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# Discovery of a potent, selective and orally active canine COX-2 inhibitor, 2-(3-difluoromethyl-5-phenyl-pyrazol-1-yl)-5-methanesulfonyl-pyridine

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**Abstract**—Structure–activity relationship (SAR) studies of 2-[3-di(and tri)fluoromethyl-5-arylpyrazol-1-yl]-5-methanesulfonylpyridine derivatives for canine COX enzymes are described. This led to the identification of **12a** as a lead candidate for further progression. The in vitro and in vivo activity of **12a** for the canine COX-2 enzyme as well as its in vivo efficacy and pharmacokinetic properties in dog are highlighted.


Nonsteroidal anti-inflammatory drugs (NSAIDs) used for the treatment of inflammatory conditions act by inhibition of cyclooxygenase (COX)[1–3] which catalyzes the first step in arachidonic acid metabolism.[4] 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, a new isozyme of COX, was reported recently and its biological significance is under evaluation.[6] It is believed that a selective COX-2 inhibitor will greatly reduce the side effect profile, including gastric ulceration, that is commonly associated with the chronic use of non-selective NSAIDs.[7–10] Several marketed COX-2 selective inhibitors,[11–14] including celecoxib (Celebrex®), valdecoxib (Bextra®), rofecoxib (Vioxx®), and etoricoxib (Arcoxia®) have shown excellent efficacy in humans with few side effects (Fig. 1).

Progressive degenerative joint disease, or osteoarthritis, is the most common cause of chronic pain in dogs.[15] It is estimated that one out of every five adult dogs, or

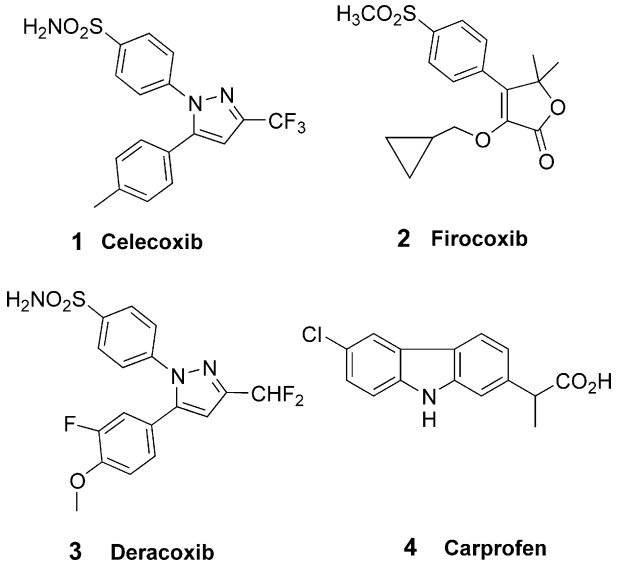


**Figure 1.** Structures of selected COX-2 inhibitors.

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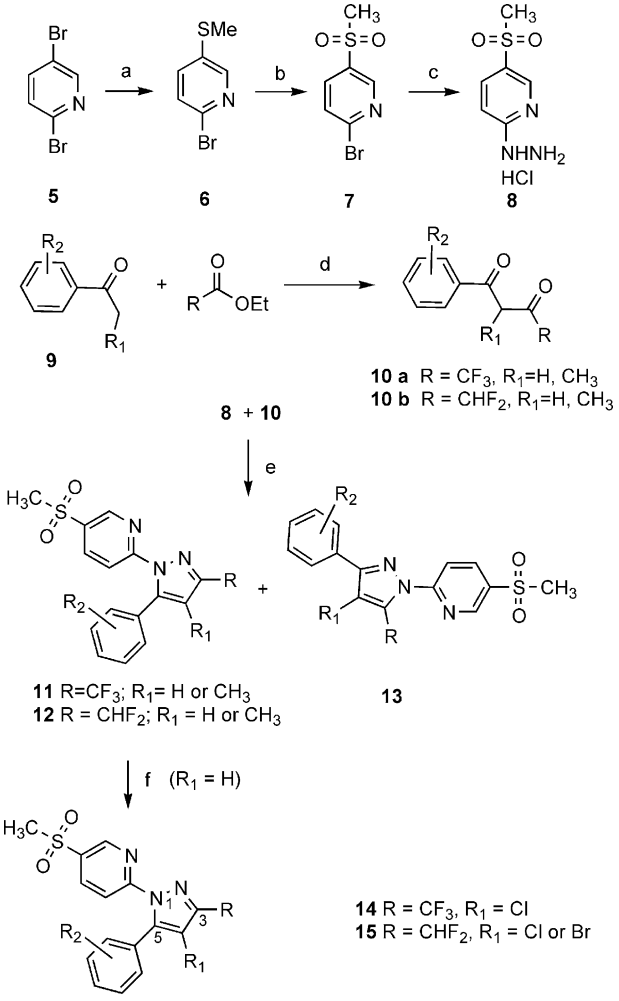
approximately 8 million animals, have osteoarthritis, yet nearly half (48%) of these patients are untreated.[16] As in people, chronic use of NSAIDs in dogs is often associated with GI side effects.[17] Carprofen (**4**) (Rimadyl®)[18] and deracoxib (**3**) (Deramaxx™),[19] two of the marketed agents for the treatment of inflammation and pain for dogs, have demonstrated moderate COX-2 selectivity.[17] Firocoxib (**2**), a new class of COX-2 inhibitor for animal health, is under further clinical evaluation.[20] In this paper, we disclose the synthesis and SAR of a novel class of canine selective COX-2 inhibitors, pyridylsulfones. This research effort culminated in the identification of **12a**. A general synthetic route for the synthesis of the pyrazole analogues[21] is described in Scheme 1.

