Communications to the Editor

3-(5-Alkyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic Acids as Orally Active Inhibitors of IL-1 Generation

Interleukin-1 (IL-1) is a polypeptide produced mainly by macrophages, which has multiple biological activities including induction of fever, induction of the production of acute phase proteins by hepatocytes, and stimulation of prostaglandins and collagenase production by synovial cells.[1] On the basis of these facts, IL-1 is thought to be an essential mediator of inflammation. In particular, the importance of IL-1 in rheumatoid arthritis (RA) has been reported by various investigators. For example, IL-1 production from RA synovium correlated not only with the degree of inflammation but also with that of joint destruction.[2] Therefore, it is thought that an inhibitor of IL-1 generation could be an useful therapeutic agent in the treatment of RA.

From published reports, it is known that some compounds such as SK&F86002,[3] CP66248,[4] and E5110[5a] reduce IL-1 generation from human monocytes in in vitro studies.

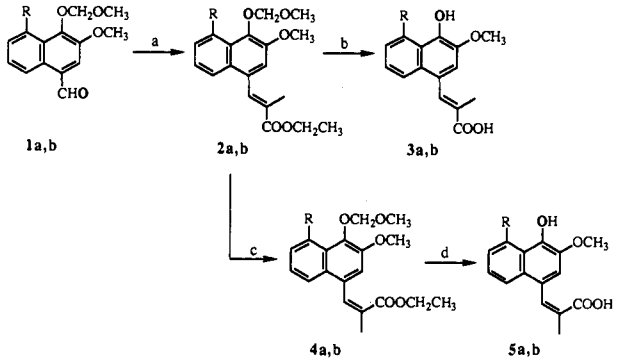
In the course of our studies on inhibitors of IL-1 generation using in vitro systems and the rat air-pouch inflammatory model, we have discovered that 3-(5-alkyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic acids **3a,b** and **5a,b** are orally active inhibitors. In particular, (*Z*)-3-(5-ethyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic acid (**5a**) was one of the most potent orally active inhibitors of IL-1 generation.

In this communication, we describe the synthesis and the pharmacological profile of compound **5a** and related compounds.

Chemistry[5b]

3-(5-Alkyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic acids **3a,b** and **5a,b** were prepared as shown in Scheme I. The Wadsworth–Emmons reaction between aldehydes **1a,b**[6] and triethyl 2-phosphonopropionate gave ethyl (*E*)-propenoates **2a,b**, which were then hydrolyzed under alkaline conditions (KOH/aqueous MeOH) followed by deprotection of the phenolic hydroxy group with HCl in acetone to yield the (*E*)-propenoic acids **3a,b**. Photochemical *E–Z* isomerization of **2a,b** was performed with a high-pressure Hg lamp in acetone to give the *Z* isomers **4a,b**,[7] which were then hydrolyzed under alkaline conditions (KOH/aqueous MeOH) followed by deprotection of the phenolic hydroxy group with HCl to yield the (*Z*)-propenoic acids **5a,b**. The *E* and *Z* configurations of the compounds were assigned on the basis of ^{1}H NMR analysis.

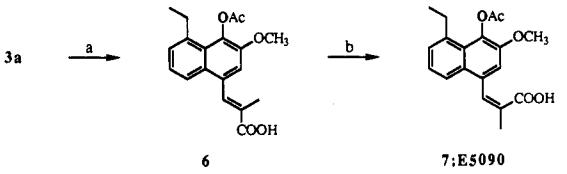
Scheme I. Preparation of 3-(5-Alkyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic Acids



a : R=Et
b : R=n-Pr

(a) NaH, $(EtO)_2P(O)CH(CH_3)COOEt$, DMF (98% for **2a**, 93% for **2b**); i) KOH, MeOH-H_2O, ii) HCl, acetone (85% for **3a**, 85% for **3b**); (c) hv, acetone (42% for **4a**, 30% for **4b**); (d) i) KOH, MeOH-H_2O, ii) HCl, acetone (87% for **5a**, 89% for **5b**)

Scheme II. Preparation of E5090



(a) Ac_2O, pyridine (78%); (b) hv, acetone (41%)

The synthetic route to (*Z*)-3-[4-(acetyloxy)-5-ethyl-3-methoxy-1-naphthalenyl]-2-methyl-2-propenoic acid (**7**) (E5090) is shown in Scheme II. Compound **3a** was acetylated with acetic anhydride in the presence of pyridine to give the (*E*)-acetate **6**, which was then photoisomerized with a high-pressure Hg lamp in acetone to yield **7**.[7]

Results and Discussion

The inhibitory activities on IL-1 generation were evaluated both by the in vitro systems using human mono-

(1) Oppenheim, J. J.; Kovacs, E. J.; Matsushima, K.; Durum, S. K. There is more than one interleukin 1. *Immunol. Today* **1986**, *7*, 45–56.

