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Hydroxyindole derivatives as inhibitors of IL-1 generation. I. Synthesis and pharmacological activities of (*E*)-3-(4-hydroxy-5-methoxyindole-7-yl)-2-methylpropenoic acid derivatives

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Summary — A series of (*E*)-3-(4-hydroxy-5-methoxyindole-7-yl)-2-methylpropenoic acids was prepared and the inhibitory activities of its members on IL-1 generation were evaluated in an *in vivo* system using the rat carboxymethyl cellulose-lipopolysaccharide (CMC-LPS) air-pouch model. Many compounds in this new series were found to be inhibitors of IL-1 generation. Structure–activity relationships indicated that a methyl substituent at the 1- and 3-positions on the indole ring are important for activity and that a 3,4,5-trimethoxy-substituted 2-phenyl group on the indole ring is suitable to give compounds exhibiting inhibition after oral administration. Among the compounds evaluated, (*E*)-3-[1,3-dimethyl-4-hydroxy-5-methoxy-2-(3,4,5-trimethoxyphenyl)indole-7-yl]-2-methylpropenoic acid (**15m**), which inhibited IL-1 generation by human monocytes stimulated with various reagents such as LPS, opsonized zymosan and immune complexes, showed inhibitory activity in the rat CMC-LPS model after oral administration.

hydroxyindole derivative / inhibitor of IL-1 generation / rat CMC-LPS air-pouch model

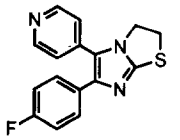
Introduction

Inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF) have recently become a focus of attention in the study of the pathology and development of the inflammatory response. In particular, because of its multiple biological activities [1–3], IL-1, a polypeptide synthesized and released by activated macrophages and other cell types, is thought to be an essential mediator of inflammation. For example, IL-1 induces fever and the synthesis of the acute-phase proteins by hepatocytes; it also stimulates prostaglandin and collagenase production by synovial cells. Furthermore, IL-1 induces synovial proliferation and bone resorption, leading to joint damage and dysfunction.

The production of IL-1 has been found to occur in various human diseases. In particular, the importance of IL-1 in rheumatoid arthritis (RA), a chronic inflammatory disease characterized by marked inflammation in the synovium, has been reported by various investigators. IL-1 activity has been detected in the synovial fluid of RA patients, and in culture supernatants from RA synovial tissues [4–10]. IL-1 production by RA synovium correlated with the degree of inflammation [6]. The level of IL-1 β in the plasma of RA patients was significantly higher than that of healthy controls and correlated with clinical disease activity [11]. High-affinity receptors for IL-1 α and β have been identified on cultured RA synovial cells [12]. On the basis of these facts, a compound that inhibits the production of IL-1 might prove to be a useful therapeutic agent for the treatment of RA.

Some synthetic compounds such as SK&F86002 (**1**) [13], E5110 (**2**) [14], CP66,248 (**3**) [15], SK&F105561 (**4**) [16], RP54745 (**5**) [17], E5090 (**6**) [18] and its pharmacologically active form (**7**) have been reported to inhibit the generation of IL-1 (chart 1). These compounds have been found to inhibit the generation of IL-1 from monocytes or macrophages in *in vitro* studies, but only a few *in vivo* studies have as yet been reported.

In the course of developing a synthetic compound to inhibit IL-1 generation, we have exchanged the naphthalene ring of a series of 3-(4-hydroxy-1-naphthalenyl)-2-propenoic acid derivatives represented by compound **7** to an indole ring, and the inhibitory activities on IL-1 generation have been evaluated in an *in vivo* system using the rat carboxymethyl cellulose-lipopolysaccharide (CMC-LPS) air-pouch



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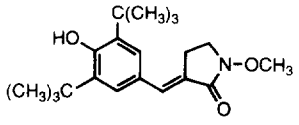

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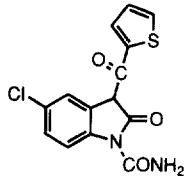

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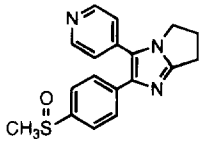

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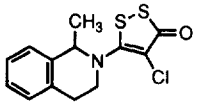

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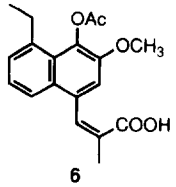

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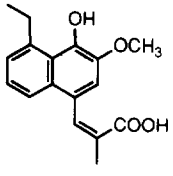


7

model. As a result, we have discovered that a series of (*E*)-3-(4-hydroxy-5-methoxyindole-7-yl)-2-methylpropenoic acid derivatives possess high inhibitory activities against IL-1 generation. Among the compounds evaluated (*E*)-3-[1,3-dimethyl-4-hydroxy-5-methoxy-2-(3,4,5-trimethoxyphenyl)indole-7-yl]-2-methylpropenoic acid (**15m**) inhibited IL-1 generation in the rat CMC-LPS model after oral administration.

In the present paper, we describe the synthesis and pharmacological properties of (*E*)-3-(4-hydroxy-5-methoxyindole-7-yl)-2-methylpropenoic acid derivatives.

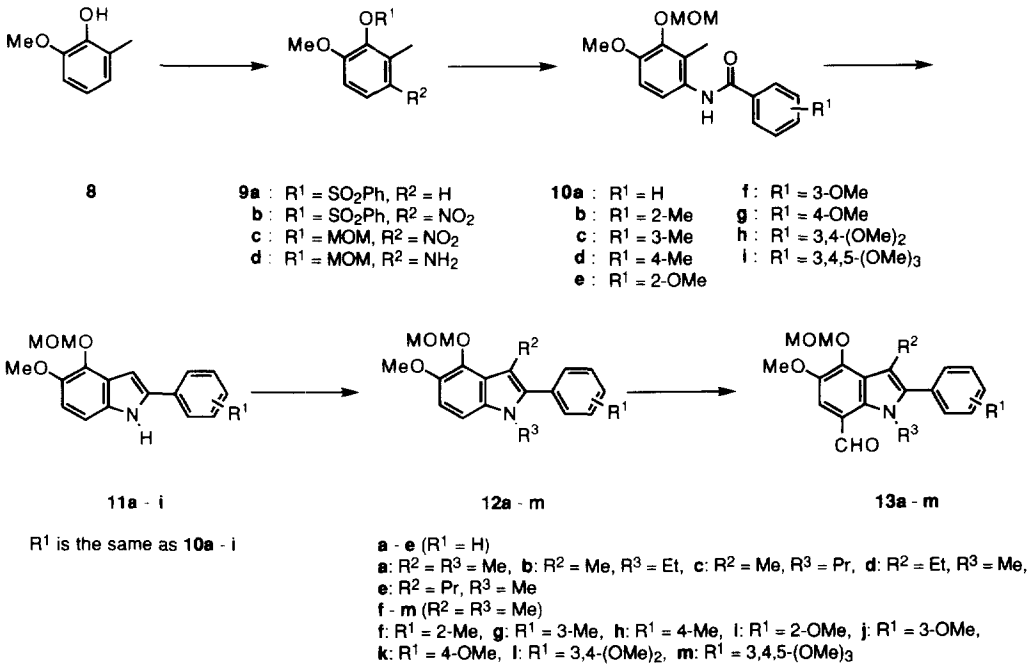
Chemistry

The requisite intermediate aldehydes for the synthesis of a series of (*E*)-3-(4-hydroxy-5-methoxyindole-7-yl)-2-methylpropenoic acid derivatives were prepared as shown in scheme 1. 2-Methoxy-6-methylphenol (**8**) was treated with benzenesulfonyl chloride in the presence of pyridine or *N,N*-diisopropylethylamine to give the sulfonate **9a**. Nitration of **9a** followed by hydrolysis of the phenolic hydroxy group gave 6-methoxy-2-methyl-3-nitrophenol, which was then treated with sodium hydride and chloromethyl methyl ether to yield the nitrobenzene derivative **9c**. Compound **9c** was hydrogenated over 10% palladium on carbon and the resulting amine **9d** was treated with benzoyl chlorides in the presence of pyridine to give the benzamide derivatives **10a–i**. Cyclization of **10a–i** according to the method of Houlihan *et al* [19] gave the 2-phenylindole derivatives **11a–i**. Formylation of **11a** with phosphorus oxychloride and DMF [20] gave 5-methoxy-4-(methoxymethoxy)-2-phenylindole-3-carbaldehyde, which was reduced with an excess of $LiAlH_4$, then methylated on the NH group at the 1-position with sodium hydride and iodomethane to yield the 1,3-dimethylindole derivative **12a**. 1-Ethyl **12b** and 1-propyl **12c** substituted indole derivatives were prepared according to the same procedure described for the preparation of **12a** using iodoethane and *n*-propyl bromide instead of iodomethane. Methylation of the NH group of 5-methoxy-4-(methoxymethoxy)-2-phenylindole-3-carbaldehyde according to the same procedure described for the preparation of **12a** followed by a Wittig reaction with methyl triphenylphosphonium bromide or ethyl triphenylphosphonium bromide gave the vinyl derivatives, which were then hydrogenated over 10% palladium on carbon to yield the 1,3-dialkylated indole derivatives **12d,e**. Vilsmeier formylation of the 7-position of the indole derivatives (**12a–e**) with a large excess of phosphorus oxychloride and DMF gave the 1,3-dialkyl-4-hydroxy-5-methoxy-2-phenylindole-7-carbaldehydes, which were then treated with sodium hydride and chloromethyl methyl ether to yield the desired aldehydes **13a–e**.

