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SYNTHESIS OF NEW POTENT LEUKOTRIENE B4 ANTAGONISTS AND THEIR BIOLOGICAL PROPERTIES. 2.

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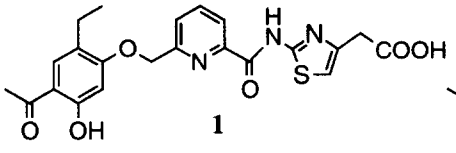
SUMMARY: The synthesis of new leukotriene B4 antagonists and their biological properties are described. Compound **10m** (SM-15178) has potent effects against human neutrophil chemotaxis, and is orally effective against LTB4-induced bronchoconstriction in the guinea pig.

INTRODUCTION AND CONCEPT: Leukotriene B4 (LTB4), a metabolite of arachidonic acid, has been reported to have various pharmacological actions on leukocytes.[1] LTB4 is thought to be an important mediator in inflammatory and allergic states, because high levels of LTB4 has been detected in the lesions of human inflammatory diseases.[2] We planned to synthesize LTB4 antagonists to try to understand the pathophysiological role of LTB4 itself and possibly to provide therapeutic value in chronic inflammatory diseases such as inflammatory bowel disease, psoriasis, and asthma.

During our own research for specific LTB4 antagonists, we already found a lead compound **1**[3] having a 5-ethyl-2,4-dihydroxyacetophenone moiety, which had been reported to be an excellent component for LTB4 antagonists by Lilly Research laboratories.[4] After a detailed pharmacokinetic evaluation of compound **1**, we found that it was easily conjugated with UDP glucuronic acid by glucuronyltransferase (about 60% of compound **1** was glucuronidated 5 min after intravenous administration to the rat). The hydroxyl group and/or the carboxylic group of compound **1** was conjugated. Further, the half life of the glucuronides of compound **1** was very short (about 10 min).[5] Therefore, we planned to synthesize potent, metabolically stable and orally active LTB4 antagonists. Oral activity is believed to be important, because LTB4 antagonists would be useful for the treatment of chronic inflammatory diseases which require the administration of anti-inflammatory drugs for long periods of time.

We planned to synthesize a series of phenoxymethylpyridinecarboxamide derivatives to investigate the structure-activity relationships. In the beginning, we modified the pyridine and the thiazolylacetic acid moieties and evaluated the in vitro activities of this series against human neutrophil chemotaxis. Next, we evaluated the



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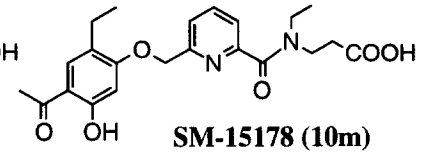
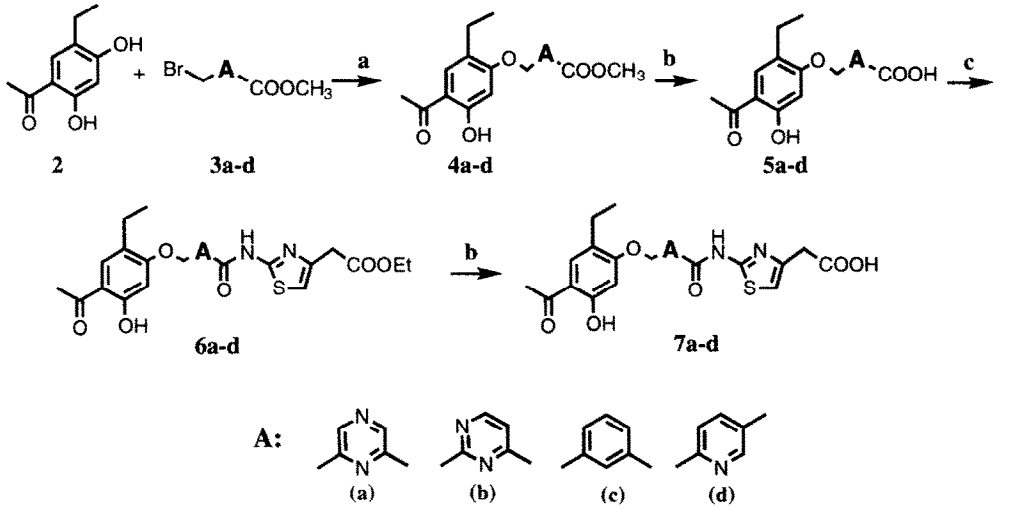
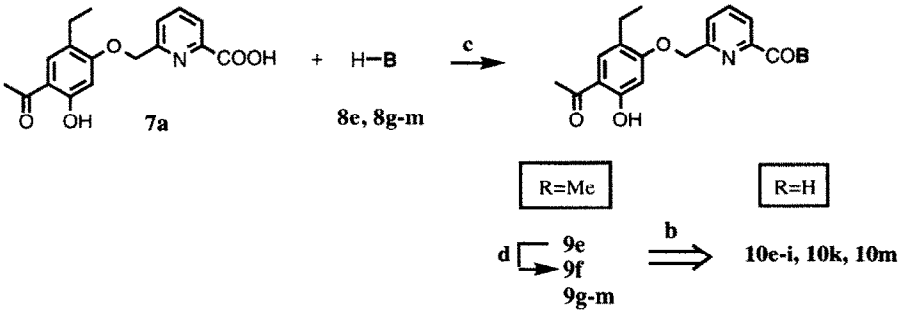