(2) Miyasaka, N.; Sato, K.; Goto, M.; Sasano, M.; Natsuyama, M.; Inoue, K.; Nishioka, K. Augmented interleukin-1 production and HLA-DR expression in the synovium of rheumatoid arthritis patients. *Arthritis Rheum.* **1988**, *31*, 480–486.

(3) Lee, J. C.; Griswold, D. E.; Votta, B.; Hanna, N. Inhibition of monocyte IL-1 production by the anti-inflammatory compound, SK&F 86002. *Int. J. Immunopharmacol.* **1988**, *10*, 835–843.

(4) McDonald, B.; Loose, L.; Rosenwasser, L. J. The influence of a novel arachidonate inhibitor, CP66,248, on the production and activity of human monocyte IL-1 (abstract). *Arthritis Rheum.* **1988**, *31*, Suppl 4, s-17.

(5) (a) Shirota, H.; Goto, M.; Hashida, R.; Yamatsu, I.; Katayama, K. Inhibitory effects of E5110 on interleukin-1 generation from human monocytes. *Agents Actions* **1989**, *27*, 322–324. (b) All melting points were determined on a YAZAWA BY-10 melting point apparatus in open capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL FX-90Q spectrometer with tetramethylsilane as an internal standard. Elemental analyses for compounds **3a**, **3b**, **5a**, **5b**, and **7** were all within ±0.4% of the calculated values.

(6) Okita, M.; Shirota, H.; Tanaka, M.; Kaneko, T.; Tagami, K.; Hibi, S.; Okamoto, Y.; Nomoto, S.; Suzuki, T.; Chiba, T.; Goto, M.; Hashida, R.; Ono, H.; Ohara, H.; Sakurai, H.; Souda, S.; Machida, Y.; Katayama, K.; Yamatsu, I. Japan Kokai Tokkyo Koho, JP 2-256645, 1990.

(7) Compounds **2a,b** and **6** were photoisomerized to yield the *E,Z* mixtures (ca. *E*/*Z* = 2/3), which were separated by flash column chromatography.

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Table I. Inhibitory Effects of 3-(5-Alkyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic Acids on IL-1 Generation

compd	IC_{50},[a] μM		in vivo air-pouch model: % inhibition[b]			
	human monocyte[c]	rat macrophage[d]	100 μM[e]	200 mg/kg[f]	50 mg/kg[f]	25 mg/kg[f]
3a[g]	4.8	2.0	85	36	NT[h]	NT[h]
3b[i]	~1	1.0	91	67	4	NT[h]
5a[j]	3.0	1.4	94	91	65	40
5b[k]	~1	1.1	87	86	33	NT[h]

[a] IC_{50} values were calculated by the least-squares method. [b] Values are the mean of five animals. [c] Values are the mean of two or more experiments. [d] Values are the mean of duplicate experiments. [e] Local administration. [f] Oral administration. [g] Mp 196 °C dec; anal. ($C_{17}H_{18}O_4$) C, H. [h] Not tested. [i] Mp 170 °C dec; anal. ($C_{18}H_{20}O_4$) C, H. [j] Mp 193 °C dec; anal. ($C_{17}H_{18}O_4$) C, H. [k] Mp 155 °C dec; anal. ($C_{18}H_{20}O_4$) C, H.