The pyrazoles were prepared via condensation of the hydrazine **8** with the respective diketone **10**. The commercially available 2,5-dibromopyridine (**5**) was converted to 2-bromo-5-methylsulfanylpyridine (**6**) by a lithium–halogen exchange reaction followed by addition of methyldisulfanylmethane. The methylsulfanyl functional group was transformed to the methanesulfonyl moiety in **7** by MCPBA mediated oxidation. Displacement of bromine with hydrazine furnished 5-methanesulfonyl-pyridin-2-yl-hydrazine which was converted to its HCl salt **8** in situ. The 1,3-diketones **10a** and **10b** were synthesized[11] using Claisen condensation of the aryl-substituted ketones **9** with either ethyl trifluoroacetate or ethyl difluoroacetate in DME. The condensation of hydrazine **8** with the 1,3-dicarbonyl **10a**/**10b** in ethanol then provided the 1,5-diarylpyrazole (**11** or **12**) as the major product,[11] which was readily separated from its 1,4-diarylpyrazole isomer **13** by flash chromatography. It was found that the ratio of the two regioisomers (1,5- vs 1,4-diarylpyrazole) was affected by different acids and solvents. Generally, a better product ratio (1,5- vs 1,4-diarylpyrazole) was obtained by refluxing the mixture of **8** and **10** in ethanol containing concentrated sulfuric acid.[22] The 4-chloro/bromo pyrazole analogues (**14**/**15**) were obtained in high yield by stirring the pyrazole (**11**/**12**) with NCS or NBS in DMF at room temperature.




**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; HCl/MeOH, 100%; (d) NaOMe, DME, rt, 60–95%; (e) EtOH, reflux, 50–95%; (f) NCS or NBS, DMF, rt, >95%.

Numerous 11/12 analogues were prepared and tested in the in vitro canine whole blood COX inhibition assays.[23] Data for a select group of compounds are listed in Tables 1–3. Generally, analogues with halogen substitution on the 4-position of the phenyl ring had good potency against canine COX-2 enzyme in both the-$CF_3$ and-$CHF_2$ analogue series. The selectivity (COX-2 vs COX-1), on the other hand, tended to decrease from F to Br (e.g., **11a–d**, **12a–d**). Analogues which contained an electron donating group at the 4 position of the phenyl ring tended to have increased COX-1 and COX-2 potency, with decreased COX-1/COX-2 selectivity (e.g., **11e–g**, **12e–f**, with the exception of **11h**). The potency against COX-1 could be modulated without affecting COX-2 potency by introducing a halogen or a methyl substituent at the 3-position of the phenyl ring in both-$CF_3$ and -$CHF_2$ analogue series (**11i–k**, **12g–i**). The selectivity and potency tended to drop with the introduction of a halogen at the 2-position (**11m**, **12j**). Limited improvement in activity was achieved in analogues with substitution at 3-position or 2-position only or di-substitution in both the 2- and 4-positions of the aryl ring. Increasing the steric bulk at the 4-position of the phenyl ring reduced the potency towards the COX-2 enzyme (**12k–n**). Celecoxib (**1**) and deracoxib (**3**) were tested in the in vitro canine whole blood COX inhibition assays as well (Table 2). In general, the pyridyl sulfone series (**11** and **12**) demonstrated improved potency and selectivity toward COX-2 compared to the benzylsulfonamide series (celecoxib and deracoxib). As illustrated in Table 3, chloro or bromo substitution at the 4-position of the pyrazole ring generally increased the potency towards both COX-1 and COX-2 enzymes and resulted in a slight decline in selectivity.