Compounds **11b–i** were treated according to the same procedure described for the preparation of **12a** and **13a** to give the other desired aldehydes **13f–m**.

(*E*)-3-(4-Hydroxy-5-methoxyindole-7-yl)-2-methylpropenoic acid derivatives were prepared as shown in scheme 2. The Wadsworth–Emmons reaction between suitable aldehydes **13a–m** and triethyl 2-phosphonopropionate gave the (*E*)-propenoates **14a–m**, which were then hydrolyzed under alkaline conditions (KOH/aqueous EtOH), followed by deprotection of the phenolic hydroxy group with concentrated HCl in acetone to yield the (*E*)-3-(4-hydroxy-5-methoxyindole-7-yl)-2-methylpropenoic acid derivatives **15a–m**.

In order to measure the effects of the double bond configuration on the activity of the propenoic acids, the preparation of the *Z* isomers was attempted according to the same procedure reported for the preparation of a series of 3-(4-hydroxy-1-naphthalenyl)-2-propenoic acid derivatives [18]. However, in the case of the indole series, deprotection of the phenolic hydroxy group of the *Z* isomers in the final step resulted in conversion to the *E* isomers.



Scheme 1. Preparation of 5-methoxy-4-(methoxymethoxy)-2-phenylindole-7-carbaldehyde derivatives.

Pharmacology

The inhibitory activities against IL-1 generation were evaluated with an *in vivo* system using the rat CMC-LPS air-pouch model [18]. At 24 h after the injection of air into the dorsum of rats, sodium carboxymethyl cellulose (CMC-Na) was injected into the air-pouch. Inflammation was induced by injection of LPS 24 h after this CMC injection. The test compounds were administered either orally (200 mg/kg) or locally (final concentration in the exudate was about 100 μM). Four or five animals were used in each group. At 4 h after the LPS injection, 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 [21]. The relative potencies are expressed as inhibitory percentages (% ± SEM).

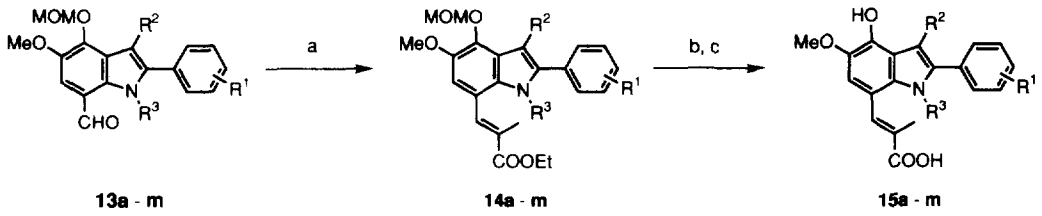
Selected compound was evaluated with an *in vitro* system using human monocytes stimulated with LPS, opsonized zymosan (OZ), and immune complexes (IC). After cultivation for 18 h with the stimulus and the compound being tested, both the extra- and intracellular amounts of IL-1α and β were determined by ELISA. Tests were run in duplicate, and the IC_{50} value determinations were performed by the least-squares method using four concentrations of compound.

In addition, the effects of selected compound on PGE_2 production and granulation tissue formation in the rat CMC-LPS air-pouch model were measured. The amounts of PGE_2 in the exudate supernatant were determined by ELISA. Granulation tissue wet weights were determined at 5 d after the injection of LPS.

Results and discussion

The inhibitory activities of the various compounds against IL-1 generation in the rat CMC-LPS air-pouch model are summarized in table I. Firstly, the effects of the substituents at the 1- and 3-positions on the indole ring were evaluated. Compound **15a** substituted with a methyl group at the 1-position showed high inhibitory activity. On the other hand, compounds **15b,c** with a larger alkyl group such as ethyl or propyl were less active. Of the 3-substituted compounds, the methyl-substituted compound **15a** inhibited IL-1 generation more strongly than the ethyl- or propyl-substituted compounds **15d,e**. From these results, 1,3-dimethyl substitution of the indole ring appeared to yield the most potent inhibitors of IL-1 generation.

Secondly, the effects of substituents on the phenyl group at the 2-position of the indole ring were evaluated. Such substitution affected the inhibitory effects on IL-1 generation. The methyl-substituted compounds **15f–h** were less active than the unsubstituted compound **15a**. On the other hand, the methoxy-substituted compounds **15i–m** showed activity comparable to, or stronger than, that of the latter. The



13a - m 14a - m 15a - m

Scheme 2. Preparation of (*E*)-3-(4-hydroxy-5-methoxyindole-7-yl)-2-methylpropenoic acid derivatives.

Table I. Inhibitory activities against IL-1 generation in the rat CMC-LPS air-pouch model.

Compound	*R¹*	*R²*	*R³*	*Mp*[b] *(°C) dec*	*Formula*[c]	*In vivo air-pouch model: % inhibition*[a]	
						100 μM[d]	*200 mg/kg*[e]
15a	H	Me	Me	188–190	$C_{21}H_{21}NO_4$	90 ± 1	
15b	H	Me	Et	191–192	$C_{22}H_{23}NO_4$	30 ± 4	
15c	H	Me	Pr	187–188	$C_{23}H_{25}NO_4$	Ia[f]	
15d	H	Et	Me	197–198	$C_{22}H_{23}NO_4$	68 ± 9	
15e	H	Pr	Me	191–192	$C_{23}H_{25}NO_4$	69 ± 9	
15f	2-Me	Me	Me	162–163	$C_{22}H_{23}NO_4 \cdot 0.2H_2O$	65 ± 5	
15g	3-Me	Me	Me	163–164	$C_{22}H_{23}NO_4 \cdot 0.3H_2O$	80 ± 5	
15h	4-Me	Me	Me	196–198	$C_{22}H_{23}NO_4$	33 ± 20	
15i	2-OMe	Me	Me	103–104	$C_{22}H_{23}NO_5 \cdot 0.3H_2O$	84 ± 1	
15j	3-OMe	Me	Me	186–188	$C_{22}H_{23}NO_5$	86 ± 1	
15k	4-OMe	Me	Me	183–185	$C_{22}H_{23}NO_5$	100	Ia[f]
15l	3,4-$(OMe)_2$	Me	Me	182–184	$C_{23}H_{25}NO_6 \cdot 0.4H_2O$	94 ± 4	Ia[f]
15m	3,4,5-$(OMe)_3$	Me	Me	192–194	$C_{24}H_{27}NO_7 \cdot 0.3H_2O$	90 ± 6	51 ± 8

[a]Percent inhibition of IL-1 generation in the rat CMC-LPS air-pouch model. Values are the mean of four animals. [b]All compounds were crystallized from water. [c]Compounds were analyzed for C, H, and N, and results agreed to ±0.4% of the calculated values. [d]Local administration. [e]Oral administration. [f]Inactive (Ia) is defined as < 25% inhibition at a screening dose.

inhibitory activities of compounds **15k–m**, which showed almost equal activity after local administration, were evaluated after oral administration. Only one compound, **15m**, inhibited IL-1 generation at a dose of 200 mg/kg. Compound **15k** did not show inhibitory activity after oral administration, in spite of its complete inhibition of IL-1 generation after local administration. In order to clarify the reason for these differences, the pharmacokinetic profiles of these three compounds were examined (table II). Compound **15m** exhibited higher plasma concentrations and AUC values than compounds **15k,l**. These results indicate that the activity of compound **15m** observed in the *in vivo* model after oral administration reflects its good pharmacokinetic profile.

From this *in vivo* study using the rat CMC-LPS air-pouch model after local administration and/or after oral administration, (*E*)-3-[1,3-dimethyl-4-hydroxy-5-methoxy-2-(3,4,5-trimethoxyphenyl)indole-7-yl]-2-methylpropenoic acid (**15m**) was chosen as an orally active inhibitor of IL-1 generation.

The pharmacological profile of compound **15m** was compared to that of **7**. *In vitro* inhibitory effects of these compounds on IL-1 generation were evaluated simultaneously using human monocytes (table III). Similarly to compound **7**, compound **15m** inhibited the generation of IL-1α and β from human monocytes caused by various stimuli such as LPS, OZ, and IC. This universality of the *in vitro* inhibitory effects on IL-1 generation was also found in a steroidal anti-inflammatory drug, prednisolone.

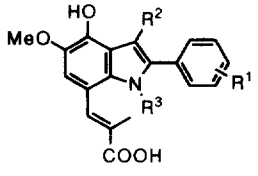
In order to investigate whether the compounds in this series inhibit protein synthesis, the inhibitory activities of compound **15m** and emetine, a protein synthesis inhibitor used as a positive control, against incorporation of [H^3]amino acid were evaluated using human monocytes (table IV). Emetine inhibited protein synthesis in parallel with its inhibition of IL-1 generation. However, compound **15m** did not inhibit protein synthesis even at a dose of 30 μM. This result shows that the inhibitory activities of this series on IL-1 generation are not due to general protein synthesis inhibition.