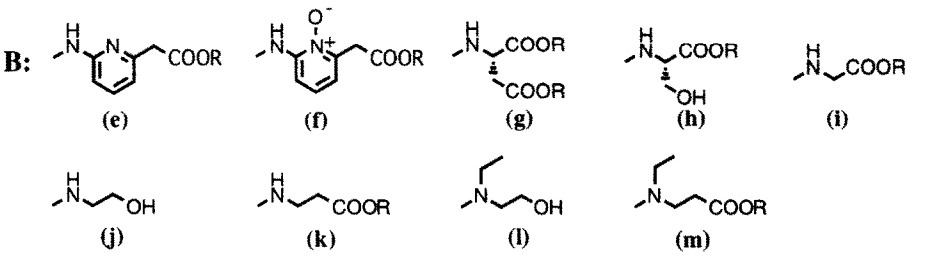
SM-15178 (10m)

Scheme 1



Scheme 2





CONDITIONS: **a:** K_2CO_3, acetone-DMF, 25 °C-reflux, 1-6 h, 30-96%; **b:** 1N-NaOH, MeOH, 0-25 °C, 10-60 min, 60-100%, **c:** ethyl 2-amino-4-thiazoleacetate, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxy-1*H*-benzotriazole monohydrate, Et_3N, CH_2Cl_2-DMF, 20-90%. **d:** *m*-CPBA, CH_2Cl_2, 25 °C, 16 h, 63%.

in vivo effects against LTB4-induced guinea pig bronchoconstriction and the plasma concentrations of the potent inhibitors in vivo after intravenous and oral administrations to the rat. Most of the compounds were effective against human neutrophil chemotaxis, and only a few compounds were active in the in vivo evaluation after oral administrations. Compound **10m** (SM-15178) was orally effective against the in vivo LTB4-induced guinea pig bronchoconstriction and showed long-lasting and high plasma concentration.

SYNTHESIS: The compounds shown in Table 1-3 were synthesized as outlined in Scheme 1 and 2. Alkylation of readily available 4-acetyl-6-ethyl-resorcinol **2**[4] with readily available halides **3a-d** in the presence of K_2CO_3, followed by saponification with aqueous NaOH, gave the acids **5a-d**. Condensation of **5a-d** with ethyl 2-amino-4-thiazoleacetate in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxy-1*H*-benzotriazole monohydrate and triethylamine, followed by saponification with aqueous NaOH gave the acids **7a-d**. The compounds **9e, 9g-m, 10e-i, 10k** and **10m** were obtained under the same conditions as described above. Oxidation of **9e** with *m*-CPBA gave the N-oxide **9f**.

