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Properties and Synthesis of 2-{2-Fluoro (or Bromo)-4-[(2-oxocyclopentyl)methyl]phenyl}propanoic Acid: Nonsteroidal Anti-inflammatory Drugs with Low Membrane Permeabilizing and Gastric Lesion-Producing Activities

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We previously proposed that membrane permeabilization activity of NSAIDs is involved in NSAID-induced gastric lesions. We here synthesized derivatives of loxoprofen that have lower membrane permeabilization activity than other NSAIDs. Compared to loxoprofen, the derivatives **10a** and **10b** have lower membrane permeabilization activity and their oral administration produced fewer gastric lesions but showed an equivalent anti-inflammatory effect. These results suggest that **10a** and **10b** are likely to be therapeutically beneficial as safer NSAIDs.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs[a]) are a useful family of therapeutics, accounting for nearly 5% of all prescribed medications.[1] Their inhibitory effect on cyclooxygenase (COX) activity and resulting decrease in prostaglandins (PGs) such as PGE_2 has been shown to be responsible for their anti-inflammatory actions. On the other hand, NSAID use is associated with gastrointestinal complications, with about 15−30% of chronic users of NSAIDs suffering from gastrointestinal ulcers and bleeding.[2−4]

In 1991, two subtypes of COX, COX-1, and COX-2, which are responsible for the majority of COX activity at the gastrointestinal mucosa and in tissues with inflammation, respectively, were identified,[5,6] and a greatly reduced incidence of gastroduodenal lesions has been reported for selective COX-2 inhibitors (such as celecoxib and rofecoxib).[7−9] However, a recently raised issue concerning the use of selective COX-2 inhibitors is their potential risk for cardiovascular thrombotic events.[10,11] This may be due to the fact that prostacyclin, a potent antiaggregator of platelets and a vasodilator, is mainly produced by COX-2 in vascular endothelial cells, while thromboxane A_2, a potent aggregator of platelets and a vasoconstrictor, is mainly produced by COX-1 in platelets.[12−14] Because of this concern, rofecoxib was withdrawn from the worldwide market. Therefore, NSAIDs exhibiting gastrointestinal safety, other than selective COX-2 inhibitors, need to be developed.

Although PGE_2 has a strong protective effect on the gastrointestinal mucosa, it is now believed that the inhibition of COX by NSAIDs is not thought to be the sole explanation for the gastrointestinal side effects of NSAIDs.[15] We have recently suggested that COX-independent NSAID-induced cell death is also involved in NSAID-induced gastric lesions and that this direct cytotoxicity of NSAIDs is due to their membrane permeabilization activity.[16,17] Thus, we proposed that NSAIDs with lower membrane permeabilization activity would be safer on stomach tissue even without the selectivity for COX-2.[17]

Loxoprofen has been used clinically for a long time as a standard NSAID in Japan, and clinical studies have suggested that it is safer than other NSAIDs, such as indomethacin.[18,19] Loxoprofen is a pro-drug, which is converted (by reduction of the cyclopentanone moiety) to its active metabolite (the *trans*-alcohol metabolite of loxoprofen) by aromatic aldehyde-ketone reductase only after absorption by the gastrointestinal tract.[20] We recently reported that loxoprofen has relatively lower membrane permeabilization activity than other NSAIDs.[21] In this study, we synthesized a series of its derivatives and examined their membrane permeabilization, ulcerogenic and anti-inflammatory activities.

Results and Discussion

We considered that loxoprofen sodium (**1**) (Scheme 1) could be a lead compound to obtain NSAIDs with even lower membrane permeabilization activity, and on this basis, we synthesized a series of its derivatives, including the position isomer which is chemically modified at an aromatic ring of lead structure by halogens or modified aryl groups (Chart 1). We then examined their membrane permeabilization activities and selected two compounds, fluoro loxoprofen (**10a**) and bromo loxoprofen (**10b**). The synthesis of these compounds is outlined in Scheme 1 (experimental details are available in Supporting Information).

We previously established an assay system for assessing the membrane permeabilization activity of NSAIDs using calcein-loaded liposomes. Calcein fluorescence is very weak at high concentrations due to self-quenching, so the addition of

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[a]Abbreviations: COX, cyclooxygenase; EIA, enzyme immunoassay; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin.