The effects of compounds **7** and **15m** in the CMC-LPS air-pouch model were also measured simultaneously after local administration (fig 1). These compounds showed almost equal inhibitory activity against IL-1 generation (**7**: 98 ± 1%; **15m**: 93 ± 2%). Moreover, both compounds suppressed granulation tissue formation (**7**: 77 ± 10%; **15m**: 70 ± 7%), which is thought to be a suitable pre-clinical index of synovium proliferation in RA. Compound **7** showed complete inhibition (99 ± 1%) of the PGE_2 generation by inflammatory tissue, but compound **15m** was less active (56 ± 9%).

Conclusion

A series of (*E*)-3-(4-hydroxy-5-methoxyindole-7-yl)-2-methylpropenoic acid derivatives was synthesized and the inhibitory activities of its members on IL-1 generation were evaluated by the *in vivo* rat CMC-LPS air-pouch model after local administration. Many of the compounds in this new series were found to be inhibitors of IL-1 generation. Regarding substituents on the indole ring, structure-activity relationships indicated that a methyl substituent at the 1- and 3-positions are important for activity. For the 2-phenyl group, the methoxy-substituted compounds had higher inhibitory activities than the methyl-substi-

Table II. Pharmacokinetic parameters of compounds **15k–m** in plasma after oral administration (50 mg/kg) to male F_{344} rats.



15k - m (R^2 = R^3 = Me)

Compound	*R^1*	*n*[a]	*C_{max}*[b] *(μg/ml)*	*AUC*[c] *(μg•h/ml)*
15k	4-OMe	3	3.93 ± 0.71	6.96 ± 0.91
15l	3,4-$(OMe)_2$	3	20.60 ± 3.29	26.52 ± 1.05
15m	3,4,5-$(OMe)_3$	3	58.00 ± 14.21	44.11 ± 1.21

[a]Number of rats. [b]Maximum concentration (mean ± SEM). [c]Area (mean ± SEM) under the concentration *vs* time course (0–8 h).

Table III. *In vitro* inhibitory effects of compounds **7** and **15m** on IL-1 generation.

Compound	*Stimulus*	IC_{50}[a] (μM)	
		IL-1α	*IL-1β*
7	LPS	4.6	6.7
	OZ	3.7	2.7
	IC	3.1	2.0
15m	LPS	6.4	8.6
	OZ	5.5	7.6
	IC	3.8	4.4

[a]Concentration of drug inhibiting IL-1 generation by 50% of control values. IC_{50} values were calculated by the least-squares method. Values are the mean of two independent determinations.

tuted compounds. Among the compounds evaluated, (*E*)-3-[1,3-dimethyl-4-hydroxy-5-methoxy-2-(3,4,5-trimethoxyphenyl)indole-7-yl]-2-methylpropenoic acid (**15m**) inhibited IL-1 generation after oral administration in the rat CMC-LPS air-pouch model. Similarly to compound **7**, compound **15m** inhibited IL-1 generation from human monocytes stimulated with various reagents such as LPS, OZ, and IC. Moreover, compound **15m** inhibited the formation of granulation tissue in the rat CMC-LPS air-pouch model.

Table IV. Inhibitory effects of compounds **15m** and emetine against IL-1 generation and protein synthesis.

Compound	*IL-1α*[b]	IC_{50}[a] (μM)	
		IL-1β[b]	*Protein*[c]
15m	6.4	8.6	>30
Emetine	0.10	0.05	0.12

[a]IC_{50} values were calculated by the least-squares method using four concentrations of compound. [b]Stimulated with LPS. [c]Values are the mean of duplicate samples.

Experimental protocols

Chemistry

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 Varian Unity 400 spectrometer with tetramethylsilane as an internal standard. All organic extracts were dried over anhydrous $MgSO_4$, and the solvent was removed with a rotary evaporator under reduced pressure. Merck silica gel 60, 70–230 mesh or 230–400 mesh, was used for flash column chromatography. Thin-layer chromatography (TLC) was developed using Merck silica gel 60F-254 precoated glass plates. Compounds were detected on TLC by UV light (254 nm).

2-Methoxy-6-methyl-1-phenyl benzenesulfonate ***9a***

To a solution of 2-methoxy-6-methylphenol (**8**) (135 g, 0.98 mol) in pyridine (240 ml) at 0°C was added dropwise benzenesulfonyl chloride (153 ml, 1.20 mol) over a period of 30 min. After being stirred at room temperature for 12 h, the mixture was poured into ice water and extracted with EtOAc. The organic extract was washed successively with dilute HCl

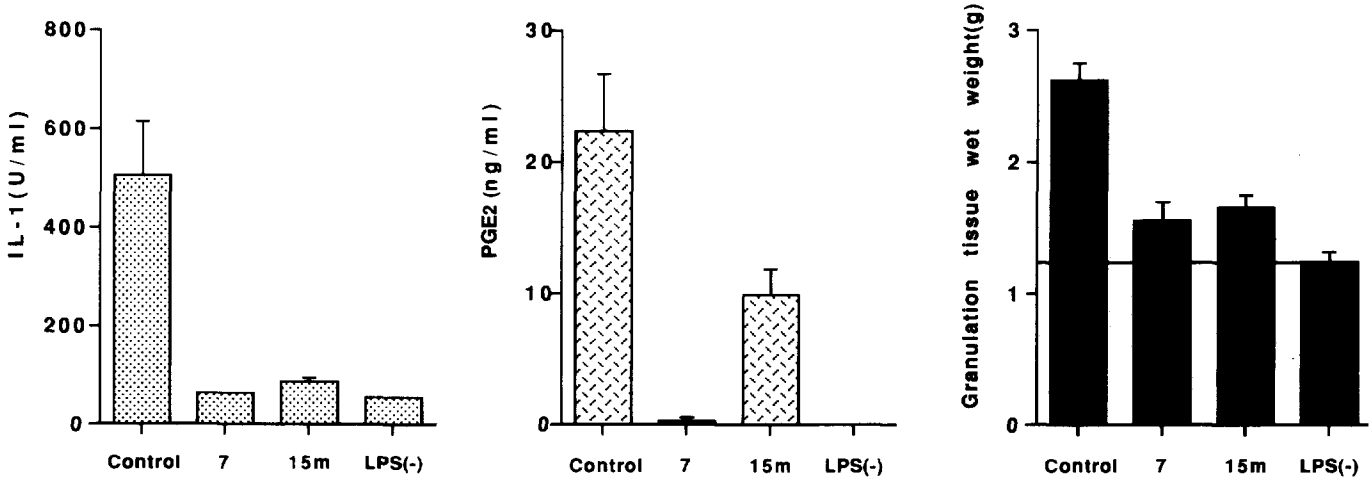


Fig 1. Inhibitory effects of compounds **7** and **15m** in the rat CMC-LPS air-pouch model after local administration. Five animals were used in each group.

and brine, dried, and evaporated. The crude residue was purified by column chromatography on silica gel eluting with EtOAc/hexane (1:9) to afford **9a** (172 g, 63%) as a colorless solid: mp 57–58°C; ^{1}H-NMR ($CDCl_3$) δ 2.31 (s, 3 H), 3.45 (s, 3 H), 6.68 (d, J = 8.0 Hz, 1 H), 6.81 (d, J = 8.0 Hz, 1 H), 7.09 (t, J = 8.0 Hz, 1 H), 7.53–7.58 (m, 2 H), 7.64–7.69 (m, 1 H), 7.96–8.00 (m, 2 H).

6-Methoxy-2-methyl-3-nitro-1-phenyl benzenesulfonate ***9b***
Concentrated HNO_3 (31.4 ml, 0.42 mol) was added dropwise to acetic anhydride (400 ml) at 0°C. After stirring of the mixture at the same temperature for 10 min, a solution of **9a** (100 g, 0.36 mol) in acetic anhydride (100 ml) was added dropwise at –20°C. The reaction mixture was allowed to warm to room temperature and stirring was continued for 12 h. The mixture was poured into ice water and extracted with EtOAc. The organic extract was washed with brine, dried, and evaporated. The crude residue was purified by column chromatography on silica gel eluting with EtOAc/hexane (1:4) to afford **9b** (60 g, 52%) as a pale-yellow solid: mp 121–122°C; ^{1}H-NMR ($CDCl_3$) δ 2.47 (s, 3 H), 3.63 (s, 3 H), 6.82 (d, J = 9.0 Hz, 1 H), 7.58–7.63 (m, 2 H), 7.70–7.75 (m, 1 H), 7.96–8.00 (m, 2 H), 8.01 (d, J = 9.0 Hz, 1 H).