RESULTS : The inhibitory activities of **7a-c** in Scheme 1 for human neutrophil chemotaxis are given in Table 1. Compounds **7a-c** having a pyrazinediyl, a pyrimidinediyl and a phenylene moiety, respectively as well as the regioisomer **7d** were less potent than the 2,6-pyridinediyl derivative **1**. Therefore, the variations and the substitution patterns of the aromatic ring were highly important. Next, we modified the thiazolylacetic acid moiety of the compound **1**. The inhibitory activities for human neutrophil chemotaxis of **10e-i, 9j, 10k, 9l** and **10m** in Scheme 2 are given in Table 2. Compound **10f** was the most potent, whereas **10i** and **9j** were less potent. The comparison of **10i** and **10k** indicated that the β-carboxylic acid of the amino acid moiety of **10k** was necessary for the inhibitory activity. Comparing **9j** with **9l**, and **10k** with **10m**, the ethyl substituent of the amide appeared to be important for the enhancement of the inhibitory activity.

Table 1. Inhibition of human PMNLs chemotaxis

compound	logP*[b]	$IC_{50}(\mu M)$[c]
1	**1.9**	**0.3**
7a	**0.6**	**10**
7b	NT[a]	**3**
7c	NT[a]	**5**
7d	NT[a]	**0.7**

[a]Not tested

[b]Measured (n-octanol / pH 7.4 phosphate buffer)

[c]Chemotaxis assay was performed in a modified Boyden chamber according to the method of Onozaki et al.[6] Briefly, human neutrophils (10^6 cells/ml) in the upper compartment of the chamber were incubated with 3 nM LTB_4 in the presence of compound in the lower compartment at 37 °C for 90 min under 5 % CO_2. After incubation, the number of cells adhering to the cover glass at the bottom of lower compartment was counted. Percentage inhibition was calculated, using the mean cell number obtained from 8 observations (4 observations/chamber x duplicate chambers) in one experiment after subtracting that for the buffer control(blank). The value of IC_{50} was calculated using percentage inhibitions for different concentrations of compound by probit analysis.

Table 2. Inhibition of human PMNLs chemotaxis

compound	logP*[b]	$IC_{50}(\mu M)$[c]
SC-41930[d]	NT[a]	3.3
10e	1.1	0.8
10f	0.8	0.02
10g	-1.8	0.9
10h	-0.3	0.3
10i	NT[a]	12
9j	NT[a]	15
10k	0.7	1.0
9l	2.8	1.0
10m	0.1	0.3

[a]Not tested

[b]Measured (n-octanol / pH 7.4 phosphate buffer)

[c]According to the method written in Table 1, the values of IC_{50} were given

[d]SC-41930 was reported as first orally active and selective LTB_4 receptor antagonist by G D. Searle and Co.[7] This compound was prepared by Sumitomo Pharmaceuticals Research Center as reference compound.

Table 3. The inhibitory activities for LTB_4-induced bronchoconstriction of guinea pig[b]

	i.v. (5 mg/kg)			p.o. (40 mg/kg)
compound	2 min	10 min	30 min	60 min
1	NT[a]	+	-	NT[a]
7a	++	+	-	NT[a]
10e	NT[a]	-	NT[a]	NT[a]
10f	++	++	++	-
10g	+	+	-	NT[a]
10h	++	++	++	+
10k	++	++	-	NT[a]
9l	++	-	NT[a]	NT[a]
10m	++	++	++	++