Table II. Pharmacokinetic Parameters of 3-(5-Alkyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic Acids in Plasma after Oral Administration (50 mg/kg) to Male F_{344} Rats

compd	n[a]	C_{max},[b] μg/mL	$t_{1/2}$,[c] h	AUC,[d] μg·h/mL
3a	3	67.2 ± 3.9	0.44 ± 0.03	107.9 ± 4.1
3b	2	29.9	1.07	60.4
5a	3	153.9 ± 4.8	1.72 ± 0.12	418.1 ± 24
5b	3	84.2 ± 11.8	1.07 ± 0.05	188.8 ± 18.3

[a] Number of rats. [b] Maximum concentration (mean ± SEM). [c] Half-life (mean ± SEM). [d] Area (mean ± SEM) under the concentration vs time course (0–4 h).

cytes[8] or rat exudated macrophages[9] stimulated with LPS, and by the in vivo system using rat CMC-LPS air-pouch model.[10]

The inhibitory effects of 3-(4-hydroxy-1-naphthalenyl)-2-propenoic acids on IL-1 generation are summarized in Table I. Compounds **3b** and **5b** had higher activities than **3a** and **5a** in the in vitro systems using human monocytes and rat exudated macrophages, but these four compounds showed almost equal activity in the in vivo system involving local administration to the air pouch. On the other hand, compounds **3a** and **3b** showed poor activity by oral administration, because the *E* isomers exhibited lower plasma concentration and AUC values than the *Z* isomers (Table II). However, compounds **5a** and **5b** dose-dependently inhibited IL-1 generation, and their minimum effective doses (MED) were estimated to be 25 and 50 mg/kg, respectively. The pharmacokinetics of compounds **5a** and **5b** in the rat air-pouch exudate was examined (Table III). The maximum concentrations of the compounds in the rat air-pouch exudate were 11.6 ± 0.8 and 5.7 ± 0.8 μg/mL, respectively, and were estimated to be less than $^1/_{10}$ of the maximal plasma concentrations. At 6 h after oral administration, which was the time of sample collection for IL-1 determination, they were 8.76 ± 0.97 and 1.94 ± 0.16 μg/mL, respectively. The half-lives of compounds **5a** and **5b** in the air-pouch exudate were longer than those in plasma.

Table III. Pharmacokinetic Parameters of Compounds **5a** and **5b** in the Rat Air-Pouch Exudate after Oral Administration (50 mg/kg) to Male F_{344} Rats

compd	n[a]	C_{max},[b] μg/mL	C_{6h},[c] μg/mL	$t_{1/2}$,[d] h
5a	5	11.6 ± 0.8	8.76 ± 0.97	4.15 ± 0.37
5b	5	5.7 ± 0.8	1.94 ± 0.16	2.05 ± 0.32

[a] Number of rats. [b] Maximum concentration (mean ± SEM). [c] Concentration at 6 h after oral administration (mean ± SEM). [d] Half-life (mean ± SEM).

Table IV. Inhibitory Effects of Compounds **5a** and **5b** on the Adjuvant Arthritis in Rats[a]

compound	dose, mg/kg	adjuvant-untreated paw	
		swelling (%)	inhibition (%)
control		55.2 ± 5.5	
5a	12.5	44.1 ± 6.3	20.1
	25	36.4 ± 4.0*	34.1
	50	33.8 ± 4.9*	38.8
	100	26.8 ± 2.8***	51.4
5b	12.5	45.8 ± 7.4	17.0
	25	50.8 ± 6.3	8.0
	50	29.5 ± 4.4**	46.6
	100	25.6 ± 4.4**	53.6
indomethacin	1	28.8 ± 4.0**	47.8

[a] The test drugs were orally administered once daily from the first day of the adjuvant treatment. The swelling of the adjuvant-untreated paw (mean ± SEM of 6–10 animals) was evaluated by using a plethysmometer at the 15th day after adjuvant treatment. Significant difference from control: (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

The antiinflammatory activity of compounds **5a** and **5b** in rat adjuvant arthritis was examined (Table IV). Both compounds **5a** and **5b** dose-dependently inhibited the paw swelling of adjuvant arthritis. The MEDs of **5a** and **5b** were 25 and 50 mg/kg, respectively, and were consistent

(8) Oppenheim, J. J.; Shneyour, A.; Kook, A. I. Enhancement of DNA synthesis and cAMP content of mouse thymocytes by mediator(s) derived from adherent cells. *J. Immunol.* **1976**, *116*, 1466–1472. The amounts of IL-1 in the test samples were calculated by a titration curve prepared with standard human recombinant IL-β (Genzyme). The IL-1 levels of LPS treated and untreated control were 24–194 and 2–16 units/mL, respectively.