With many potent compounds in hand, we next focused on identifying the best pharmacokinetic (PK) profile. In vivo pharmacokinetic evaluations of the analogues were carried out in beagle dogs by oral gavage at 5 mg/kg.

**Table 1.** In vitro COX-1 and COX-2 inhibition data of **11a–m** ($CF_3$ analogues, $R_1 = H$)

| Compd | $R_2$ | Ratio COX 1/2 | $IC_{50}$ (μM) | |
|---|---|---|---|---|
| | | | COX-1 | COX-2 |
| **11a** | H | >122 | >50 | 0.41 |
| **11b** | 4-F | 116 | 27.9 | 0.24 |
| **11c** | 4-Cl | 77 | 19.4 | 0.25 |
| **11d** | 4-Br | 64 | 28.6 | 0.45 |
| **11e** | 4-OMe | 19 | 0.83 | 0.044 |
| **11f** | 4-SMe | 28 | 1.9 | 0.069 |
| **11g** | 4-OEt | 0.38 | 0.05 | 0.13 |
| **11h** | $NMe_2$ | 202 | 24.2 | 0.12 |
| **11i** | 3-Me, 4-OMe | 151 | 40.7 | 0.27 |
| **11j** | 3-F, 4-OMe | 154 | 47.8 | 0.31 |
| **11k** | 3-Cl, 4-OMe | >167 | >50 | 0.30 |
| **11l** | 3-Br, 4-OMe | >33 | >50 | 1.50 |
| **11m** | 2-F, 4-OMe | 47 | 2.8 | 0.06 |

**Table 2.** In vitro COX-1 and COX-2 inhibition data of **12a–n** ($CHF_2$ analogues, $R_1 = H$), celecoxib (**1**) and deracoxib (**3**)

| Compd | $R_2$ | Ratio COX 1/2 | $IC_{50}$ (μM) | |
|---|---|---|---|---|
| | | | COX-1 | COX-2 |
| **12a** | H | 155 | 48.0 | 0.31 |
| **12b** | 4-F | 47 | 14.0 | 0.30 |
| **12c** | 4-Cl | 173 | 20.7 | 0.12 |
| **12d** | 4-Br | 3.4 | 1.0 | 0.29 |
| **12e** | 4-OMe | 3.1 | 0.2 | 0.064 |
| **12f** | 4-SMe | 7.5 | 0.9 | 0.12 |
| **12g** | 3-Me, 4-OMe | >294 | >50 | 0.17 |
| **12h** | 3-F, 4-OMe | 198 | 9.9 | 0.05 |
| **12i** | 3-Cl, 4-OMe | >152 | >50 | 0.33 |
| **12j** | 2-F, 4-OMe | 93 | 36.4 | 0.39 |
| **12k** | 4-Me | 56 | 10 | 0.18 |
| **12l** | 4-Et | 30 | 12 | 0.4 |
| **12m** | 4-*n*-pr | >25 | >50 | 2 |
| **12n** | 4-*i*-pr | >6.2 | >50 | 8.1 |
| Celecoxib (**1**) | | 6.2 | 5.57 | 0.90 |
| Deracoxib (**3**) | | 36.5 | 23 | 0.63 |

**Table 3.** In vitro COX-1 and COX-2 inhibition data

| Compd | $R_1$ | $R_2$ | Ratio COX 1/2 | $IC_{50}$ (μM) | |
|---|---|---|---|---|---|
| | | | | COX-1 | COX-2 |
| **14a** | Cl | H | 132 | 7.9 | 0.06 |
| **15a** | Cl | H | 7.5 | 0.33 | 0.044 |
| **15b** | Cl | 3-Cl, 4-OMe | 24 | 1.5 | 0.062 |
| **15c** | Br | H | 25 | 2 | 0.08 |
| **11n** | Me | H | >294 | >50 | 0.17 |
| **12o** | Me | H | 48 | 9.1 | 0.19 |

PK data for select analogues and celecoxib (**1**) are summarized in Table 4. The $–CHF_2$ analogues showed higher AUCs values compared to the $–CF_3$ analogues (e.g., **12a** and **b** vs **11a** and **b**), presumably due to increased polarity (clogP of the $–CHF_2$ analogues were 1 unit lower compared to the $–CF_3$ analogues). Either unsubstituted phenyl or mono-substituted 4-phenyl had better PK profiles overall than di-substituted analogues (e.g., **11a–c** vs **11j–k**, **12a–c** vs **12g–i**). Halogen or methyl substitution at the 4-pyrazole position did not improve the PK of the analogues (e.g., **14a**, **15b**, **11n**, **12o**). Thus,