2-(Methoxymethoxy)-3-methyl-4-nitroanisole ***9c***
To a solution of **9b** (60 g, 186 mmol) in EtOH (500 ml) was added a solution of KOH (26 g, 464 mmol) in H_2O (100 ml) and this mixture was then stirred at 60°C for 1 h. The cooled solution was acidified with 2 N HCl and extracted with EtOAc. The organic extract was washed with brine, dried, and evaporated. The resulting precipitate was washed with diisopropyl ether to afford 6-methoxy-2-methyl-3-nitrophenol (25.0 g, 74%) as a pale-yellow solid: mp 113–114°C; ^{1}H-NMR ($CDCl_3$) δ 2.49 (s, 3 H), 3.98 (s, 3 H), 5.94 (s, 1 H), 6.77 (d, J = 8.8 Hz, 1 H), 7.63 (d, J = 8.8 Hz, 1 H).

To a solution of this phenol (42.9 g, 234 mmol) in DMF (300 ml) at 0°C was added in portions sodium hydride (55% dispersion in mineral oil; 11.2 g, 257 mmol), followed by addition of chloromethyl methyl ether (21.3 ml, 280 mmol) at the same temperature. After being stirred at room temperature for 30 min, the mixture was poured into water and extracted with EtOAc. The organic extract was washed successively with water and brine, dried, and evaporated. The crude residue was purified by column chromatography on silica gel eluting with EtOAc/hexane (1:9) to afford **9c** (48.4 g, 91%) as a pale-yellow solid: mp 64–65°C; ^{1}H-NMR ($CDCl_3$) δ 2.56 (s, 3 H), 3.61 (s, 3 H), 3.93 (s, 3 H), 5.10 (s, 2 H), 6.83 (d, J = 8.8 Hz, 1 H), 7.85 (d, J = 8.8 Hz, 1 H).

4-Methoxy-3-(methoxymethoxy)-2-methylaniline ***9d***
A solution of **9c** (48.4 g, 213 mmol) in MeOH/EtOAc (150 ml/150 ml) was hydrogenated over 10% palladium on carbon (water content ~50%; 2.0 g) at 1 atm for 48 h. The catalyst was filtered off and the filtrate was evaporated to afford **9d** (41.7 g, 99%) as a pale-brown solid: mp 48–49°C; ^{1}H-NMR ($CDCl_3$) δ 2.15 (s, 3 H), 3.39 (br, s, 2 H), 3.59 (s, 3 H), 3.76 (s, 3H), 5.08 (s, 2H), 6.43 (d, J = 8.8 Hz, 1 H), 6.64 (d, J = 8.8 Hz, 1 H).

4'-Methoxy-3'-(methoxymethoxy)-2'-methylbenzamide ***10a***
To a solution of **9d** (41.7 g, 211 mmol) in CH_2Cl_2 (400 ml) at 0°C was added pyridine (25.6 ml, 317 mmol) and benzoyl chloride (27.0 ml, 233 mmol). This mixture was stirred at room temperature for 10 min, water and dilute HCl were added, and the mixture was extracted with MeOH/CH_2Cl_2 (1:9). The organic extract was washed successively with water and brine, dried, and evaporated. Diisopropyl ether was added to the residue and the crystalline precipitate was collected by filtration to afford **10a** (54.3 g, 85%) as a colorless solid: mp 185–186°C; ^{1}H-NMR (DMSO-d_6) δ 2.11 (s, 3 H), 3.46 (s, 3 H), 3.78 (s, 3 H), 5.01 (s, 2 H), 6.90 (d, J = 8.8 Hz, 1 H), 7.02 (d, J = 8.8 Hz, 1 H), 7.46–7.60 (m, 3H), 7.95 (d, J = 7.2 Hz, 2 H), 9.81 (br, s, 1 H).

5-Methoxy-4-(methoxymethoxy)-2-phenylindole ***11a***
To a suspension of **10a** (45.0 g, 149 mmol) in anhydrous THF (300 ml) at 13–16°C under a nitrogen atmosphere was added dropwise *n*-BuLi (1.6 M in hexane; 205 ml, 328 mmol) over a period of 1 h. After being stirred at room temperature for 12 h, the mixture was poured into water and extracted with EtOAc. The organic extract was washed successively with water and brine, dried, and evaporated. The crude residue was purified by column chromatography on silica gel eluting with EtOAc/hexane (1:9) to afford **11a** (28.6 g, 68%) as a colorless solid: mp 91–92°C; ^{1}H-NMR ($CDCl_3$) δ 3.66 (s, 3 H), 3.90 (s, 3 H), 5.35 (s, 2 H), 6.91 (dd, J = 1.2, 2.4 Hz, 1 H), 6.95 (d, J = 8.8 Hz, 1 H), 7.09 (dd, J = 1.2, 8.8 Hz, 1 H), 7.30–7.35 (m, 1 H), 7.41–7.46 (m, 2 H), 7.63–7.68 (m, 2 H), 8.25 (br, s, 1 H).

1,3-Dimethyl-5-methoxy-4-(methoxymethoxy)-2-phenylindole ***12a***
Phosphorus oxychloride (2.2 ml, 23.6 mmol) was added dropwise to DMF (30 ml) at 0°C. After stirring of the mixture at room temperature for 30 min, a solution of **11a** (5.5 g, 19.4 mmol) in DMF (20 ml) was added dropwise and stirring was continued for 30 min. The mixture was poured into 5 N NaOH (50 ml), the resulting precipitate was collected by filtration and then washed with water to afford 5-methoxy-4-(methoxymethoxy)-2-phenylindole-3-carbaldehyde (5.6 g, 92%) as a yellow solid: mp 171–172°C; ^{1}H-NMR ($CDCl_3$) δ 3.61 (s, 3 H), 3.92 (s, 3 H), 5.32 (s, 2 H), 7.00 (d, J = 8.8 Hz, 1 H), 7.18 (d, J = 8.8 Hz, 1 H), 7.44–7.52 (m, 3 H), 7.72–7.78 (m, 2 H), 10.43 (s, 1 H), 10.82 (br, s, 1 H).

To a suspension of $LiAlH_4$ (2.68 g, 70 mmol) in THF (100 ml) at 0°C was added in portions the above indole-3-carbaldehyde derivative (5.5 g, 17.7 mmol) and the reaction mixture was refluxed for 1 h. After cooling of the reaction mixture to 0°C, 1 N HCl was added and the mixture was extracted with EtOAc. The organic extract was washed successively with water and brine, dried, and evaporated. The crude residue was purified by column chromatography on silica gel eluting with EtOAc/hexane (1:4) to afford 5-methoxy-4-(methoxymethoxy)-3-methyl-2-phenylindole (4.9 g, 93%) as a colorless solid: mp 107–108°C; ^{1}H-NMR ($CDCl_3$) δ 2.62 (s, 3 H), 3.64 (s, 3 H), 3.89 (s, 3 H), 5.28 (s, 2 H), 6.91 (d, J = 8.8 Hz, 1 H), 7.04 (d, J = 8.8 Hz, 1 H), 7.32–7.39 (m, 1 H), 7.41–7.49 (m, 2 H), 7.50–7.57 (m, 2 H), 7.87 (br, s, 1 H).

To a suspension of this 3-methyl-2-phenylindole derivative (1.78 g, 6.0 mmol) in DMF (30 ml) at 0°C was added sodium hydride (60% dispersion in mineral oil; 264 mg, 6.6 mmol), followed by addition of iodomethane (0.45 ml, 7.2 mmol) at the same temperature. After stirring this mixture at room temperature for 10 min, water was added and the mixture was extracted with EtOAc. The organic extract was washed successively with water and brine, dried, and evaporated. The crude residue was purified by column chromatography on silica gel eluting with EtOAc/hexane (1:9) to afford **12a** (1.79 g, 96%) as a yellow solid: mp 75–76°C; ^{1}H-NMR ($CDCl_3$) δ 2.43 (s, 3 H), 3.53 (s, 3 H), 3.64 (s, 3 H), 3.90 (s, 3H), 5.27 (s, 2H), 6.97 (d, J = 8.8 Hz, 1 H),7.01 (d, J = 8.8 Hz, 1 H), 7.35–7.44 (m, 3 H), 7.46–7.51 (m, 2 H).

1-Ethyl-5-methoxy-4-(methoxymethoxy)-3-methyl-2-phenylindole ***12b***

5-Methoxy-4-(methoxymethoxy)-3-methyl-2-phenylindole (2.3 g, 7.7 mmol) was treated according to the same procedure described in the preparation of **12a** using iodoethane (0.74 ml, 9.2 mmol) instead of iodomethane to afford **12b** (2.34 g, 93%) as a pale-yellow oil; ^{1}H-NMR ($CDCl_3$) δ 1.18 (t, J = 7.2 Hz, 3 H), 2.38 (s, 3 H), 3.64 (s, 3 H), 3.90 (s, 3 H), 3.97 (q, J = 7.2 Hz, 2 H), 5.27 (s, 2 H), 6.95 (d, J = 8.8 Hz, 1 H), 7.02 (d, J = 8.8 Hz, 1 H), 7.34–7.52 (m, 5 H).

