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Pyridazinones as Selective Cyclooxygenase-2 Inhibitors

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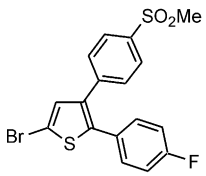
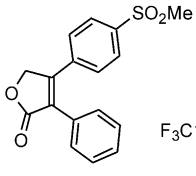
Abstract—Pyridazinone was found to be an excellent core template for selective COX-2 inhibitors. Two potent, selective and orally active COX-2 inhibitors, which were highly efficacious in rat paw edema and rat pyresis models, have been obtained.

Selective cyclooxygenase-2 (COX-2) inhibitors represent a new generation of anti-inflammatory drugs. In clinical trial, these compounds have demonstrated less gastrointestinal side effects than non-steroidal anti-inflammatory drugs (NSAIDs), which also inhibit the cytoprotective action of COX-1 in the GI tract.[1] During the last decade, several selective inhibitors based on Dup-697 have been reported.[2] A general modification of leads based on Dup-697 is the replacement of the central thiophene ring with other carbocylic or heterocyclic ring system. Five membered rings were frequently studied systems and were the most produtive area. Three compounds derived from five membered heterocycles have reached the market, such as furanone of rofecoxib,[3] pyrazole of celecoxib[4] and isoxazole of valdecoxib.[5] For the six-member ring systems, only benzene[6,7] and pyridine[7,8] were reported. The pyridine analogue etoricoxib[8] is now in advanced clinical trial. Herein, we describe our SAR studies on a new class of orally active COX-2 inhibitors based on the six-member heterocyclic pyridazinone system.



Dup-697



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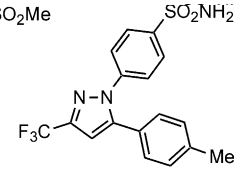
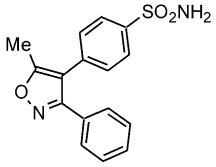
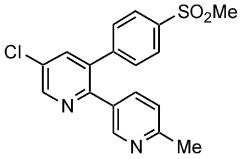


Celecoxib



Valdecoxib



Etoricoxib

The *N*-substituted pyridazinone analogue **3** could be readily synthesized from furanone **1.** Bromination of **1** followed by hydrolysis gave the 5-hydroxy intermediate **2**, which was subsequently reacted with an appropriately substituted hydrazine, or with hydrazine to give **3**. Alternatively, **3** can be obtained by reaction of **2** with hydrazine following by an alkylation reaction with the desired electrophile (Scheme 1). SAR at the 4-position of the pyridazinone was most conveniently investigated with the 4-bromo-substituted pyridazinone intermediate **8**, which can be obtained from 4-bromo-furan-2-one **4**[9] in 7 steps (Scheme 2). Suzuki coupling of **4** with 4-methylthiophenylboronic acid, followed by bromination and oxidiation yielded the 3-bromo-furan-2-one intermediate **5**. This intermediate was then converted to the pyridazinone intermediate **8** using chemistry as described in Scheme 1. Suzuki reaction of **8** with a boronic acid intermediate gave **3**. Substitution reaction of **8** with a nucleophile afforded **9**.

The COX-2 inhibition was determined in stably transfected chinese hamster ovary (CHO) cells expressing human COX-2[10] as well as a human whole blood (HWB) COX-2 assay.[11] The COX-1 inhibition was evaluated with a sensitive U-937 microsomal COX-1