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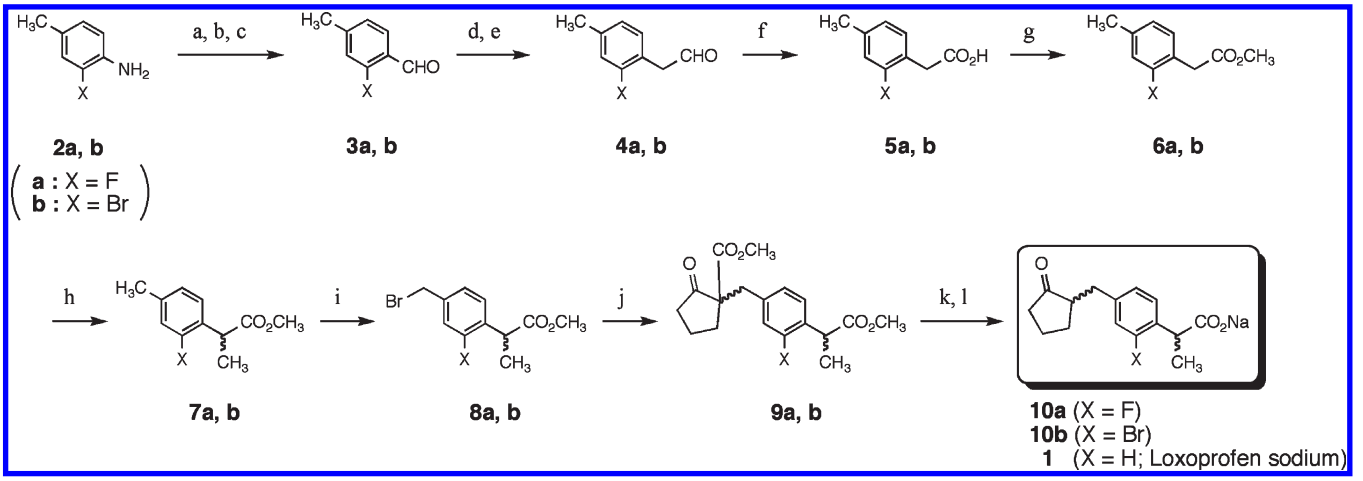
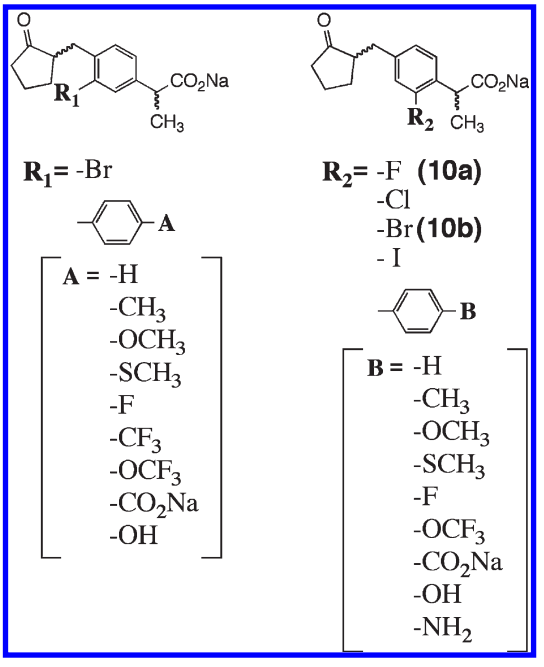
Scheme 1. Synthesis of Halogeno-Loxoprofen Derivatives (**10a**,**b**)[a]



[a] Reagents and conditions: (a) 3 m HCl aq, $NaNO_2$, $CuSO_4$, Na_2SO_3, AcONa, H_2O, 0 °C; (b) $NH_2OH \cdot HCl$, $(HCHO)_n$, AcONa, H_2O; (c) conc HCl, reflux; (d) $MeOCH_2P(Ph_3)Cl$, $C_6H_{18}KNSi_2$, toluene; (e) 3 M HCl aq, acetone, reflux; (f) PFC (2 mol %), H_5IO_6, acetonitrile; (g) conc HCl, CH_3OH, reflux; (h) 2.0 M LDA, CH_3I, dry THF, −78 °C to −40 °C; (i) NBS, AIBN, CCl_4, reflux; (j) dry Na_2CO_3, methyl 2-oxocyclopentanecaroxylate, dry acetone, reflux; (k) conc HCl, AcOH, reflux; (l) 1 M NaOH aq, C_2H_5OH, reflux.