5-Methoxy-4-(methoxymethoxy)-3-methyl-2-phenyl-1-propylindole ***12c***

5-Methoxy-4-(methoxymethoxy)-3-methyl-2-phenylindole (2.0 g, 6.7 mmol) was treated according to the same procedure described in the preparation of **12a** using 1-bromopropane (0.73 ml, 8.0 mmol) instead of iodomethane to afford **12c** (2.14 g, 94%) as a pale-yellow oil; ^{1}H-NMR ($CDCl_3$) δ 0.72 (t, J = 7.2 Hz, 3 H), 1.54–1.66 (m, 2 H), 2.37 (s, 3 H), 3.64 (s, 3 H), 3.85–3.92 (m, 2 H), 3.90 (s, 3 H), 5.27 (s, 2 H), 6.94 (d, J = 8.8 Hz, 1 H), 7.01 (d, J = 8.8 Hz, 1 H), 7.34–7.50 (m, 5 H).

3-Ethyl-5-methoxy-4-(methoxymethoxy)-1-methyl-2-phenylindole ***12d***

5-Methoxy-4-(methoxymethoxy)-2-phenylindole-3-carbaldehyde (5.0 g, 16.0 mmol) was treated according to the same procedure described in the preparation of **12a** (in part) to afford 5-methoxy-4-(methoxymethoxy)-1-methyl-2-phenylindole-3-carbaldehyde (4.75 g, 91%) as a pale-yellow solid: mp 91–92°C; ^{1}H-NMR ($CDCl_3$) δ 3.57 (s, 3 H), 3.62 (s, 3 H), 3.95 (s, 3 H), 5.31 (s, 2 H), 7.09 (d, J = 8.8 Hz, 1 H), 7.12 (d, J = 8.8 Hz, 1 H), 7.43–7.48 (m, 2 H), 7.51–7.55 (m, 3 H), 10.13 (s, 1 H).

A mixture of methyl triphenylphosphonium bromide (1.1 g, 3.1 mmol), sodium hydride (55% dispersion in mineral oil; 134 mg, 3.1 mmol), and this indole-3-carbaldehyde derivative (500 mg, 1.55 mmol) in dimethoxyethane (15 ml) was refluxed for 5 h. After cooling of the reaction mixture to 0°C, water was added and the mixture was extracted with EtOAc. The organic extract was washed successively with water and brine, dried, and evaporated. The crude residue was purified by column chromatography on silica gel eluting with EtOAc/hexane (1:19) to afford 5-methoxy-4-(methoxymethoxy)-1-methyl-2-phenyl-3-vinylindole (460 mg, 92%) as a pale-yellow oil; ^{1}H-NMR ($CDCl_3$) δ 3.49 (s, 3 H), 3.61 (s, 3 H), 3.92 (s, 3 H), 4.98 (dd, J = 2.4, 11.6 Hz, 1 H), 5.18 (s, 2 H), 5.22 (dd, J = 2.4, 18.0 Hz, 1 H), 6.96–7.06 (m, 3 H), 7.38–7.52 (m, 5 H).

A solution of this indole (460 mg, 1.4 mmol) in EtOAc (20 ml) was hydrogenated over 10% palladium on carbon (water content ~50%; 20 mg) at 1 atm for 30 min. The catalyst was filtered off and the filtrate was evaporated. The residue was purified by column chromatography on silica gel eluting with EtOAc/hexane (1:19) to afford **12d** (420 mg, 91%) as a colorless oil; ^{1}H-NMR ($CDCl_3$) δ 1.22 (t, J = 7.2 Hz, 3 H), 2.77 (q, J = 7.2 Hz, 2 H), 3.50 (s, 3 H), 3.63 (s, 3 H), 3.90 (s, 3 H), 5.28 (s, 2 H), 6.97 (d, J = 8.8 Hz, 1 H), 7.01 (d, J = 8.8 Hz, 1 H), 7.35–7.39 (m, 2 H), 7 40–7.50 (m, 3 H)

5-Methoxy-4-(methoxymethoxy)-1-methyl-2-phenyl-3-propylindole ***12e***

5-Methoxy-4-(methoxymethoxy)-1-methyl-2-phenylindole-3-carbaldehyde (3.0 g, 9.2 mmol) was treated according to the same procedure described in the preparation of **12d** using ethyl triphenylphosphonium bromide (6.85 g, 18.4 mmol) instead of methyl triphenylphosphonium bromide to afford **12e** (2.98 g, 95%) as a colorless oil; ^{1}H-NMR ($CDCl_3$) δ 0.83 (t, J = 7.6 Hz, 3 H), 1.58–1.70 (m, 2 H), 2.68–2.74 (m, 2 H), 3.49 (s, 3 H), 3.64 (s, 3 H), 3.90 (s, 3 H), 5.27 (s, 2 H), 6.97 (d, J = 8.8 Hz, 1 H), 7.00 (d, J = 8.8 Hz, 1 H), 7.34–7.38 (m, 2 H), 7.40–7.50 (m, 3 H).

1,3-Dimethyl-5-methoxy-4-(methoxymethoxy)-2-phenylindole-7-carbaldehyde ***13a***

Phosphorus oxychloride (5.4 ml, 58 mmol) was added dropwise to DMF (20 ml) at 0°C. After stirring of the mixture at the same temperature for 30 min, a solution of **12a** (1.79 g, 5.8 mmol) in DMF (10 ml) was added dropwise and stirring was continued at room temperature for 12 h. The mixture was poured into water and extracted with EtOAc. The organic extract was washed successively with water and brine, dried, and evaporated to afford crude 1,3-dimethyl-4-hydroxy-5-methoxy-2-phenylindole-7-carbaldehyde, which was used in the next step without further purification.

This crude aldehyde was treated according to the same procedure described in the preparation of **9c** to afford **13a** (1.4 g, 72%) as a yellow solid: mp 92–93°C; ^{1}H-NMR ($CDCl_3$) δ 2.43 (s, 3 H), 3.60 (s, 3 H), 3.74 (s, 3 H), 3.95 (s, 3 H), 5.40 (s, 2 H), 7.38–7.54 (m, 5 H), 7.51 (s, 1 H), 10.36 (s, 1 H).

1-Ethyl-5-methoxy-4-(methoxymethoxy)-3-methyl-2-phenylindole-7-carbaldehyde ***13b***

Compound **12b** (2.34 g, 7.2 mmol) was treated according to the same procedure described in the preparation of **13a** to afford **13b** (1.52 g, 60%) as a yellow oil; ^{1}H-NMR ($CDCl_3$) δ 1.01 (t, J = 7.2 Hz, 3 H), 2.35 (s, 3 H), 3.60 (s, 3 H), 3.94 (s, 3 H), 4.35 (q, J = 7.2 Hz, 2 H), 5.40 (s, 2 H), 7.36–7.39 (m, 2 H), 7.41–7.51 (m, 4 H), 10.22 (s, 1 H).

5-Methoxy-4-(methoxymethoxy)-3-methyl-2-phenyl-1-propylindole-7-carbaldehyde ***13c***

Compound **12c** (2.14 g, 6.3 mmol) was treated according to the same procedure described in the preparation of **13a** to afford **13c** (1.72 g, 74%) as a yellow oil; ^{1}H-NMR ($CDCl_3$) δ 0.56 (t, J = 7.2 Hz, 3 H), 1.40–1.52 (m, 2 H), 2.36 (s, 3 H), 3.60 (s, 3 H), 3.95 (s, 3H), 4.26 (t, J = 7.2 Hz, 2 H), 5.40 (s, 2 H), 7.36–7.39 (m, 2 H), 7.42–7.52 (m, 4 H), 10.19 (s, 1 H).

3-Ethyl-5-methoxy-4-(methoxymethoxy)-1-methyl-2-phenylindole-7-carbaldehyde ***13d***

Compound **12d** (1.40 g, 4.3 mmol) was treated according to the same procedure described in the preparation of **13a** to afford **13d** (1.26 g, 83%) as a yellow oil; ^{1}H-NMR ($CDCl_3$) δ 1.20 (t, J = 7.2 Hz, 3H), 2.77 (q, J = 7.2 Hz, 2 H), 3.59 (s, 3 H), 3.71 (s, 3 H), 3.95 (s, 3 H), 5.42 (s, 2 H), 7.38–7.41 (m, 2 H), 7.43–7.54 (m, 3 H), 7.52 (s, 1 H), 10.37 (s, 1 H).

5-Methoxy-4-(methoxymethoxy)-1-methyl-2-phenyl-3-propylindole-7-carbaldehyde ***13e***

Compound **12e** (2.98 g, 8.8 mmol) was treated according to the same procedure described in the preparation of **13a** to afford **13e** (2.60 g, 80 %) as a yellow oil; ^{1}H-NMR ($CDCl_3$) δ 0.83 (t, J = 7.6 Hz, 3 H), 1.56–1.66 (m, 2 H), 2.69–2.75 (m, 2 H), 3.60 (s, 3 H), 3.71 (s, 3 H), 3.94 (s, 3 H), 5.41 (s, 2 H), 7.36–7.40 (m, 2 H), 7.42–7.53 (m, 3 H), 7.52 (s, 1 H), 10.37 (s, 1 H).