**Table 4.** Pharmacokinetic data

| Compd | $C_{max}$ (ng/mL) | $T_{max}$ (h) | AUC (ng h/mL) | Oral $T_{1/2}$ (h) | $C_{24}$ (ng/mL) |
|---|---|---|---|---|---|
| **12a** | 1089 | 1.5 | 16541 | 26 | 443 |
| **12b** | 509 | 1.3 | 7652 | 17 | 329 |
| **11b** | 540 | 0.8 | 4817 | 17.5 | 134 |
| **12c** | 412 | 1 | 4285 | 14 | 138 |
| **11a** | 611 | 0.8 | 4277 | 13.1 | 77 |
| **14a** | 473 | 0.75 | 2654 | 14.7 | 34 |
| **11n** | 338 | 1.5 | 2098 | 13.3 | 28 |
| **11c** | 95 | 1 | 913 | 16.2 | 26 |
| **12g** | 89 | 1 | 594 | 6.3 | 4.6 |
| **12o** | 208 | 0.5 | 516 | 18.5 | 3.6 |
| **11j** | 69 | 1 | 396 | 10.4 | 5 |
| **12l** | 45 | 2.5 | 249 | 7 | 0 |
| **12i** | 36 | 1 | 185 | 12.4 | 2 |
| **12h** | 27 | 1 | 145 | 17.7 | 2.1 |
| **11k** | 42 | 0.8 | 115 | 2.6 | 4 |
| **15b** | 13 | N/A | 26 | 0.8 | 0 |
| **1** | 540 | N/A | 3100 | 3.3 | N/A |

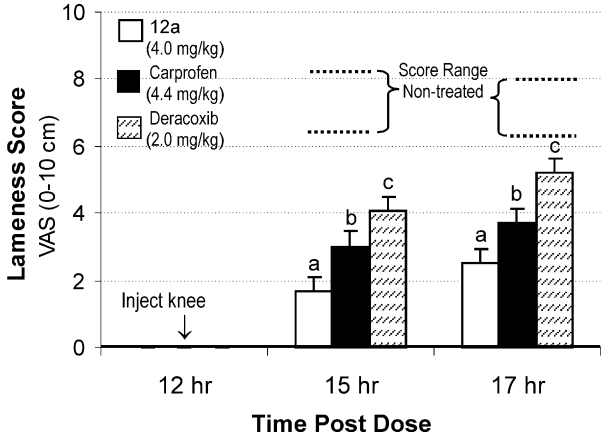



**Figure 2.** Comparison of lameness scores (bars show mean + standard error of the mean) for dogs given **12a**, carprofen or deracoxib prior to induction of acute knee joint synovitis in one hind leg.[24] Different letters over the error bars indicate statistical differences in the means ($p < 0.10$). The range of scores for non-treated dogs falls between the parallel dashed lines above each time point.

**12a** was identified for further in vivo profiling based on its potency and PK properties. The analgesic effect of **12a** was evaluated in a beagle acute pain model in which lameness develops following induced synovitis of the knee joint. Dogs were dosed orally for 5 consecutive days to achieve steady-state plasma concentration and synovitis induced 12 h after the last drug dose. Compared to nontreated dogs, lameness was improved in all groups of drug-treated animals. A once-a-day 4.0 mg/kg dose of **12a** demonstrated efficacy superior to carprofen dosed once daily at 4.4 mg/kg and deracoxib dosed once daily at 2.0 mg/kg (Fig. 2).

In summary, a novel class of potent and selective canine COX-2 inhibitors were synthesized and evaluated. Analogue **12a** demonstrated good COX-2 selectivity as well as desirable potency and PK properties, and was selected for in vivo efficacy testing. Excellent control of pain was shown in a canine synovitis model.