Chart 1. Synthesized Loxoprofen Derivatives



membrane-permeabilizing drugs to a medium containing calcein-loaded liposomes causes an increase in fluorescence by diluting the calcein.[17] Using this assay system, we compared the membrane permeabilization activity of **10a** and **10b** to **1**. Neither **10a** nor **10b** apparently released calcein into the medium at concentrations less than 1 M (Figure 1), showing that these compounds have much lower membrane permeabilization activity than **1**.

We then examined their inhibitory effects on COX-1 (or COX-2), using a human whole blood COX assay. As shown in Table 1, both **10a** and **10b** showed IC_{50} values for COX-1-derived PG biosynthesis that are similar to **1**. For COX-2-derived PG biosynthesis, **10a** showed a similar result, but **10b** showed a little weaker inhibitory effect compared to that of **1**. These results suggest that, as for **1**, **10a** and **10b** did not exhibit apparent selectivity for COX-2 (The COX-1/COX-2 value of

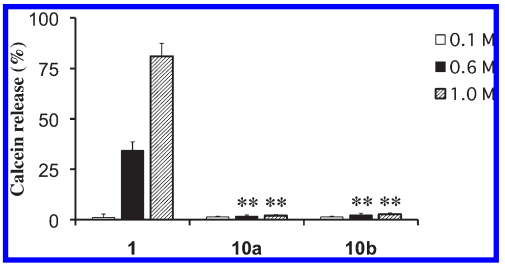


Figure 1. Membrane permeabilization by loxoprofen sodium and its derivatives. Calcein-loaded liposomes were incubated in the presence of indicated concentrations of **1**, **10a** and **10b**. The release of calcein from the liposomes was determined by measuring fluorescence intensity. Triton X-100 (10 μM) was used to establish the 100% level of membrane permeabilization. Values are mean ± SEM ($n = 3$).

Table 1. In Vitro Human Whole Blood Assay for Inhibition of COX-1- and COX-2-Derived PG Biosynthesis[a]

	IC_{50} (μM)		
compd	COX-1	COX-2	COX-1/COX-2
1	23.5 ± 4.8	10.1 ± 1.3	2.5 ± 0.5
10a	24.2 ± 8.6	14.3 ± 6.8	2.5 ± 1.5
10b	30.3 ± 4.1	65.3 ± 11.4	0.5 ± 0.1
17	6.3 ± 1.2	12.2 ± 3.9	0.6 ± 0.1
18	1.3 ± 0.3	2.4 ± 0.4	0.6 ± 0.1
19a	7.7 ± 0.3	48.0 ± 3.2	0.2 ± 0.02
19b	7.2 ± 0.9	34.0 ± 4.9	0.2 ± 0.1
20a	0.6 ± 0.1	3.1 ± 1.5	0.4 ± 0.2
20b	2.2 ± 1.6	8.5 ± 0.4	0.3 ± 0.2

[a] Inhibitory effect of each compound on COX-1- and COX-2-derived PG biosynthesis was measured and the IC_{50} value (concentration of each compound required for 50% inhibition), and the COX-1/COX-2 ratio of that for COX-1 and COX-2 are shown. We estimated the values of IC_{50} from the sigmoid-like dose-response curve (four-parameter logistic curve model) drawn by the logistic-curve fitting software (ImageJ 1.43u; National Institutes of Health, USA). Values are mean ± SEM ($n = 3$).

celecoxib was reported to be 22.7 with the same method as that used in Table 1[22]).

As described above, **1** is a pro-drug and the *trans*-alcohol form is the active metabolite. To test whether or not **10a** and

10b maintain this characteristic, we examined the COX-inhibitory activities of the *trans*-alcohol forms of **10a** and **10b** (**20a** and **20b**, respectively). The synthesis of these compounds is outlined in Scheme 2 (experimental details are available in Supporting Information). The *trans*-alcohol form of **1** (**18**) showed a more potent inhibitory effect on both COX-1- and COX-2-derived PG biosynthesis than **1** or its *cis*-alcohol form (**17**) (Table 1), as described previously.[20] Both **20a** and **20b** also showed more potent inhibitory effects on both COX-1- and COX-2-derived PG biosynthesis than **10a** and **10b** or their *cis*-alcohol forms (**19a** and **19b**, respectively) (Table 1). These results suggest that **10a** and **10b** may also act as pro-drugs and that their *trans*-alcohol forms are the active for inhibition of COX.