1,3-Dimethyl-5-methoxy-4-(methoxymethoxy)-2-phenylindole-7-carbaldehyde derivatives ***13f–m***

Compounds **11b–i** were treated according to the same procedure described for the preparation of **12a** and **13a** to afford the following aldehydes **13f–m**.

1,3-Dimethyl-5-methoxy-4-(methoxymethoxy)-2-(2-methylphenyl)indole-7-carbaldehyde ***13f***. Yellow solid: mp 67–68°C; ^{1}H-NMR ($CDCl_3$) δ 2.13 (s, 3 H), 2.27 (s, 3 H), 3.59 (s, 3 H), 3.65 (s, 3 H), 3.95 (s, 3 H), 5.41 (s, 2 H), 7.22–7.41 (m, 4 H), 7.52 (s, 1 H), 10.37 (s, 1 H).

1,3-Dimethyl-5-methoxy-4-(methoxymethoxy)-2-(3-methylphenyl)indole-7-carbaldehyde ***13g***. Yellow solid: mp 58–59°C; ^{1}H-NMR ($CDCl_3$) δ 2.42 (s, 3 H), 2.44 (s, 3 H), 3.60 (s, 3 H), 3.73 (s, 3 H), 3.94 (s, 3 H), 5.40 (s, 2 H), 7.19 (d, J = 8.0 Hz, 1 H), 7.20 (s, 1 H), 7.24–7.28 (m, 1 H), 7.39 (t, J = 8.0 Hz, 1 H), 7.51 (s, 1 H), 10.36 (s, 1 H).

1,3-Dimethyl-5-methoxy-4-(methoxymethoxy)-2-(4-methylphenyl)indole-7-carbaldehyde ***13h***. Yellow solid: mp 79–80°C; ^{1}H-NMR ($CDCl_3$) δ 2.42 (s, 3 H), 2.45 (s, 3 H), 3.60 (s, 3 H), 3.73 (s, 3 H), 3.94 (s, 3 H), 5.39 (s, 2 H), 7.28 (d, J = 8.0 Hz, 2 H), 7.32 (d, J = 8.0 Hz, 2 H), 7.50 (s, 1 H), 10.35 (s, 1 H).

1,3-Dimethyl-5-methoxy-4-(methoxymethoxy)-2-(2-methoxyphenyl)indole-7-carbaldehyde ***13i***. Yellow solid: mp 80–81°C; ^{1}H-NMR ($CDCl_3$) δ 2.35 (s, 3 H), 3.60 (s, 3 H), 3.70 (s, 3 H), 3.82 (s, 3 H), 3.93 (s, 3 H), 5.38 (m, 2 H), 7.03–7.11 (m, 2 H), 7.25–7.28 (m, 1 H), 7.44–7.49 (m, 1 H), 7.53 (s, 1 H), 10.43 (s, 1 H).

1,3-Dimethyl-5-methoxy-4-(methoxymethoxy)-2-(3-methoxyphenyl)indole-7-carbaldehyde ***13j***. Yellow solid: mp 58–59°C; ^{1}H-NMR ($CDCl_3$) δ 2.43 (s, 3 H), 3.60 (s, 3 H), 3.74 (s, 3 H), 3.87 (s, 3 H), 3.94 (s, 3 H), 5.40 (s, 2 H), 6.91–6.94 (m, 1 H), 6.96–7.01 (m, 2 H), 7.42 (t, J = 8.8 Hz, 1 H), 7.51 (s, 1 H), 10.35 (s, 1 H).

1,3-Dimethyl-5-methoxy-4-(methoxymethoxy)-2-(4-methoxyphenyl)indole-7-carbaldehyde ***13k***. Yellow solid: mp 67–68°C; ^{1}H-NMR ($CDCl_3$) δ 2.41 (s, 3 H), 3.60 (s, 3 H), 3.73 (s, 3 H), 3.89 (s, 3 H), 3.94 (s, 3 H), 5.39 (s, 2 H), 7.04 (d, J = 8.8 Hz, 2 H), 7.32 (d, J = 8.8 Hz, 2 H), 7.50 (s, 1 H), 10.36 (s, 1 H).

2-(3,4-Dimethoxyphenyl)-1,3-dimethyl-5-methoxy-4-(methoxymethoxy)indole-7-carbaldehyde ***13l***. Yellow solid: mp 102–103°C; ^{1}H-NMR ($CDCl_3$) δ 2.42 (s, 3 H), 3.60 (s, 3 H), 3.73 (s, 3 H), 3.92 (s, 3 H), 3.94 (s, 3 H), 3.96 (s, 3 H), 5.39 (s, 2 H), 6.89 (d, J = 2.0 Hz, 1 H), 6.95 (dd, J = 2.0, 8.0 Hz, 1 H), 7.00 (d, J = 8.0 Hz, 1 H), 7.49 (s, 1 H), 10.34 (s, 1 H).

1,3-Dimethyl-5-methoxy-4-(methoxymethoxy)-2-(3,4,5-trimethoxyphenyl)indole-7-carbaldehyde ***13m***. Yellow solid: mp 105–106°C; ^{1}H-NMR ($CDCl_3$) δ 2.45 (s, 3 H), 3.60 (s, 3 H), 3.75 (s, 3 H), 3.90 (s, 6 H), 3.94 (s, 3 H), 3.95 (s, 3 H), 5.40 (s, 2 H), 6.60 (s, 2 H), 7.50 (s, 1 H), 10.33 (s, 1 H).

Ethyl (E)-3-[1,3-dimethyl-5-methoxy-4-(methoxymethoxy)-2-(3,4,5-trimethoxyphenyl)indole-7-yl]-2-methylpropenoate ***14m***
To a suspension of sodium hydride (60% dispersion in mineral oil; 51 mg, 1.28 mmol) in DMF (15 ml) at 0°C was added a solution of triethyl 2-phosphonopropionate (333 mg, 1.40 mmol) in DMF (5 ml). After stirring of the mixture at the same temperature for 10 min, a solution of **13m** (500 mg, 1.16 mmol) in DMF (10 ml) was added and stirring was continued at room temperature for 2 h. The mixture was poured into water and extracted with EtOAc. The organic extract was washed successively with water and brine, dried, and evaporated. The crude residue was purified by column chromatography on silica gel eluting with EtOAc/hexane (1:4) to afford **14m** (560 mg, 93%) as a yellow solid: mp 101–102°C; ^{1}H-NMR ($CDCl_3$) δ 1.35 (t, J = 7.2 Hz, 3 H), 2.07 (d, J = 1.6 Hz, 3 H), 2.42 (s, 3 H), 3.61 (s, 3 H), 3.64 (s, 3 H), 3.87 (s, 3 H), 3.88 (s, 6 H), 3.93 (s, 3 H), 4.28 (q, J = 7.2 Hz, 2 H), 5.28 (s, 2 H), 6.56 (s, 2 H), 6.75 (s, 1 H), 8.21 (s, 1 H).

(E)-3-[1,3-Dimethyl-4-hydroxy-5-methoxy-2-(3,4,5-trimethoxyphenyl)indole-7-yl]-2-methylpropenoic acid ***15m***
To a solution of **14m** (540 mg, 1.05 mmol) in EtOH (20 ml) was added a solution of KOH (118 mg, 2.10 mmol) in H_2O (5 ml) and this mixture was then stirred at 60°C for 30 min. The cooled solution was acidified with 1 N HCl and extracted with EtOAc. The organic extract was washed successively with water and brine, dried, and evaporated to afford (*E*)-3-[1,3-dimethyl-5-methoxy-4-(methoxymethoxy)-2-(3,4,5-trimethoxyphenyl)indole-7-yl]-2-methylpropenoic acid (510 mg, 100%) as a yellow solid: mp 194–195°C; ^{1}H-NMR ($CDCl_3$) δ 2.12 (d, J = 1.2 Hz, 3 H), 2.43 (s, 3 H), 3.63 (s, 3 H), 3.64 (s, 3 H), 3.89 (s, 9 H), 3.94 (s, 3 H), 5.30 (s, 2 H), 6.56 (s, 2 H), 6.80 (s, 1 H), 8.38 (s, 1 H).

To a solution of this acid (200 mg, 0.41 mmol) in acetone (10 ml) at room temperature was added concentrated HCl (0.5 ml). After being stirred at the same temperature for 2 h, the mixture was poured into water. The resulting precipitate was collected by filtration and then washed with water to afford **15m** (170 mg, 93%) as a yellow solid: mp 192–194°C dec; ^{1}H-NMR ($CDCl_3$) δ 2.14 (d, J = 1.6 Hz, 3 H), 2.46 (s, 3 H), 3.64 (s, 3H), 3.89 (s, 6H), 3.92 (s, 3H), 3.93 (s, 3H), 5.97 (br, s, 1 H), 6.57 (s, 2 H), 6.81 (s, 1 H), 8.41 (s, 1 H).