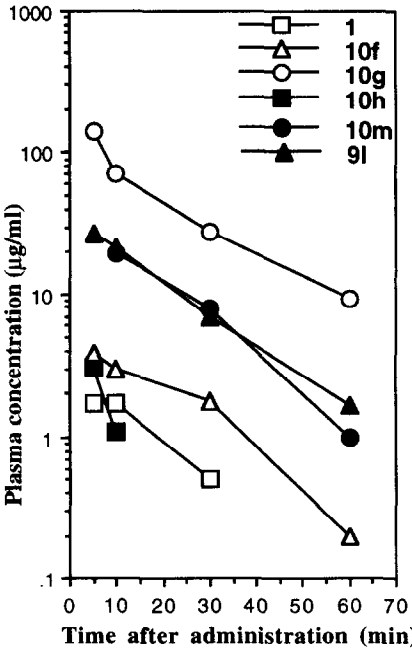
[a]Not tested

[b]Guinea pigs were anesthetized with pentobarbital sodium (30 mg/kg, i.p.) and phenobarbital (120 mg/kg, i.p.) and ventilated artificially through a tracheal cannula (4-6 ml, 60 strokes/min). Spontaneous respiration was stopped by intramuscular injection of gallamine triethiodide (10 mg/kg) Thirty minutes after anesthetic administration, bronchoconstriction was induced by injecting 8 μg/kg of LTB_4 through the catheter inserted into the jugular vein Drugs were given intravenously 2 min, 10 min or 30 min, or orally 1 h prior to the LTB_4 injection. Guinea pigs were fasted for 24 h before drug administration. Airway resistance was measured from the beginning of the ventilation to 5 min after the LTB_4-injection through a bronchospasm transducer according to the method of Konsett and Rossler. On the basis of the results of measurements (N = 2), inhibitory activities of compounds were roughly classified into three categories : Active -- ++, relatively active -- +, negative -- -. A detailed method of this evaluation will be reported.[8]

For selected compounds in Table 1 and 2, we evaluated their in vivo effects against LTB4-induced guinea pig bronchoconstriction as shown in Table 3. Most of these compounds showed potent effects 2 min after intravenous injection, but only a few compounds were effective 30 min after intravenous injection. This could be due to differences in pharmacokinetic profile such as distribution, metabolism and excretion. Then, we evaluated the oral activity of compounds which were active 30 min after intravenous injection. Compound **10h** was relatively active, and **10g** was not active. Finally, we found that **10m** was orally active against LTB4-induced guinea pig bronchoconstriction.

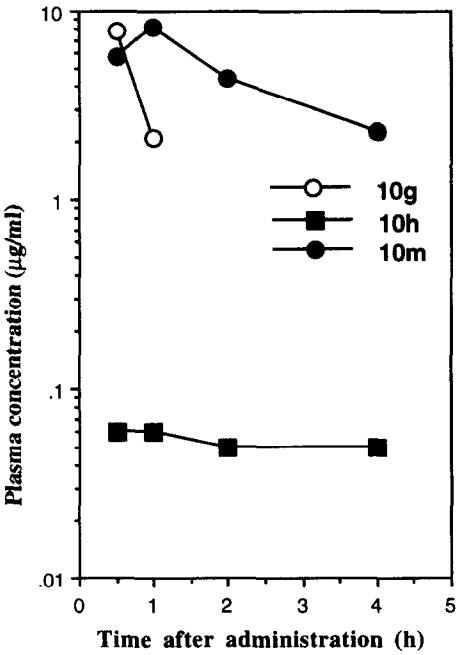
In order to investigate the relationship between oral administration and intravenous administration, we measured the plasma concentrations of the test compounds as shown in Fig. 1 and 2. The most potent compound in vitro **10f** showed low plasma concentration after intravenous administration to the rat. While compound **10g** showed the highest plasma concentration (Fig. 1). The plasma concentrations of **10g**, **10h**, and **10m** after oral administrations to the rat are shown in Fig. 2. Compound **10g** would be expected to be rapidly excreted owing to the relative low distribution constant (logP* = -1.8) as described in Table 2. Compound **10h** showed a low plasma concentration indicating poor absorption after oral administration. On the contrary,

Fig. 1 Plasma concentrations of drugs after intravenous administrations to the rat (dose: 5 mg/kg)



Compound **1** and **10h** were not detected in plasma at 60 min and at 30 min, 60 min, respectively.

Fig. 2 Plasma concentrations of the drugs after oral administrations to the rat (dose: 50 mg/kg, vehicle: 0.5%MC)



Compound **10g** was not detected at 2 h and 4 h.

10m showed long-lasting and high plasma levels indicating high absorption after oral administration, metabolic stability, and relatively low excretion. In addition, pharmacokinetic evaluation of **10h** showed that it was easily glucuronidated similarly to **1**. On the contrary, **10m** was only slightly glucuronidated. In summary, **10m** (SM-15178) showed excellent bioavailability and metabolic stability Detailed biological evaluations of the compound **10m** will be reported separately.[8]

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5) Plasma concentrations of **1** and its glucuronides after intravenous administration of **1** (5 0 mg/kg) to the rat were respectively as follows: 5 min (5 50 and 7.80 μM), 30 min (0.50 and 1.89 μM). The plasma concentrations of its glucuronides was evaluated from the plasma level with and without β-glucuronidase.

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