We then evaluated the activities of **10a** and **10b** in vivo. Compound **1** (40 or 50 mg/kg) and equivalent molar amounts of **10a** and **10b** were orally administered to rats and the lesion index was calculated. Administration of **1** produced gastric lesions in a dose-dependent manner (Figure 2A), as described previously.[18,19] Both **10a** and **10b** produced fewer gastric lesions than **1** (Figure 2A). We also measured the gastric level of PGE_2 by enzyme immunoassay (EIA) after oral administration of these compounds. As shown in Figure 2B, administration of **10a** decreased the level to an extent similar to that of **1**. On the other hand, administration of **10b** decreased the gastric level of PGE_2, although not to the extent of that seen with **1** (Figure 2B). Considering our hypothesis that both a decrease in the gastric level of PGE_2 and gastric mucosal damage due to membrane permeabilization activity of NSAIDs are involved in the production of NSAID-induced gastric lesions, the lower lesion-producing activity of **10a** seems to be due to its lower membrane permeabilizing activity. On the other hand, that of **10b** may involve both its lower membrane permeabilization activity and decreased inhibitory effect on the gastric production of PGE_2.

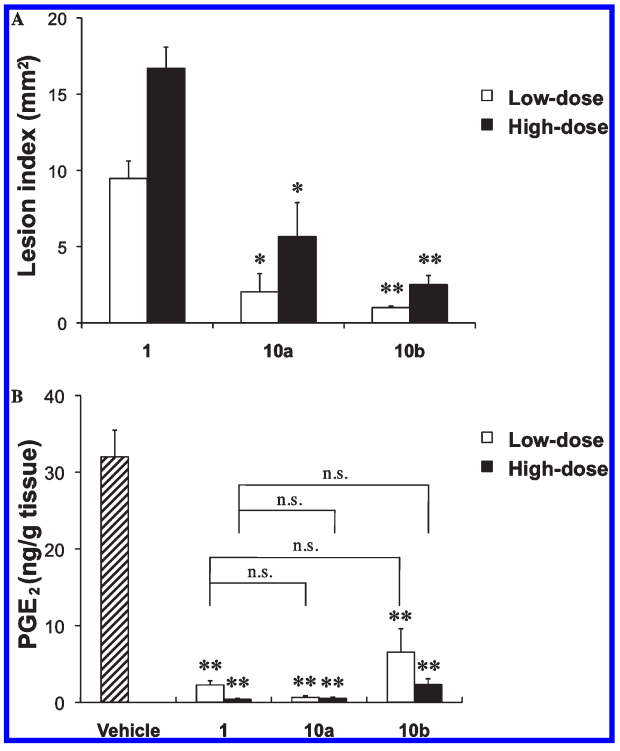


Figure 2. (A) Production of gastric lesions or (B) gastric PGE_2 level in the presence of loxoprofen sodium and its derivatives. (A,B) Rats were orally administered a low (40, 43, or 52 mg/kg) or high (50, 53, or 65 mg/kg) dose of **1**, **10a**, or **10b**, respectively, and their stomachs were removed after 8 h. (A) Stomachs were scored for hemorrhagic damage. (B) Gastric PGE_2 level was determined by EIA. Values are mean ± SEM (n = 3). $^{**}P < 0.01$; $^{*}P < 0.05$; n.s., not significant (vs, **1** (A); vs, vehicle (B)).