Pharmacology

In vitro IL-1 generation using human monocytes

Human mononuclear cells were isolated from the peripheral blood of healthy volunteers using Ficoll–Hypaque density gradient centrifugation and then washed with Hanks' balanced salt solution (HBSS). The cells were adjusted to 4 x 10^6 cells/ml in RPMI 1640 medium (Gibco) containing 10% heat-inactivated autologous serum, and this suspension was seeded into 48-well plastic culture plates (2 x 10^6 cells/0.5 ml/well). The cells were allowed to adhere for 2 h, and non-adherent cells were removed by rinsing, the remaining cells were used as the monocytes preparation. The monocytes were cultured in the presence or absence of test drugs for 18 h in RPMI 1640 medium (500 μl) containing 1% heat-inactivated autologous serum and 0.1% DMSO along with various stimuli such as LPS (10 ng/ml, Sigma), OZ (1 mg/ml) and IC (100 μl/ml + 10 μg/ml of polymyxin B). After cultivation, the supernatants were collected for extracellular assay. The remaining adherent cells in the well were suspended in RPMI 1640 medium (500 μl) and lysed by freeze-thawing and sonication for intracellular assay. All samples were stored at –80°C until assay. Both the extra- and intracellular amounts of IL-1α and β were determined by using a human IL-1α and β enzyme immunoassay kit (Cayman). The potencies were expressed as the mean of the IC_{50} values determined by two independent experiments. The amounts for stimuli-treated control in two independent experiments were as follows, while the amounts for stimuli-untreated control were negligible. LPS: (α) 6.35 ± 0.66 and 2.81 ± 0.50 ng/ml, (β) 1.12 ± 0.12 and 0.693 ± 0.098 ng/ml; OZ: (α) 129 ± 15 and 126 ± 12 pg/ml, (β) 313 ± 25 and 50.2 ± 5.3 pg/ml; IC: (α) 1092 ± 208 and 388 ± 85 pg/ml, (β) 562 ± 113 and 222 ± 37 pg/ml. Intracellular percentages of IL-1α and β with each stimuli were approximately as follows. LPS: (α) 95%, (β) 40%; OZ: (α) 55%, (β) 5%; IC: (α) 25%, (β) 10%.

Preparation of immune complexes
A mixture of chicken egg albumin (0.64 mg, Sigma) dissolved in saline (0.8 ml) and rabbit IgG fraction to chicken egg albumin (0.8 ml, Cappel) was incubated at 37°C for 30 min. After leaving at 4°C for 18 h, the mixture was centrifuged (3000 rpm) for 5 min, and then washed three times with RPMI 1640 medium (1.6 ml) to afford immune complexes.

Incorporation of [^{3}H]amino acid using human monocytes
Human monocytes were prepared according to the same procedure described for *in vitro* IL-1 generation. The monocytes were cultured in the presence or absence of test drugs for 18 h in RPMI 1640 medium (500 µl) containing 1% heat-inactivated autologous serum and 0.1% DMSO along with [^{3}H]amino-acid mixtures (1 µCi/well). After cultivation, chloroacetic acid precipitable radiolabeled materials were prepared from both the supernatants and the cell lysates and determined in a liquid scintillation counter. Tests were run in duplicate and the mean of control levels was 1915 dpm.

Rat CMC-LPS air-pouch inflammation model
A volume of 10 ml of air was injected subcutaneously into the dorsum of rats. At 24 h after the injection of air, 6 ml of a sterilized 2% (w/v) sodium carboxymethyl cellulose (CMC-Na, Cellogen F-3H, Dai-ichikogyo Seiyaku Co) in saline was injected into the air-pouch. Inflammation was induced by injecting LPS (5 ng, Sigma) dissolved in 0.5 ml of saline 24 h after the CMC injection. 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. Four or 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. RPMI 1640 medium (500 µl) was added to inflammatory exudate and this suspension was centrifuged. The supernatants were collected for extracellular assay. The remaining cells were suspended in RPMI 1640 medium (500 µl) and lysed by sonication for intracellular assay. All samples were stored at –80°C until assay. The extra- and intracellular IL-1 activities were determined by the standard LAF assay [21]. The IL-1 activities were mainly detected in the intracellular fraction (70–80%), and were completely inhibited by polyclonal anti-rat-IL-1α antibody prepared in our laboratories. The amounts of IL-1 in the test samples were calculated by a titration curve prepared with standard human recombinant IL-1α (Genzyme). The amounts of PGE_2 in the exudate supernatant were determined by using a enzyme immunoassay kit (Amersham). Granulation tissue wet weights were determined 5 d after the LPS injection.

Pharmacokinetic study
Plasma concentrations of the compounds were determined by the following HPLC method. Male Fischer rats were fasted for 16 h before and 8 h after administration, and were allowed free access to water. The compounds, suspended in 0.5% methyl cellulose solution, were administered orally to rats at a dose of 50 mg/kg. Blood samples were taken from the jugular vein periodically (15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h), and were centrifuged to plasma at 10 000 rpm for 5 min. After deproteinization of plasma with an equal volume of acetonitrile and centrifugation at 10 000 rpm for 5 min, supernatants were injected onto a LiChrospher RP-SelectB column (4 x 250 mm, Kanto Chemical). The column was eluted with a mobile phase, consisting of 0.1 M phosphoric acid/methanol/acetonitrile (40:30:30 or 50:20:30) containing 5 mM sodium dodecyl sulfate at a flow rate of 1 ml/min, and the compounds were detected at 254 nm. The HPLC system was equipped with a 880-PU pump (Jasco), 875-UV detector (Jasco) and WISP 710B autoinjector (Waters).

References

1 Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK (1986) *Immunol Today* 7, 45–56
2 di Giovine FS, Duff GW (1990) *Immunol Today* 11, 13–20
3 Dinarello CA (1991) *Blood* 77, 1627–1652
4 Fontana A, Hengartner H, Weber E, Fehr K, Grob PJ, Cohen G (1982) *Rheumatol Int* 2, 49–53
5 Wood DD, Ihrie EJ, Dinarello CA, Cohen PL (1983) *Arthritis Rheum* 26, 975–983
6 Miyasaka N, Sato K, Goto M *et al* (1988) *Arthritis Rheum* 31, 480–486
7 Nouri AME, Panayi GS, Goodman SM (1984) *Clin Exp Immunol* 55, 295–302
8 Westacott CI, Whicher JT, Barnes IC, Thompson D, Swan AJ, Dieppe PA (1990) *Ann Rheum Dis* 49, 676–681
9 Rooney M, Symons JA, Duff GW (1990) *Rheumatol Int* 10, 217–219
10 Kahle P, Saal JG, Schaudt K, Zacher J, Fritz P, Pawelec G (1992) *Ann Rheum Dis* 51, 731–734
11 Eastgate JA, Symons JA, Wood NC, Grinlinton FM, di Giovine FS, Duff GW (1988) *Lancet II*, 706–709
12 Chin J, Rupp E, Cameron PM *et al* (1988) *J Clin Invest* 82, 420–426
13 Lee JC, Griswold DE, Votta B, Hanna N (1988) *Int J Immunopharmacol* 10, 835–843
14 Shirota H, Goto M, Hashida R, Yamatsu I, Katayama K (1989) *Agents Actions* 27, 322–324
15 Otterness IG, Bliven ML, Downs JT, Natoli EJ, Hanson DC (1991) *Cytokine* 3, 277–283
16 Marshall PJ, Griswold DE, Breton J *et al* (1991) *Biochem Pharmacol* 42, 813–824
17 Folliard F, Bousseau A, Terlain B (1992) *Agents Actions* 36, 119–126
18 Tanaka M, Chiba K, Okita M *et al* (1992) *J Med Chem* 35, 4665–4675
19 Houlihan WJ, Parrino VA, Uike Y (1981) *J Org Chem* 46, 4511–4515
20 James PN, Snyder HR (1963) *Org Synth Coll Vol IV*, 539–542
21 Oppenheim JJ, Shneyour A, Kook AI (1976) *J Immunol* 116, 1466–1472

Appendix

(E)-3-(1,3-Dimethyl-4-hydroxy-5-methoxy-2-phenylindole-7-yl)-2-methylpropenoic acid ***15a***. ^{1}H-NMR ($CDCl_3$) δ 2.13 (d, J = 1.6 Hz, 3 H), 2.44 (s, 3 H), 3.62 (s, 3 H), 3.92 (s, 3 H), 5.98 (br, s, 1 H), 6.81 (s, 1 H), 7.35–7.51 (m, 5 H), 8.41 (s, 1 H). Anal calcd for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.48; H, 5.97; N, 3.97.

(E)-3-(1-Ethyl-4-hydroxy-5-methoxy-3-methyl-2-phenylindole-7-yl)-2-methylpropenoic acid ***15b***. ^{1}H-NMR ($CDCl_3$) δ 0.98 (t, J = 6.8 Hz, 3 H), 2.12 (d, J = 1.2 Hz, 3 H), 2.38 (s, 3 H), 3.92 (s, 3 H), 4.02 (q, J = 6.8 Hz, 2 H), 5.95 (br, s, 1 H), 6.76 (s, 1 H), 7.35–7.51 (m, 5 H), 8.31 (s, 1 H). Anal calcd for $C_{22}H_{23}NO_4$: C, 72.31; H, 6.34; N, 3.83. Found: C, 72.14; H, 6.26; N, 3.78.

