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1,3-Diarylcycloalkanopyrazoles and Diphenyl Hydrazides as Selective Inhibitors of Cyclooxygenase-2

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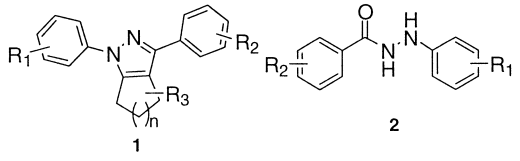
Abstract—Novel 1,3-diarylcycloalkanopyrazoles **1**, and diphenyl hydrazides **2** were identified as selective inhibitors of cyclooxygenase-2. The 1,3-diaryl substitution pattern of the pyrazole ring in **1** differentiates these compounds from most of the known selective COX-2 inhibitors that contain two aryl rings at the adjacent positions on a heterocyclic or a phenyl ring. Similarly, the two phenyl rings in **2** are also separated by three atoms. SAR of both phenyl rings in **1** and **2**, and the aliphatic ring in **1** will be discussed.

The principal pharmacological effects of nonsteroidal anti-inflammatory drugs (NSAIDs) are due to their ability to inhibit prostaglandin synthesis by blocking cyclooxygenase activities.[1–7] The gastrointestinal (GI) and renal side effects of NSAIDs, however, frequently limit their therapeutic use. These side effects were believed to be inseparable from the pharmacological effects since prostaglandins have cytoprotective effects in the gastrointestinal tract and also regulate renal blood flow. The discovery of two isozymes, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), that catalyze the second step in prostaglandin synthesis has provided the possibility of separating the pharmacological effects from the side effects of NSAIDs. Research results suggest that COX-1 and COX-2 belong to separate prostaglandin-forming systems. COX-1 is expressed constitutively in most cells and tissues.[8–10] The COX-1-dependent pathway can respond instantaneously and produces prostaglandins that regulate acute events such as vascular homeostasis. The synthesis of prostaglandins by COX-1 also helps maintain normal stomach and renal function. COX-2 is only expressed following mitogenic or inflammatory stimuli. Since it is not expressed in most resting tissues and must be induced, prostaglandins produced by COX-2 are probably involved only secondarily in chronic physiological reactions. This discovery suggests that the gastrointestinal and renal side effects of the nonsteroidal antiinflammatory drugs might be avoided.

Recently, many COX-2 selective inhibitors have been reported, including Celebrex and Vioxx, the first COX-2 inhibitors on the market. The pharmacophore for most of these inhibitors consists of a central template, most commonly cyclic (five- or six-membered, carbocyclic or heterocyclic), which is 1,2-disubstituted by aryl moieties. We wish to report that the 1,3-disubstitution pattern, such as in structures **1** and **2**, is also an effective pharmacophore for selective COX-2 inhibition.[11]



Compounds **1** were synthesized in two ways. The first synthetic route is based on conventional 1,3-dipolar cycloaddition chemistry (Scheme 1).[12] Thus, compounds **2** were prepared from the corresponding benzoic acids by standard procedures and transformed to α-chlorohydrazones with phorsphorus pentachloride. Cycloaddition of the nitrileimines generated from **3** to cycloalkenes **4** gave the precursors **5** which underwent oxidation to provide the desired products **1**. The second method involves the condensation of β-diketones **8** with arylhydrazines. The intermediates **8** were prepared by

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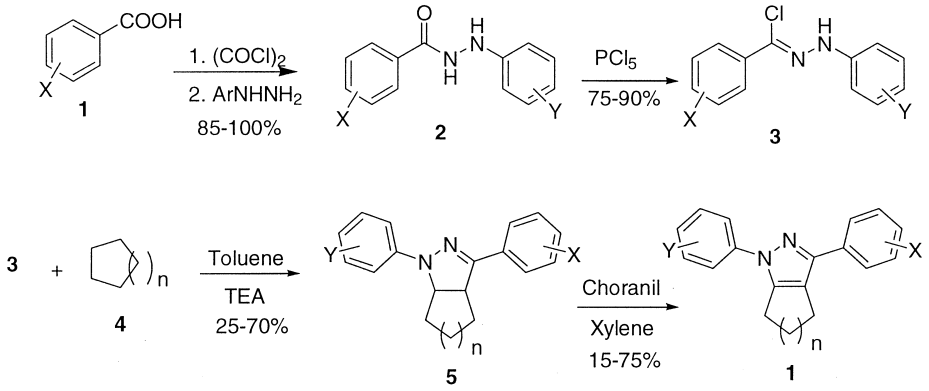