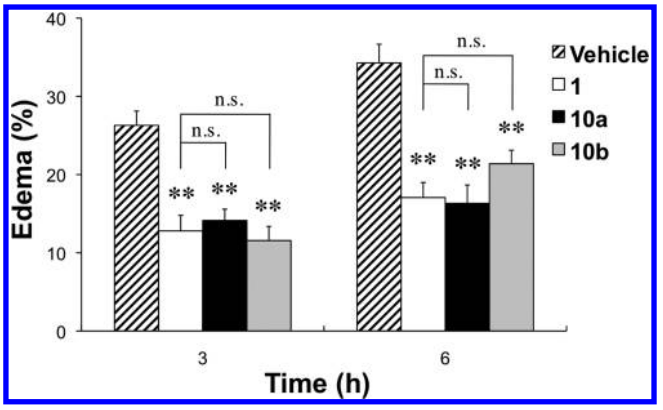
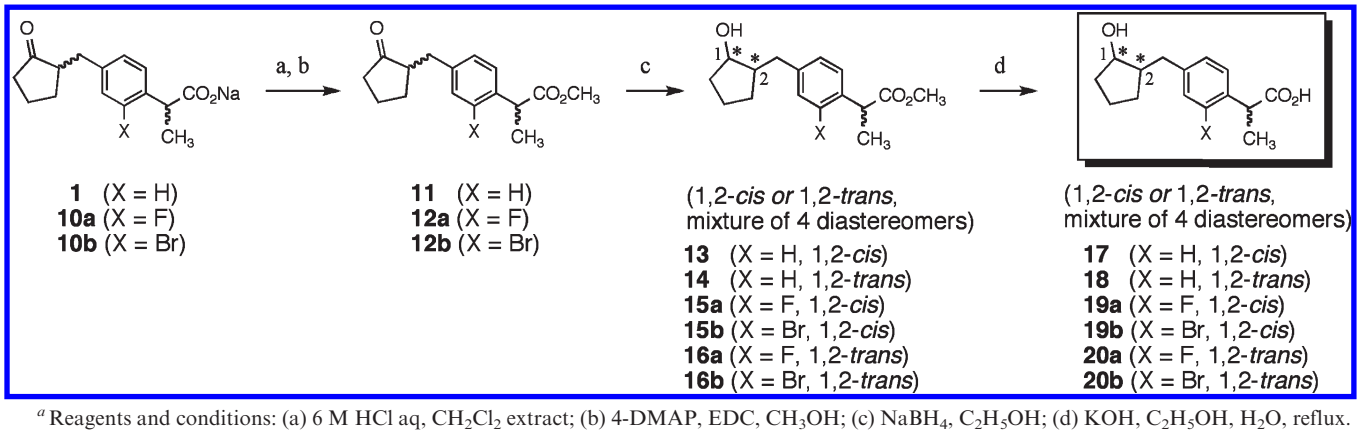


Figure 3. Anti-inflammatory activities of loxoprofen and its derivatives. Rats were orally administered 10, 11, or 13 mg/kg of **1**, **10a**, or **10b**, respectively, and 1 h later they received an intradermal injection of carrageenan (1%) into the left hindpaw. Paw edema were measured 3 h and 6 h after the administration of carrageenan. Values are mean ± SEM (n = 3−4). $^{**}P < 0.01$; n.s., not significant (vs, vehicle).

Scheme 2. Synthesis of Loxoprofen Alcohol (**17**, **18**) and its Halogeno-Derivatives (**19a**,**b**, **20a**,**b**)[a]



[a] Reagents and conditions: (a) 6 M HCl aq, CH_2Cl_2 extract; (b) 4-DMAP, EDC, CH_3OH; (c) $NaBH_4$, C_2H_5OH; (d) KOH, C_2H_5OH, H_2O, reflux.

Finally, we compared the anti-inflammatory effects of **10a** and **10b** to **1** by employing a rat carrageenan-induced footpad edema assay. As shown in Figure 3, the volume of carrageenan-induced footpad edema was significantly decreased after oral administration of **1**, confirming its previously described anti-inflammatory activity.[20,23] The effects of **10a** and **10b** were much the same as that of **1**, showing that **10a** and **10b** have anti-inflammatory activity equivalent to **1**.

Conclusions

In this study, we showed that two derivatives of **1**, **10a** and **10b**, have even lower membrane permeabilization activity and produced fewer gastric lesions after oral administration to rats, compared to **1**. This is chemical evidence in support of our proposal that the membrane permeabilization activity of NSAIDs is involved in their induction of gastric lesions.

We also found that **10a** and **10b** showed anti-inflammatory effects equivalent to that of **1**, suggesting that the membrane permeabilization activity of NSAIDs is not involved in their anti-inflammatory effect. Compounds **10a** and **10b** showed very low gastric lesion-inducing activity in rats, although they have no apparent selectivity for COX-2. Thus, we consider that **10a** and **10b** are likely to be therapeutically beneficial NSAIDs in terms of gastrointestinal and cardiovascular safety.

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Supporting Information Available: Experimental details and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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