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STRUCTURAL OPTIMIZATION OF ALKYL-SUBSTITUTED 2-(7-5*H*-[1]BENZOPYRANO[2,3-*b*]PYRIDYL)PROPIONIC ACID AS ANTI-INFLAMMATORY AGENTS

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Abstract: Several alkyl-analogues of pranoprofen were synthesized and their cyclooxygenase inhibitory activities were evaluated in a screening test. The chosen compounds were analyzed on the relationships between structure, lipophilicity (log P) and cell binding affinity. 2-Methyl analogue (**4b**) was confirmed to be an optimal compound in this series.

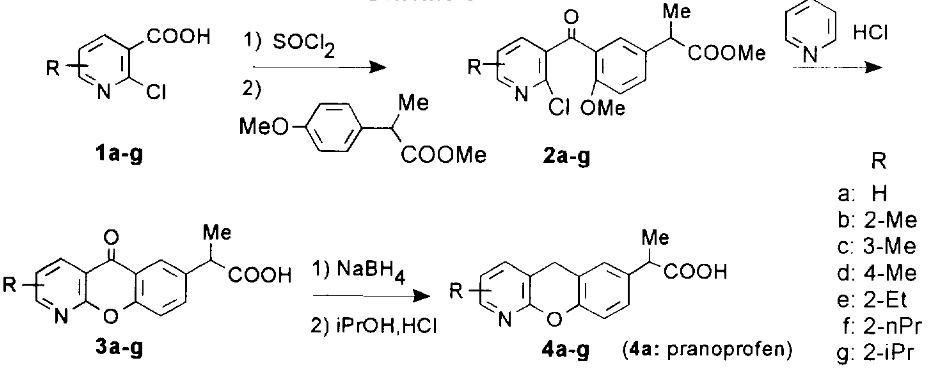
In the presence of cyclooxygenase (COX), arachidonic acid is converted to oxyeicosanoids including prostaglandins. Among them, prostaglandin E_2 (PGE_2) is believed to be responsible for pathophysiological processes of inflammation, algesia and pyrexia. Nonsteroidal anti-inflammatory drugs (NSAIDs) is confirmed to inhibit the biocatalytic action of COX. Thus, such an inhibitory activity to COX has been regarded as one of the indications for the pharmacological evaluation of NSAIDs[1]. There is a couple of COX isozymes, namely COX-1 and COX-2, and they are differentially sensitive to NSAIDs[2].

It is also known that membrane permeability of drugs depends on their lipophilicity. The lipophilicity can usually be represented by the partition coefficient (log P). In the structural optimization of NSAIDs, therefore, the log P value should be an important parameter because these drugs can interact with COX only in the interior of the inflammatory cells e.g. polymorphonuclear leukocytes (PMNLs). In addition, the penetration rate of NSAIDs into the PMNLs should depend not only on the lipophilicity of these drugs but also on their binding ability with intracellular COX. However, the relationships between such three parameters have not yet been investigated intensively on NSAIDs.

These situation prompted us to reexamine the adequacy of structural optimization on pranoprofen[3], 2-(7-5*H*-[1]benzopyrano[2,3-*b*] pyridyl)propionic acid, and its analogues. Here, pranoprofen is one of NSAIDs marketed in Japan. In order to enhance the lipophilicity of pranoprofen, we adopted to introduce one alkyl substituent onto its pyridine ring. In a screening stage of test, the synthesized compounds were evaluated on their inhibitory activities to COX-1 at enzyme level. Then, the log P value and penetration rate into the PMNLs in vitro were determined for each selected compound, and the results were plotted on graph. The test compound that occupied a maximum point in the graph was tentatively regarded as an optimal compound in the pranoprofen series. Instead of the PMNLs, the erythrocytes, which never contain COX, were used in a control experiment.

Various alkylated derivatives (**4**) were prepared according to the procedure[4,5] already reported for pranoprofen (**Scheme 1**). Alkylated-2-chloro-3-pyridinecarboxylic acids **1** were converted to acid chlorides and the intermediated chlorides were treated with methyl (4-methoxyphenyl)propionate under a condition of Friedel Crafts reaction to form compounds **2**. In the presence of pyridine hydrochloride, **2** were concertedly demethylated and cyclized to afford compounds **3**. Carbonyl groups of **3** were reduced by usual manner and the intermediated carbinols were reductively dehydroxylated to give the desired compounds **4** in practical overall yields.

Scheme 1



All the synthesized compounds listed in **Table 1** were tested for their inhibitory activity to COX-1 at enzyme level. In comparison with pranoprofen, **4b** exhibited an extremely improved activity, nevertheless both **4c** and **4d** showed diminished activity. These results suggest a pharmacological superiority of 2-alkyl analogues of pranoprofen. We therefore selected compounds **4b**, **4e**, **4f** and **4g** as candidates for further investigations. The log P values of **4a**, **4b**, **4e**, **4f** and **4g** were shown in **Table 1**. The rates of binding to both the PMNLs and the erythrocytes were measured respectively for these five compounds. In the case of erythrocytes, which never

Table 1. log P value and Inhibitory activity to COX-1

Entry	R	log P[a]	COX-1[b] IC_{50} (μM)
4a	H	-0.72	3.1
4b	2-Me	-0.11	0.32
4c	3-Me	0.03	11
4d	4-Me	-0.17	>100
4e	2-Et	0.45	0.85
4f	2-nPr	1.08	>100
4g	2-iPr	0.67	>100

a. Measured (n-octanol / pH 7.4 phosphate buffer)
b. COX-1 assay in enzyme level was performed according to the method of Futaki et al[6]. Briefly, COX-1 purified from ram seminal vesicles was incubated with arachidonic acid in the presence of various concentrations of compounds and PGE_2 produced was determined by enzyme immunoassay. IC_{50} value was calculated using percentage inhibitions for different concentrations of compounds by probit analysis.

contained COX-1, the rate of binding increased with the log P value as expected, and the membrane permeability of these five compounds was directly proportional to their physicochemical parameter (**Figure 1**). With respect to the PMNLs, however, the rate of binding showed significantly a second order relationship with the log P value, and **4b** occupied a maximal point in the negative parabola (**Figure 2**). Such an phenomenon can reasonably explained by posturating the participation of a specific binding of these compounds with COX-1 or cell membrane of the PMNLs. Thereby, we tentatively singled out **4b** as the optimized compoud to be examined still more.