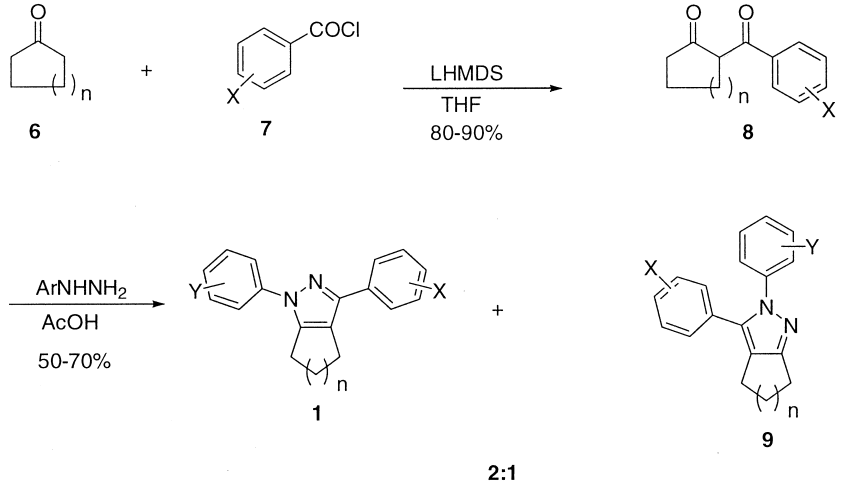
Scheme 1.

reactions of cycloalkanones **6** with benzoyl chlorides **7** using similar procedures reported by our laboratory (Scheme 2).[13] Both regioisomers were isolated in the condensation reactions. The activities of the regioisomer with the adjacent diaryl substitution (**9**) will be discussed elsewhere.

In general, the cycloalkanopyrazoles possess potent inhibitory activity against COX-2 and are selective versus COX-1 except when both phenyl rings are unsubstituted (Table 1). Seven-membered cycloalkanes ($n=3$) were most potent against COX-2, followed by the six-membered ring compounds. Five-membered cycloalkanes were the least active series (IC_{50}: **1a** > **1d** > **1h**, **1b** > **1e** > **1i**). While substitution of the A-ring with chloro at the 4-position did not significantly change the activity against COX-2 (**1b**, **1e** and **1i**), an additional substitution with chloro at 4-position of B-ring abolished activity (**1c** and **1j**). The most common combination of substituents of 1,2-diarylsubstituted selective COX-2 inhibitors in the literature,[14] Cl/$MeSO_2$ or F/$MeSO_2$, did not show inhibition in the 1,3-diaryl compounds synthesized in our study (**1f**, **1g** and **1n**). When X = Cl, Y = Me or OMe, the compounds (**1k** and **1m**) showed potent COX-2 activity and moderate selectivity versus COX-1. In addition, none of the dihydropyrazole precursors (**5**) showed inhibitory activity against COX-2.

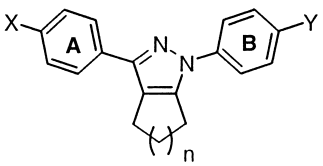
Furthermore, we studied the SAR of the alkane ring modifications. A series of 5-substituted cyclohexanopyrazoles were synthesized from the 5-keto precursor. As shown in Table 2, substitution of the cyclohexane ring with fluoro did not change the activity profile (**1q**). Replacing the fluoro with OH increased the activity against COX-2 and hence enhanced the selectivity (**1r**). While substitution with *N*-acetylhydroxylamino did not significantly alter the activity, *O*-acetylhydroxylamino abolished the activity against COX-2 (**1s** and **1t**).

Surprisingly, the diphenylhydrazides, intermediates for the synthesis of the cycloalkanopyrazoles, showed potent inhibition of COX-2 and are also selective versus COX-1 (Table 3). The comparative SAR of the diphenylhydrazides to that of the cycloalkanopyrazoles suggests that the two series share similar binding mode. Analogous to the cycloalkanopyrazole series, substitution on one or both phenyl rings with chloro, methyl or methoxy generally provided potent and selective COX-2 inhibitors (except **2m**). When methylsulfonyl is present on B-ring, the activity was abolished. The best compound in



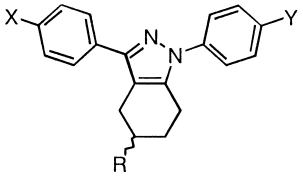
Scheme 2.