(9) Exudated macrophages of rats (Fisher) were prepared by the intraperitoneal injection of 2.5% glycogen solution. After 4 days, peritoneal exudated cells were collected. The cells suspended in RPMI 1640 containing 1% heat-inactivated serum derived from normal rats were seeded to 48-well culture plates (0.5×10^6 cells/0.5 mL) and incubated at 37 °C for 1.5 h. Nonadherent cells were removed by rinsing, and the remaining cells were used as the exudated macrophage preparation. The macrophages were cultured with LPS (1 ng/mL) for 4 h in the presence or absence of test compounds. After the cultivation, the intracellular IL-1 activity was determined by the standard LAF assay.[8] The amounts of IL-1 in the test samples were calculated by a titration curve prepared with standard human recombinant IL-1α (Genzyme). The IL-1 level of LPS-treated control was 399 units/mL, and the levels of untreated control were negligible.

(10) Chiba, K.; Goto, M.; Shirota, H. A novel inhibitor of IL-1 generation, E5090: In vivo inhibitory effect on the generation of IL-1-like factor and on granuloma formation in an air-pouch model. *Agents Actions* **1991**, *32*, Suppl., 231–235. The test compounds were administered either orally suspended in 0.5% methyl cellulose solution or locally dissolved in 5% EtOH-RPMI 1640 medium containing 30% heat-inactivated autologous serum at a final concentration in the exudate of 100 μM. Five animals were used in each group. Oral administration was performed at 2 h before the LPS injection, and local administration was performed at 30 min and just before the LPS injection. At 4 h after the LPS injection, 50 μL of inflammatory exudate was collected from the air pouch for measurements of IL-1 activity. The extra- and intracellular IL-1 activities were determined by the standard LAF assay.[8] The amounts of IL-1 in the test samples were calculated by a titration curve prepared with standard human recombinant IL-1α (Genzyme). The IL-1 level of LPS-treated control was 1175–4856 units/mL, and the levels of untreated control were negligible.

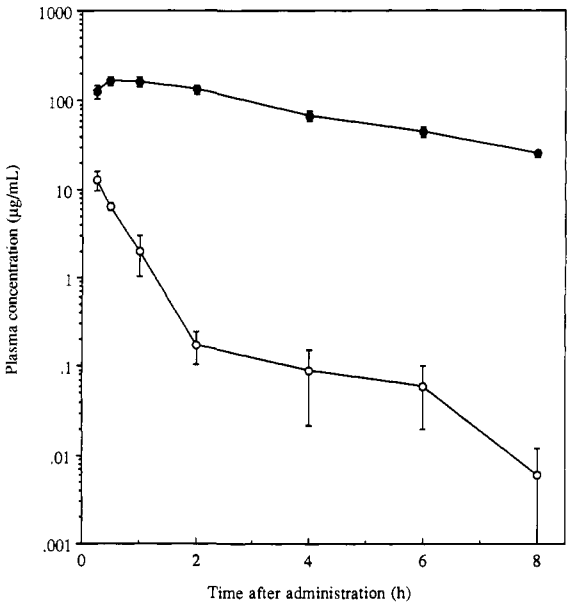

Figure 1. Plasma concentration of compounds **7** (○) and **5a** (●) after oral administration of **7** (50 mg/kg) to male F_{344} rats. Each point represents mean with SEM of three rats.

with those in the rat air-pouch model. In addition, both compounds **5a** and **5b** also inhibited the paw swelling arising from type II collagen-induced arthritis in rats (data not shown). In these models, prostaglandins are usually accepted to be the main mediator. However, compounds **5a** and **5b** exhibited no inhibitory effects on cycloxygenase activity in sheep seminal vesicles even at 100 μM. The antiinflammatory activities in these arthritic models may suggest that both the compounds **5a** and **5b** exert their effects by a novel mechanism of action.