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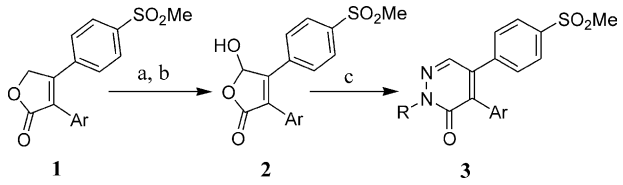
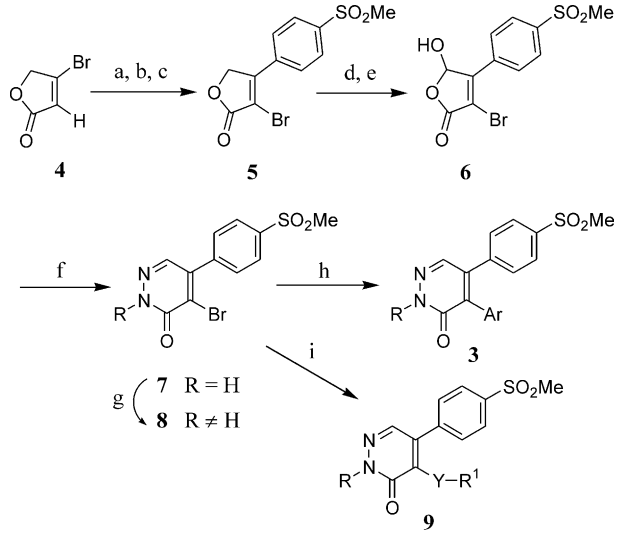


Scheme 1. (a) NBS; (b) HOAc, THF-H_2O, ~45% for 2 steps; (c) $RNHNH_2$ or (i) NH_2NH_2; (ii) R-X, NaOH, DMF, 30–75%.



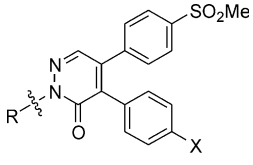
Scheme 2. (a) 4-methylthiophenylboronic acid, $PdCl_2(PPh_3)_2$, Na_2CO_3, benzene, EtOH, quantitative; (b) Br_2, CH_2Cl_2; (c) MMPP, 79% for 2 steps; (d) NBS; (e) HOAc, THF-H_2O; 73% for 2 steps; (f) NH_2NH_2, 84%; (g) base, R-X; (h) Ar-$B(OH)_2$, Pd(0); (i) K_2CO_3 or Cs_2CO_3, R^1-YH, DMF, 8–71%.

assay[12] at low arachidonic acid concentration. Selected compounds were also tested in the rat paw edema[13] and rat pyresis[13] assays for their in vivo efficacies.

Various *N*-substituted analogues were initially prepared to evaluate the effect of *N*-substitution (Table 1). It was very clear that *N*-substitution was absolutely required for good in vitro COX-2 inhibitory potency since the unsubstituted analogue **3a** was not potent. An increase in size of the nitrogen substitutent such as **3b**, **3c** and **3d** improved COX-2 inhibitory potency, especially in the human whole blood assay. However, a tertiary alkyl susbstituent such as *tert*-butyl in **3e** was not tolerated. Cyclopropylmethyl substituent **3f** and benzyl substitutent **3g**, which exhibited excellent COX-2 potency and selectivity, have the potential for further optimization. A wide range of other large alkyl, alkenyl (**3h**), cycloalkyl (**3i**), and substituted benzyl substituents have also been investigated. Many of them retained COX-2 inhibitory potency and reasonable selectivity, but none showed properties that were clearly superior to **3f** and **3g**. Heterocyclic substituents (**3j**, **3k** and **3l**), except for the 2-thienylmethyl analogue **3l**, were less potent. Compound **3l**, somehow suffered from the loss of selectivity and poor pharmacokinetic. Substitution at the 6 position of the pyridazinone template (**3m**), surprisingly abolished the human whole blood COX-2 inhibitory potency.

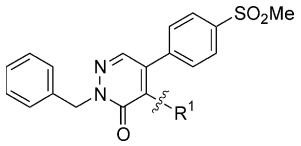
Preliminary pharmacokinetic studies in rats indicated that *N*-benzyl derivatives showed poorer pharmacokinetic than the corresponding *N*-cyclopropylmethyl derivatives. For example, compound **3g** has a C_{max} of 1 μM at 20 mg/kg oral dosing, while the corresponding cyclopropylmethyl analogue **3f** has a C_{max} of 2.2 μM. One of the key objectives in the *N*-benzyl series was then to improve the pharmacokinetic and maintain the COX-2 inhibitory potency and selectivity. This can be achieved by varying substitutents at position 4. Several analogues based on experiences with other templates were synthesized (Table 2). Compound **10** with the celecoxib 4-methylphenyl substituent showed moderate improvement in the human whole blood COX-2 inhibitory potency as well as oral plasma level. Compound **10** has good in vivo efficacy in the rat paw edema assay (ED_{50} = 2.2 mg/kg), but the compound was not potent