## References and notes


1. Carter, J. S. *Exp. Opin. Ther. Pat.* **1998**, *8*, 21.
2. Laneuville, O.; Breuer, D. K.; Dewitt, D. L.; Hla, T.; Funk, C. D.; Smith, W. L. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 927.
3. O'Neill, G. P.; Mancini, J. A.; Kargman, S.; Yergey, J.; Kwan, M. Y.; Falgueyret, J. P.; Abramovitz, M.; Kennedy, B. P.; Ouellet, M.; Cromlish, W. *Mol. Pharmacol.* **1994**, *45*, 245.
4. Otta, J. C.; Smith, W. L. J. *Lipid. Mediat. Cell Signal* **1995**, *12*, 139.
5. Prasit, P.; Riendeau, D. *Ann. Rep. Med. Chem.* **1997**, *32*, 211.
6. Warner, T. D.; Mitchell, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 13371, and references cited herein.
7. Clive, D. M.; Stoff, J. S. *New Engl. J. Med.* **1984**, *310*, 563.
8. Allison, M. C.; Howatson, A. G.; Torrance, C. J.; Lee, F. D.; Russell, R. I. *New Engl. J. Med.* **1992**, *327*, 749.
9. Griswold, D. E.; Adams, J. L. *Med. Res. Rev.* **1996**, *16*, 181.
10. Cryer, B.; Dubois, A. *Prostag. Oth. Lipid Mediat.* **1998**, *56*, 341.
11. Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Doctor, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.
12. Talley, J. J.; Brown, D. L.; Carter, J. S.; Graneto, M. J.; Koboldt, C. M.; Masferrer, J. L.; Perkins, W. E.; Rogers, R. S.; Shaffer, A. F.; Zhang, Y. Y.; Zweifel, B. S.; Seibert, K. *J. Med. Chem.* **2000**, *43*, 775.
13. Prasit, P.; Wang, Z.; Brideau, C.; Chan, C.-C.; Charleson, S.; Cromlish, W.; Ethier, D.; Evans, J. F.; Ford-Hutchinson, A. W.; Gauthier, J. Y.; Gordon, R.; Guay, J.; Gresser, M.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Léger, S.; Mancini, J.; O'Neill, G. P.; Ouellet, M.; Percival, M. D.; Perrier, H.; Riendeau, D.; Rodger, I.; Tagari, P.; Thérien, M.; Vickers, P.; Wong, E.; Xu, L.-J.; Young, R. N.; Zamboni, R.; Boyce, S.; Rupniak, N.; Forrest, M.; Visco, D.; Patrick, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1773.
14. Chauret, N.; Yergey, J. A.; Brideau, C.; Friesen, R. W.; Mancini, J.; Riendeau, D.; Silva, J.; Styhler, A.; Trimble, L. A.; Nicoll-Griffith, D. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1059.
15. Fox, S. M.; Johnston, S. A. *J. Am. Vet. Med. Assoc.* **1997**, *210*, 1493.
16. Pfizer Animal Health Data File.
17. Ricketts, A. P.; Lundy, K. M.; Seibel, S. B. *Am. J. Vet. Res.* **1998**, *59*, 1441.
18. Streppa, H. K.; Jones, C. J.; Budsberg, S. C. *Am. J. Vet. Res.* **2002**, *63*, 91.
19. Millis, D. L.; Weigel, J. P.; Moyers, T.; Buonomo, F. C. *Vet. Ther.* **2002**, *3*, 453.
20. Black, W. C.; Anderson, D. R.; Brideau, C.; Chauret, N.; Chern, R. T.; Chen, J.; Drag, M.; Fortin, R.; Hanson, P.; Haven, M.; Hickey, G. J.; McCann, M. E.; Patrick, D.; Wang, Z.-Y.; Young, R. N. *Drugs Future* **2002**, *27* (Suppl. A), 142.
21. Cheng, H. M.; Li, J.; Lundy, K. M.; Minich, M. L.; Sakya, S. M. WO 0140126, A1, 2001.
22. A detailed report in this regard will be published later.
23. Whole blood was collected by venal puncture into two tubes with and without heparin. All analogues 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 millilitres of whole blood without heparin was immediately added to the tubes with drug. Following incubation for an h at 37 °C, COX-1 activity was determined by measuring the thromboxane $B_2$ ($TXB_2$) synthesized from platelets, using an EIA kit. Samples without drug were included as controls for maximum production of $TXB_2$. Five hundred millilitres of heparinized blood was added to tubes containing drug and 10 μg/mL of LPS that stimulates production of $PGE_2$ for COX-2 activity. LPS and vehicle only controls were included 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 $PEG_2$).
24. 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 analogue scale (VAS) scoring system where 0 cm represents no lameness and 10 cm the most severe lameness. Animals were anesthetized during the injection procedure. Capsules of powdered compound were prepared to provide the following doses: **12a** at 4.0 mg/kg, carprofen at 4.4 mg/kg (crushed Rimadyl® tablet) or deracoxib at 2.0 mg/kg. Different letters over the error bars indicate statistical differences in the means ($p < 0.10$). The range of lameness scores in dogs that were not treated with a drug prior to knee injection fall between the parallel dashed lines above the bars at each time point.