Table 1. Inhibitory activities of representative compounds against COX-1 and COX-2



Cmpd	X	Y	*n*	COX-2[15] IC_{50} (μM)	COX-1[16] IC_{50} (μM)
1a	H	H	1	6.20	>10
1b	Cl	H	1	2.59	10
1c	Cl	Cl	1	>10	>10
1d	H	H	2	0.57	0.49
1e	Cl	H	2	0.62	10
1f	Cl	SO_2Me	2	>10	>10
1g	F	SO_2Me	2	>10	>10
1h	H	H	3	0.17	0.12
1i	Cl	H	3	0.14	1.0
1j	Cl	Cl	3	10	>10
1k	Cl	Me	3	0.64	>10
1m	Cl	OMe	3	1.56	>10
1n	Cl	SO_2Me	3	>10	10
1p	OMe	H	3	1.24	10

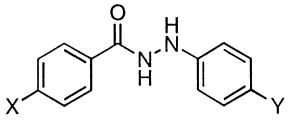
Table 2. Examples of cycloalkane ring modifications



Cmpd[a]	X	Y	R	COX-2 IC_{50} (μM)	COX-1 IC_{50} (μM)
1m	Cl	OMe	H	1.56	>10
1q	Cl	OMe	F	2.10	>10
1r	Cl	OMe	OH	0.46	>10
1s	Cl	OMe	-N(OH)Ac	2.40	>10
1t	Cl	OMe	-NHOAc	>10	>10

[a]Compounds **1q**–**1t** are racemic.

Table 3. Inhibitory activities of representative hydrazides against COX-1 and COX-2



RWJ	X	Y	COX-2 IC_{50} (μM)	COX-1 IC_{50} (μM)
2a	H	H	10	>10
2b	Cl	H	0.33	>10
2c	Cl	Cl	0.34	>10
2d	H	Me	0.33	>10
2e	H	Cl	0.53	>10
2f	H	SO_2Me	>10	>10
2g	H	OMe	0.18	>10
2h	Cl	Me	0.01	>10
2i	Cl	OMe	0.13	>10
2j	Cl	SO_2Me	>10	>10
2k	OMe	Cl	0.47	>10
2m	OMe	H	>10	>10

this series (**2h**) has an IC_{50} of 10 nM against COX-2 and greater than 1000 fold selectivity versus COX-1.

In summary, 1,3-diarylpyrazoles fused with a cycloalkane were identified as selective COX-2 inhibitors. SAR revealed the importance of the size and substitution of the cycloalkane ring. *N*′-Phenylarylhydrazides were also found to be potent and selective COX-2 inhibitors. The best compound **2h** has an IC_{50} of 10 nM against COX-2 and >10,000 nM against COX-1. The two phenyl rings displayed distinct SARs from those in 1,2-diphenyl substituted analogues found in most of the selective COX-2 inhibitors reported in the literature. The results indicate that the 1,3-diarylsubstitution pattern is an alternative pharmacophore to the more common 1,2-diarylsubstitution pattern. The fact that none of the dihydro precursors (**5**) demonstrates activity indicates the importance of the planarity of the designed compounds.

Acknowledgement

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15. Human cyclooxygenase type II whole cell assay: ECV-304 (human, endothelial, umbilical cord) cells are maintained in culture in a suitable medium, and are trypsinized and plated at a density of 9×10^4 cells per well of a 96 well plate prior to assay. Approximately 28 hours later, 50 μg/mL PMA and 1 μM ionomycin (both final concentrations) are added to each well. Approximately 24 hours later, cells are incubated in the presence of vehicle or drug and the presence of PGE_2 is determined via RIA after the addition of 30 μM arachidonic acid. The % inhibition of products as compared to vehicle is calculated.

16. Human cyclooxygenase type I whole cell assay: Peripheral blood is drawn in heparinized tubes and platelet-rich plasma (PRP) collected by centrifugation ($200\times g$) for 20 min at room temperature. The PRP is removed and diluted 1:20 in 0.9% NaCl. Test compound or vehicle is added to a 180 μL aliquot in a 96-well plate. After a 15 min incubation at 37 °C, 27 μM calcium ionophore A-23187 (free acid) is added and the samples are incubated at 37 °C for another 15 min. Following the incubation, the samples are placed on ice for 5 min. An aliquot of the supernatant is assayed for the presence of thromboxane B_2 by standard radioimmunoassay techniques.