Table 1. In vitro activity of *N*-substituted analogues



Compd	X	R	COX-2 IC_{50} (μM)		COX-1 IC_{50} (μM)
			CHO	HWB	U-937
3a	H	H	> 5	> 33	> 10
3b	H	Me	0.3	21	> 10
3c	H	2,2,2-Trifluoroethyl	0.5	11	> 10
3d	H	Phenyl	0.08	4.7	> 10
3e	H	*tert*-Butyl	> 5	> 33	> 10
3f	H	Cyclopropylmethyl	0.07	1.3	> 10
3g	H	Benzyl	0.03	0.9	~10
3h	F	3-Methyl-2-butenyl	0.1	0.3	1–3
3i	F	Cyclohexylmethyl	0.04	0.41	0.3–1
3j	H	3-Pyridylmethyl	1.3	7.1	~10
3k	F	2-Thiazoylmethyl	0.6	5.9	~10
3l	F	2-Thienylmethyl	0.05	< 0.41	~1
3m	H	Benzyl (6-Me)	0.35	> 33	> 10

Table 2. In vitro and in vivo activity of *N*-benzyl analogues

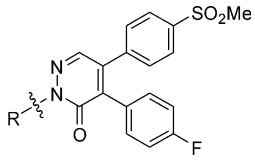


Compd	R^1	COX-2 IC_{50} (μM)		COX-1 IC_{50} (μM)	C_{max} (μM)	Rat paw ED_{50}, mg/kg	Rat pyresis ED_{50}, mg/kg
		CHO	HWB	U-937	20 mg/kg, PO		
3g	Phenyl	0.03	0.9	>10	1.0		
10	4-Methylphenyl	0.02	0.35	>10	2.7	2.2	>10
11	2-Methyl-5-pyridyl	1.2	1.1	>10	4.2	>10	
12	4-Fluorophenoxy	0.02	0.09	0.3–1		>10	
13	5-Chloro-2-pyridyloxy	0.26	0.9	3–10	0		
14	Isopropoxy	0.02	1.7	>10	2.8	<0.3	<0.3
15	Cyclopropylmethoxy	0.003	0.36	~10	0.8	>10	
16	Phenylthio	0.37	5.9	3–10			

in the rat pyresis assay (ED_{50} > 10 mg/kg). Compound **11** with the etoricoxib 2-methyl 5-pyridyl substituent was moderately potent in the CHO COX-2 assay and the compound was not shift in the HWB COX-2 assay, but this compound was not efficacious in the rat paw edema assay (ED_{50} > 10 mg/kg). We have demonstrated in the furanone series that an oxygen linked or sulfur-linked substituents would provide COX-2 inhibitors with excellent potency and selectivity. Indeed, all the oxygen-linked derivatives **12**, **13**, **14** and **15** were potent and selective COX-2 inhibitors. Unfortunately, the 4-fluorophenoxy compound **12** was not efficacious in vivo (ED_{50} > 10 mg/kg in the rat paw edema assay) and the 5-chloro-2-pyridyloxy compound **13** showed very poor pharmacokinetic in rats. On the other hand, the alkoxy derivatives **14** and **15** showed moderate oral plasma levels with C_{max} of 2.8 μM for **14**, 0.8 μM for **15**. While compound **14** was only moderately potent in the HWB COX-2 assay, the in vitro activity of **14** translated extremely well into excellent in vivo activity in the rat paw edema (ED_{50} < 0.3 mg/kg) and rat pyresis (ED_{50} < 0.3 mg/kg) assays. Surprisingly, compound **15** was not potent in the rat paw edema assay (ED_{50} > 10 mg/kg) and the poor oral plasma level might be partly responsible for the lack of in vivo activity. The sulfur-linked derivative **16** was not very potent in the human whole blood COX-2 assay. Overall, the isopropoxy analogue **14** represents the most promising compound in the *N*-benzyl series of pyridazinone selective COX-2 inhibitors.