(E)-3-(4-Hydroxy-5-methoxy-3-methyl-2-phenyl-1-propylindole-7-yl)-2-methylpropenoic acid ***15c***. ^{1}H-NMR ($CDCl_3$) δ 0.61 (t, J = 7.6 Hz, 3 H), 1.35–1.45 (m, 2 H), 2.11 (d, J = 1.6 Hz, 3 H), 2.38 (s, 3 H), 3.90–3.95 (m, 2 H), 3.92 (s, 3 H),

5.94 (br, s, 1 H), 6.75 (s, 1 H), 7.34–7.50 (m, 5 H), 8.29 (s, 1 H). Anal calcd for $C_{23}H_{25}NO_4$: C, 72.80; H, 6.64; N, 3.69. Found: C, 72.56; H, 6.63; N, 3.57.

(E)-3-(3-Ethyl-4-hydroxy-5-methoxy-1-methyl-2-phenylindole-7-yl)-2-methylpropenoic acid ***15d***. ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, J = 7.6 Hz, 3 H), 2.14 (d, J = 1.6 Hz, 3 H), 2.76 (q, J = 7.6 Hz, 2 H), 3.61 (s, 3 H), 3.93 (s, 3 H), 6.04 (br, s, 1 H), 6.82 (s, 1 H), 7.35–7.51 (m, 5 H), 8.41 (s, 1 H). Anal calcd for $C_{22}H_{23}NO_4$: C, 72.31; H, 6.34; N, 3.83. Found: C, 72.04; H, 6.34; N, 3.80.

(E)-3-(4-Hydroxy-5-methoxy-1-methyl-2-phenyl-3-propylindole-7-yl)-2-methylpropenoic acid ***15e***. ^{1}H-NMR ($CDCl_3$) δ 0.86 (t, J = 7.2 Hz, 3 H), 1.60–1.72 (m, 2 H), 2.14 (d, J = 1.2 Hz, 3 H), 2.68–2.74 (m, 2 H), 3.60 (s, 3 H), 3.92 (s, 3 H), 6.03 (br, s, 1 H), 6.82 (s, 1 H), 7.34–7.50 (m, 5 H), 8.42 (s, 1 H). Anal calcd for $C_{23}H_{25}NO_4$: C, 72.80; H, 6.64; N, 3.69. Found: C, 72.50; H, 6.64; N, 3.61.

(E)-3-[1,3-Dimethyl-4-hydroxy-5-methoxy-2-(2-methylphenyl)indole-7-yl]-2-methylpropenoic acid ***15f***. ^{1}H-NMR ($CDCl_3$) δ 2.13 (d, J = 1.6 Hz, 3 H), 2.14 (s, 3 H), 2.28 (s, 3 H), 3.50 (s, 3 H), 3.92 (s, 3 H), 5.97 (br, s, 1 H), 6.80 (s, 1 H), 7.19–7.38 (m, 4 H), 8.42 (s, 1 H). Anal calcd for $C_{23}H_{23}NO_4 \cdot 0.2H_2O$: C, 71.60; H, 6.39; N, 3.79. Found: C, 71.71; H, 6.37; N, 3.69.

(E)-3-[1,3-Dimethyl-4-hydroxy-5-methoxy-2-(3-methylphenyl)indole-7-yl]-2-methylpropenoic acid ***15g***. ^{1}H-NMR ($CDCl_3$) δ 2.13 (d, J = 1.6 Hz, 3 H), 2.43 (s, 3 H), 2.44 (s, 3 H), 3.62 (s, 3 H), 3.92 (s, 3 H), 5.97 (br, s, 1 H), 6.81 (s, 1 H), 7.15–7.19 (m, 2 H), 7.20–7.24 (m, 1 H), 7.34–7.39 (m, 1 H), 8.41 (m, 1 H). Anal calcd for $C_{23}H_{23}NO_4 \cdot 0.3H_2O$: C, 71.26; H, 6.41; N, 3.78. Found: C, 71.26; H, 6.41; N, 3.78.

(E)-3-[1,3-Dimethyl-4-hydroxy-5-methoxy-2-(4-methylphenyl)indole-7-yl]-2-methylpropenoic acid ***15h***. ^{1}H-NMR ($CDCl_3$) δ 2.13 (d, J = 1.6 Hz, 3 H), 2.43 (s, 3 H), 2.44 (s, 3 H), 3.61 (s, 3 H), 3.92 (s, 3 H), 5.97 (br, s, 1 H), 6.80 (s, 1 H), 7.26 (d, J = 8.0 Hz, 2 H), 7.29 (d, J = 8.0 Hz, 2 H), 8.41 (s, 1 H). Anal calcd for $C_{22}H_{23}NO_4$: C, 72.31; H, 6.34; N, 3.83. Found: C, 72.05; H, 6.34; N, 3.75.

(E)-3-[1,3-Dimethyl-4-hydroxy-5-methoxy-2-(2-methoxylphenyl)indole-7-yl]-2-methylpropenoic acid ***15i***. ^{1}H-NMR ($CDCl_3$) δ 2.13 (d, J = 1.6 Hz, 3 H), 2.37 (s, 3 H), 3.57 (s, 3 H), 3.81 (s, 3 H), 3.90 (s, 3 H), 5.96 (br, s, 1 H), 6.79 (s, 1 H), 7.00–7.09 (m, 2 H), 7.23–7.27 (m, 1 H), 7.40–7.46 (m, 1 H), 8.44 (s, 1 H). Anal calcd for $C_{22}H_{23}NO_5 \cdot 0.3H_2O$: C, 68.31; H, 6.15; N, 3.62. Found: C, 68.12; H, 6.08; N, 3.49.

(E)-3-[1,3-Dimethyl-4-hydroxy-5-methoxy-2-(3-methoxylphenyl)indole-7-yl]-2-methylpropenoic acid ***15j***. ^{1}H-NMR ($CDCl_3$) δ 2.13 (d, J = 1.2 Hz, 3 H), 2.45 (s, 3 H), 3.63 (s, 3 H), 3.86 (s, 3 H), 3.92 (s, 3 H), 5.98 (br, s, 1 H), 6.81 (s, 1 H), 6.89–6.92 (m, 1 H), 6.95 (d, J = 8.0 Hz, 1 H), 6.96 (d, J = 8.0 Hz, 1 H), 7.40 (t, J = 8.0 Hz, 1 H), 8.41 (s, 1 H). Anal calcd for $C_{22}H_{23}NO_5$: C, 69.28; H, 6.08; N, 3.67. Found: C, 68.99; H, 6.15; N, 3.54

(E)-3-[1,3-Dimethyl-4-hydroxy-5-methoxy-2-(4-methoxylphenyl)indole-7-yl]-2-methylpropenoic acid ***15k***. ^{1}H-NMR ($CDCl_3$) δ 2.13 (d, J = 1.2 Hz, 3 H), 2.42 (s, 3 H), 3.61 (s, 3 H), 3.88 (s, 3 H), 3.92 (s, 3 H), 5.96 (br, s, 1 H), 6.80 (s, 1 H), 7.02 (d, J = 8.8 Hz, 2 H), 7.29 (d, J = 8.8 Hz, 2 H), 8.41 (s, 1 H). Anal calcd for $C_{22}H_{23}NO_5$: C, 69.28; H, 6.08; N, 3.67. Found: C, 68.90; H, 6.12; N, 3.48.

(E)-3-[2-(3,4-Dimethoxylphenyl)-1,3-dimethyl-4-hydroxy-5-methoxyindole-7-yl]-2-methylpropenoic acid ***15l***. ^{1}H-NMR ($CDCl_3$) δ 2.14 (d, J = 1.2 Hz, 3 H), 2.44 (s, 3 H), 3.63 (s, 3 H), 3.91 (s, 3 H), 3.92 (s, 3 H), 3.96 (s, 3 H), 5.97 (br, s, 1 H), 6.80 (s, 1 H), 6.87 (d, J = 1.6 Hz, 1 H), 6.93 (dd, J = 1.6 Hz, 8.0 Hz, 1 H), 6.99 (d, J = 8.0 Hz, 1 H), 8.41 (s, 1 H). Anal calcd for $C_{23}H_{25}NO_6 \cdot 0.4H_2O$: C, 65.90; H, 6.22; N, 3.34. Found: C, 65.90; H, 6.15; N, 3.19.

(E)-3-[1,3-Dimethyl-4-hydroxy-5-methoxy-2-(3,4,5-trimethoxyphenyl)indole-7-yl]-2-methylpropenoic acid ***15m***. ^{1}H-NMR ($CDCl_3$) δ 2.14 (d, J = 1.6 Hz, 3 H), 2.46 (s, 3 H), 3.64 (s, 3 H), 3.89 (s, 6 H), 3.92 (s, 3 H), 3.93 (s, 3 H), 5.97 (br, s, 1 H), 6.57 (s, 2 H), 6.81 (s, 1 H), 8.41 (s, 1 H). Anal. calcd for $C_{24}H_{27}NO_7 \cdot 0.3H_2O$: C, 64.50; H, 6.22; N, 3.13. Found: C, 64.55; H, 6.22; N, 2.87.