On the basis of its inhibitory effects on IL-1 generation and the relative efficacies in animal models of inflammation, compound **5a** was selected as the most potent orally active inhibitor of IL-1 generation. However, compound **5a** was slightly unstable.[11] This physical instability of **5a** suggested that the development of a stable formulation would be difficult. The best way to achieve a stable formulation would be to protect the 4-hydroxy substituent. Fortunately, we found that the 4-acetyloxy compound **7**(E5090)[12] was quite stable. Compound **7** itself had little or no inhibitory effect on IL-1 generation in vitro (IC_{50} > 30 μM for both human monocytes and rat exudated macrophages), but orally administered **7** was rapidly absorbed and immediately transformed to the deacetylated form **5a** (Figure 1), which was pharmacologically active. The effectiveness of compound **7** was quite equal to compound **5a** in the rat air-pouch model (MED, 25 mg/kg, po)[13] and in the arthritic models of rats.[14]

(11) This compound was isomerized to the *E* form **3a** by irradiation by light (1000 lux, 1 week, ca. *Z*/*E* = 4/1) and mainly converted to the *E* form **3a** in acidic solutions or in organic solvents such as EtOH.

(12) Mp 148–150 °C; anal. ($C_{19}H_{20}O_5$) C, H. This compound was stable to heat (55 °C, 3 months, no change) and irradiation by light (1000 lux, 1 month, *Z*/*E* = 30/1).

(13) This compound dose-dependently inhibited IL-1 generation by oral administration. The inhibition percents were 41% (25 mg/kg), 56% (50 mg/kg), and 95% (200 mg/kg).

(14) Compounds **5a** and **7** were simultaneously evaluated in adjuvant arthritis of rats. These compounds inhibited the swelling of the adjuvant-untreated paw at the 18th day after adjuvant treatment. The inhibition percents of **5a** were 44% (25 mg/kg), 24% (50 mg/kg), and 64% (100 mg/kg), and those of **7** were 36% (25 mg/kg), 48% (50 mg/kg), and 66% (100 mg/kg).

In conclusion, 3-(5-alkyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic acids showed potent inhibitory activity against IL-1 generation. Among the compounds evaluated, (*Z*)-3-(5-ethyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic acid (**5a**) was chosen as the most potent orally active inhibitor of IL-1 generation. Compound **5a** inhibited transcription of IL-1α and β mRNA of LPS-treated human monocytes.[15] But the precise details of this mechanism are unknown and are now under investigation. Further details of the medicinal chemistry of this series will be described in forthcoming publications.

Supplementary Material Available: Analytical data for compounds **3a,b**, **5a,b**, and **7** (2 pages). Ordering information is given on any current masthead page.

(15) Goto, M.; Chiba, K.; Hashida, R.; Shirota, H. A novel inhibitor of IL-1 generation, E5090: In vitro inhibitory effects on the generation of IL-1 by human monocytes. *Agents Actions* **1991**, *32*, Suppl., 225–229.

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Probing the Bradykinin Receptor: Mapping the Geometric Topography Using Ethers of Hydroxyproline in Novel Peptides

The approach of preparing conformationally constrained peptide analogues of a natural peptide ligand in order to obtain insight about its bioactive conformation has become widely accepted in pharmaceutical research. The rationale is particularly appropriate in those cases where neither X-ray crystallographic nor NMR data pertaining to the receptor or ligand–receptor complex are available as is the case for the nonapeptide hormone bradykinin (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9). Since bradykinin has been implicated in such a variety of pathophysiological processes[1,2] including pain[3] and symptoms of the common cold,[4] a bradykinin-receptor antagonist could have significant therapeutic value. Despite the recent developments toward improved peptide antagonists, there are no potent and selective nonpeptide antagonists of the bradykinin receptor.[5] The challenge of deriving one ad hoc may ultimately rely on precise knowledge about the receptor-binding environment. A portion of this knowledge

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(3) Proud, D.; Reynolds, C. J.; Lacapra, S.; Kagey-Sobotka, A.; Lichtenstein, L. M.; Naclerio, R. M. Nasal Provocation with bradykinin induces symptoms of rhinovirus and a sore throat. *Am. Rev. Respir. Dis.* **1988**, *137*, 613–616.

(4) Naclerio, R. M.; Proud, D.; Lichtenstein, L. M.; Sobotka-Kagey, A.; Hemdley, J. O.; Sorrentino, J.; Gwaltney, J. M. Kinins are generated during experimental rhinovirus colds. *J. Infect. Dis.* **1988**, *157*, 133–142.

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