The *N*-cyclopropylmethyl series was usually less potent than the corresponding *N*-benzyl series in the COX-2 assays. Our initial observation suggested that increasing the size of the nitrogen substituent might improve the COX-2 potency. Several substituted *N*-cyclopropyl-methyl analogues[14] were subsequently prepared (Tables 3 and 4). Although most of these modifications maintained or indeed slightly improved the COX-2 potency, pharmacokinetics of these derivatives seemed to highly depend on the substitution patterns. Extension or reduction of the linker between the cyclopropyl ring and the nitrogen led to compounds with reduced COX-2 potency (**18** and **19**). The (1-methylcyclopropyl)methyl analogue **20**, (2,2-dimethylcyclopropyl)methyl analogue **21**, (2,3-dimethylcyclopropyl)methyl analogue **22** and the (*cis*-2-methylcyclopropyl)methyl analogue **23,** though were potent and moderately selective, they showed no oral plasma levels in rats. The α-methyl substituted analogue **24** has excellent plasma levels in

Table 3. In vitro activity of *N*-cyclopropylmethyl analogues



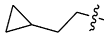
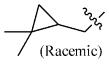
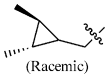
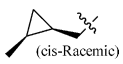
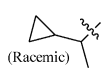
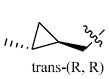
Compd	R	COX-2 IC_{50} (μM)		COX-1 IC_{50} (μM)
		CHO	HWB	U-937
17		0.2	1.7	>10
18		0.2	2.9	>10
19		0.11	>33	~10
20		0.2	0.7	3–10
21	 (Racemic)	0.03	0.8	1–3
22	 (Racemic)	0.05	0.7	1–3
23	 (cis-Racemic)	0.05	2.4	3–10
24	 (Racemic)	0.2	0.7	3–10
25	 trans-(R, R)	0.06	1.7	3–10
26	 trans-(S, S)	0.06	0.3	~3

Table 4. Plasma levels and in vivo activity of *N*-cyclopropylmethyl analogues

Compd	C_{max} (μM)	Rat paw	Rat pyresis
	20 mg/kg, PO	ED_{50}, mg/kg	ED_{50}, mg/kg
17	8.5	3–10	
24	13	1.8	>10
25	4.8	40% @ 10	
26	16	0.7	1.1

rats and showed a C_{max} of 13 μM. Compound **24** has good in vivo activity in the rat paw edema assay (ED_{50} = 1.8 mg/kg), but it was not potent in the rat pyresis assay (ED_{50} > 10 mg/kg). Interestingly, the two optically pure (*trans*-2-methylcyclopropyl)methyl analogues (*R*,*R*)-**25** and (*S*,*S*)-**26** showed 6-fold difference in the HWB COX-2 potency although both compounds have similar CHO COX-2 activity. While the racemic *cis* isomer **23** has poor oral plasma level in rats, the *trans*-(*S*,*S*)-**25** and the *trans*-(*R*,*R*)-**26** both showed decent oral plasma levels[15] with a C_{max} 4.8 μM for **25** and 16 μM for **26**. Compound **25** was not potent in the rat paw edema assay (40% @ 10 mg/kg). Compound **26** on the other hand showed excellent in vivo activity in the rat paw edema assay (ED_{50} = 0.7 mg/kg) and the rat pyresis assay (ED_{50} = 1.1 mg/kg). Compound **26** is so far the optimal compound in the *N*-cyclopropylmethyl series of pyridazinone selective COX-2 inhibitors.

In conclusion, two potent and selective COX-2 inhibitors **14** and **26** have been identified from the pyridazinone template. These two compounds also showed excellent efficacy in animal models of anti-inflammation, the rat paw edema and rat pyresis assays.

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14. The chiral cyclopropylmethyl alcohols for **25** and **26** were prepared according to Charette's condition: Charette, A. B.; Prescott, S.; Brochu, C. *J. Org. Chem.* **1995**, *60*, 1081. Other substitutents were eithier commercially available or prepared from the corresponding olefins.
15. The oral samples were prepared by the addition of 0.5 mL of a PEG 200 solution of compounds to 9.5 mL of 1% methocel under vigorously stirring. Otherwise, plasma levels would be expected much lower (e.g., racemic **26** has a C_{max} = 2.4 μM).