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Hydroxyindole derivatives as inhibitors of IL-1 generation. II. Synthesis and pharmacological activities of (*E*)-3-(7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid derivatives

M Tanaka, M Okita, H Akamatsu, K Chiba, H Obaishi, N Nagakura, H Sakurai, I Yamatsu

Tsukuba Research Laboratories, Eisai Co Ltd, 1–3, Tokodai 5-chome, Tsukuba-shi, Ibaraki 300-26, Japan

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Summary — A series of (*E*)-3-(7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid derivatives was prepared and the inhibitory activities of its members on IL-1 generation were evaluated in an in vitro system using exudate cells from the rat carboxymethyl cellulose (CMC) induced air-pouch model. All the compounds in this new series were found to be inhibitors of IL-1 generation. In particular, the methoxy-substituted 2-phenyl compounds **28d–f** were the most potent inhibitors of IL-1 generation (eg, **28d**: IC_{50} = 0.8 μM). The compounds in this series also inhibited IL-1α and IL-1β generations in an in vitro system using human monocytes stimulated with LPS (eg, **28b**: IC_{50} = 1.4 μM (IL-1α) and 0.9 μM (IL-1β)).

hydroxyindole derivative / inhibitor of IL-1 generation / IL-1α / IL-1β

Introduction

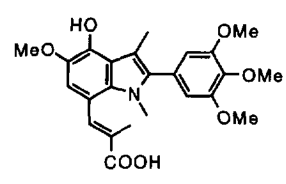
Inflammatory cytokine interleukin-1 (IL-1) consists of two structurally related polypeptides, IL-1α and IL-1β, and is produced in response to various injurious stimuli by a number of cell types such as monocytes, macrophages, and endothelial cells. Both forms of IL-1 bind the same receptor and they share many biological activities which are relevant to inflammation [1–3]. For example, IL-1 induces fever and the synthesis of 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. In view of its potent biological effects it is thought that IL-1 is an essential mediator of inflammation.

Much current evidence from clinical studies suggests that IL-1 has a significant role in chronic inflammatory diseases and in particular in rheumatoid arthritis (RA). High levels of IL-1 are found in the synovial fluid of RA patients and in culture supernatants from RA synovial tissues [4–10], the level of IL-1β in the plasma of RA patients is significantly higher than that of healthy controls and correlates with clinical disease activity [11], and high-affinity receptors for IL-1α and Il-1β have been identified on cultured RA synovial cells [12]. On the basis of these facts, a compound which inhibits the production of IL-1 could be a useful tool for the control of inflammatory responses.

In a previous paper, we reported the synthesis and the inhibitory activities on IL-1 generation of a series of (*E*)-3-(4-hydroxy-5-methoxyindole-7-yl)-2-methylpropenoic acid derivatives represented by compound **1** [13].

Compound **1** showed inhibitory activities in an in vitro system using human monocytes stimulated with various reagents such as lipopolysaccharide (LPS), opsonized zymosan, and immune complexes. Moreover, compound **1** inhibited the generation of IL-1, and the formation of granulation tissue in the in vivo rat carboxymethyl cellulose-lipopolysaccharide (CMC-LPS) air-pouch inflammatory model.

In order to find other indole derivatives inhibiting the generation of IL-1, we have moved the propenoic



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acid moiety at the 7-position to the 4-position, and evaluated the inhibitory activities of these new compounds on IL-1 generation in an in vitro system using exudate cells from the rat CMC-induced air-pouch model. As a result, we have discovered that a new series of (*E*)-3-(7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid derivatives also possess high inhibitory activities against IL-1 generation. In the present paper, we describe the synthesis and pharmacological properties of these (*E*)-3-(7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid derivatives.

Chemistry

The requisite intermediate aldehydes for the synthesis of the (*E*)-3-(7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid derivatives were prepared as shown in scheme 1.

