 4-cyano versus 4-H is weaker with 5-aryl substitution (3.4X in **3b** vs **3a**).

Our COX-2 binding models of *N*-2-pyridylmethyl-sulfonyl also show a potential hydrogen bond of 2-pyridyl nitrogen with Tyr 355 with a distance of approximately 3.0 Å. However, the hydroxyl angle with respect to the Tyr 355 phenyl ring is not ideal from a molecular mechanics minimization perspective. We attribute this to the fact that there are strong electrostatic fields in the region formed by Arg 120, Glu 524, and Arg 513, and because our modeling method did not include explicit water molecules.

Modeling of **5g** (Fig. 4) provided insight into the potency gain of having 3-methyl piperidine at the 5-position. As Ser 530 hydroxyl moves to form a hydrogen bond with 4-cyano nitrogen, a small hydrophobic pocket is formed near Phe 381, Tyr 385, and Ser 530. The methyl group of 3-methyl piperidine fits well into the pocket, potentially providing better binding potency for **5g**. Other substitution like *cis*-2,5-dimethylmorpholine might be slightly too big to fit because of di-substitution, thus, reducing the binding potency of the compound.

In summary, we have highlighted some potent *N*-2-pyridylmethylsulfonyl 4-nitrile pyrazoles with 5-aryl, 5-alkylamino, and 5-alkylether substituents that have improved CWB COX-2 activity compared to parent 4-H *N*-2-pyridylmethylsulfonyl and *N*-phenylmethylsulfonyl pyrazoles. The improved activity can be explained by the potential gain in binding energy due to favorable interactions with the appropriate hydrogen bond donors in the active site. Based on the data we present, the strong hydrophobic binding of aryl substituents in the 5-position of the pyrazole ring can outweigh other electrostatic/hydrogen bond contributions from the 4-nitrile or the *N*-2-pyridyl nitrogen. However, for weakly binding sidechains at the 5-position, electrostatic/hydrogen bond contribution from 4-cyano or/and *N*-2-pyridyl nitrogen is required for activity in the CWB COX-2 assay. The SAR also strongly suggests that the electrostatic/hydrogen bond interactions are similar between the COX-1 and COX-2 enzymes with the ligand, which explains improvement in activity against the COX-1 as well. However, we cannot rule out the possibility that the enhanced activity, especially for COX-1, may be due to other modes of binding. While our modeling studies confirm the SAR of our compounds, it would be beneficial to confirm the modeling studies with ligand bound X-ray structures.[32]

Acknowledgment

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26. 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 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).
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29. The molecular minimization was performed using MacroModel[30] software. The partial flexible binding site was constructed from the co-crystal structure of SC-588 with COX-2 (PDB: 6COX). Inhibitors and protein binding site residues were fully flexible and protein residues further away from the ligand (>5 Å) were harmonically constrained to their crystallographic positions during the minimization. Ab initio partial atomic charges were computed and assigned to all ligands and AMBER* atomic charges were assigned to protein residues. The complexes were energetically minimized using the Batchmin program of MacroModel V8.6 with the AMBER* force field and the GB/SA solvation model.
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32. Part of this paper has been presented at 2006 Fall ACS meeting in San Francisco.