Figure 1. Relationship between log P of 2-alkyl analogues and erythrocytes binding

$\text{fmol} = 76.8(\log P) + 140$
$R^2 = 0.644$

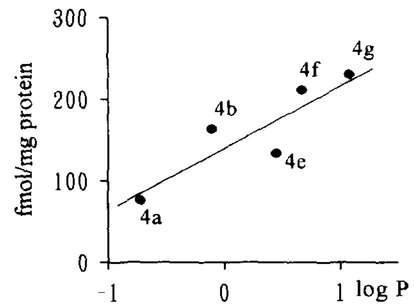
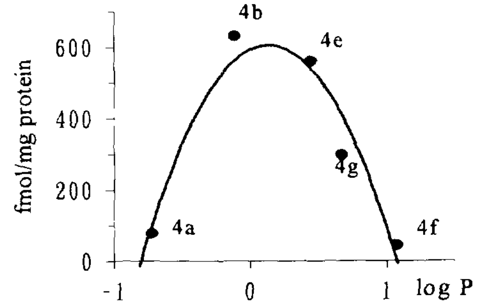


Figure 2. Relationship between logP of 2-alkyl analogues and PMNLs specific binding

$\text{fmol} = -685(\log P)^2 + 186(\log P) + 592$
$R^2 = 0.927$

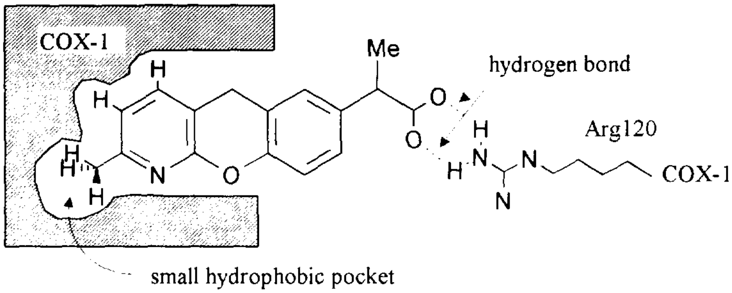


The rat PMNLs were harvested from the abdominal cavity according to the method of Smith[7]. The washed PMNLs and erythrocytes were suspended in HANKS solution. The suspension containing 5×10^7 cells for the PMNLs and 1×10^{11} cells for the erythrocytes were incubated with test compounds (final concentration 30 nM) for 10 min at 37℃. After incubation, cells were rapidly separated from medium by centrifugation at 4℃ for 5 min at 3000 rpm. Concentrations of compounds in the supernatant were determined by high performance liquid chromatography method[8] and the amount of cell associated compounds was calculated. Protein content of cells was determined by the Lowry[9] method using bovine serum albumin as a standard. The PMNLs specific binding was calculated according to the equation ; S = % bound at 37 ℃ - % bound at 4 ℃

Next, we determined the COX-1 inhibitory activity of **4b** comparing with those of **4a** and **4e** in cell level according to the method of Di Rosa et al[10]. Briefly, the rat peritoneal leukocytes were incubated with killed bacteria (*Bordetella pertussis*) in the presence of **4a**, **4b** or **4e** at various concentrations and PGE_2 produced was determined by enzyme immunoassay. The activity of each test compound was indicated as IC_{50} value. Confirming of our structural optimization *loc, cit.*, the activity of **4b** (IC_{50}: 0.03 μM) was significantly superior to those of **4a** (IC_{50}: 0.39 μM) and **4e** (IC_{50}: 0.11 μM).

Methyl substitution on the pyridine ring (**4b, 4c** and **4d**) caused dramatically different activity in enzyme level. All substitutions result in almost same effects on the value of log P (**Table 1**). Furthermore, propyl substitution at the 2 position (**4f** and **4g**) markedly decreased inhibitory activity (>100 μM) and increased the value of log P from -0.72 to 1.08 and 0.67 respectively, which were still lower than that of diclofenac (1.47). Considering the circumstance around the pyridine ring in this series docked into COX-1, these results suggest that a small hydrophobic pocket exists around the 2 position of the pyridine ring and no extra space exists around the 4 position (Figure 3). This hypothesis is consistent with the crystal structure[11] of COX-1 complexed with an inhibitor (flurbiprofen), which occupies a middle point of a long and narrow channel (8 x 25 Å) Pranoprofen and its analogues would bind to the active site of COX-1 as a same manner as flurbiprofen, for both compounds resemble each other in functional structures, propionic acid and two aromatic rings. (A long and narrow channel of the active site restricts a shape of inhibitors to slim rectangle.)

Figure 3. Proposed interactions between COX-1 and compound 4b



In the structural optimization of NSAIDs, the quantitative analysis of relationships between the structure, the log P value and the rate of binding with the PMNLs should supply an alternative methodology. By such a method, compound **4b** was selected as an optimal candidate for further pharmacological evaluations. A prodrug of **4b** (*N,N*-dimethylcarbamoylmethyl ester) was synthesized[12] to reduce its gastrointestinal side effects and is now under phase Ⅲ trial.

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