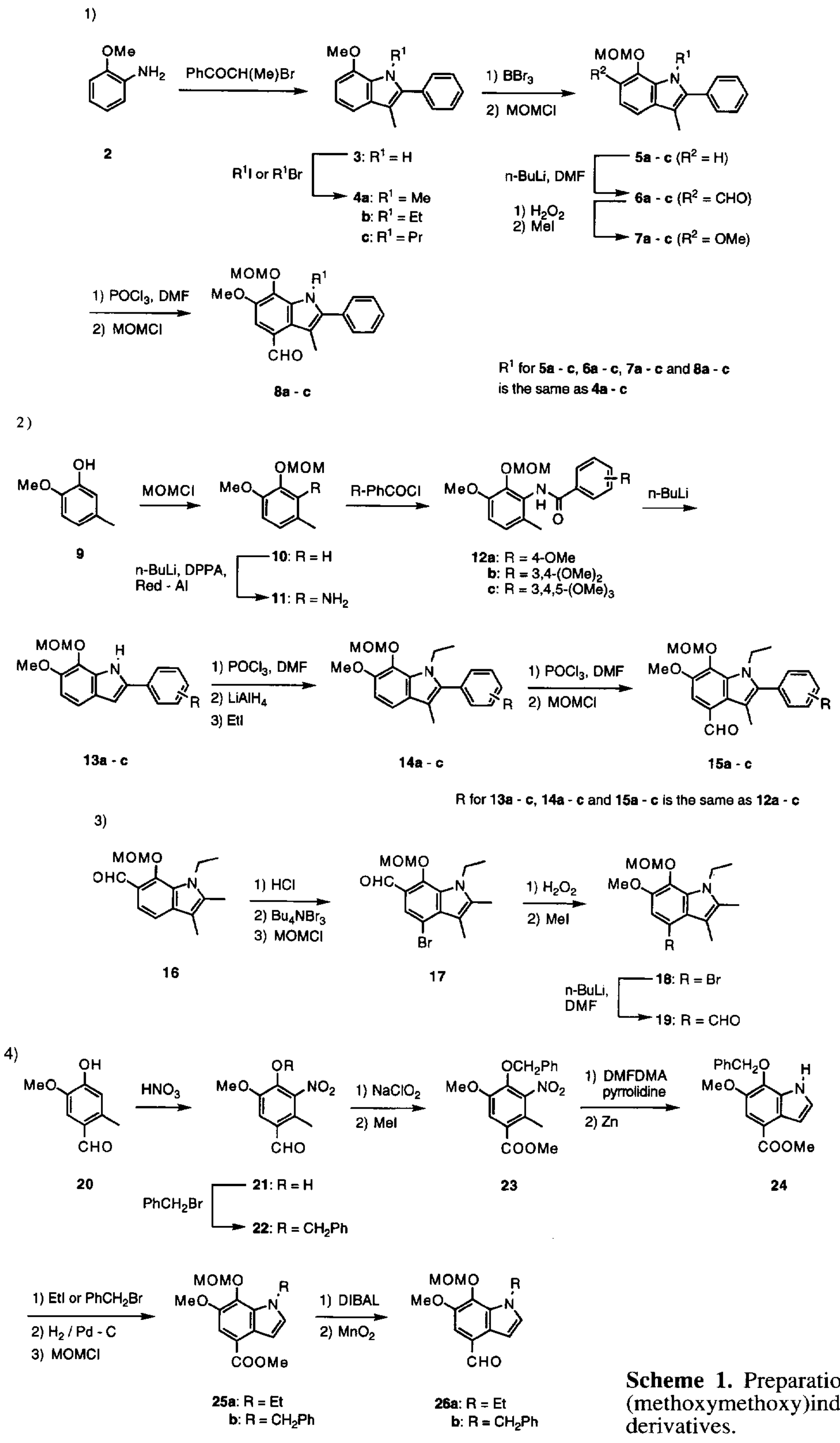
2-Methoxyaniline **2** was treated with 2-bromopropiophenone to give the 2-phenylindole derivative **3** [14]. The NH group of **3** was alkylated using sodium hydride and alkyl halides to give the 1,3-dialkylindole derivatives **4a–c**. Demethylation of **4a–c** with boron tribromide followed by protection with the methoxymethoxy group gave the 7-(methoxymethoxy)indole derivatives **5a–c**. Formylation of **5a–c** with *n*-BuLi and DMF gave the indole-6-carbaldehyde derivatives **6a–c**, which were then treated with H_2O_2 in the presence of a catalytic amount of potassium hydrogen sulfate [15], followed by methylation of the phenolic hydroxy group at 6-position with iodomethane and sodium hydride to yield the 6-methoxyindole derivatives **7a–c**. Vilsmeier formylation of the 4-position of **7a–c** with phosphorus oxychloride and DMF gave the 1,3-dialkyl-7-hydroxy-6-methoxy-2-phenylindole-4-carbaldehydes, which were then treated with sodium hydride and chloromethyl methyl ether to yield the desired aldehyde derivatives **8a–c**.

Protection of the phenolic hydroxy group of **9** using sodium hydride and chloromethyl methyl ether gave the 2-(methoxymethoxy)-4-methylanisole **10**. Reaction of the lithium salt of **10**, prepared from **10** and *n*-BuLi, and diphenylphosphoryl azide gave the phosphoryltriazene, which was then treated with sodium bis(2-methoxyethoxy)aluminum hydride to yield the aniline derivative **11** [16]. Compound **11** was treated with benzoyl chloride derivatives in the presence of pyridine to give the benzamide derivatives **12a–c**. Cyclization of **12a–c** according to the method of Houlihan et al [17] gave the indole derivatives **13a–c**. Formylation of **13a–c** with phosphorus oxychloride and DMF [18], followed by reduction of the 3-formyl group with an excess of $LiAlH_4$, gave the 3-methylindole derivatives, which were then ethylated on the NH group at the 1-position using sodium hydride and iodoethane to yield the 1-ethyl-3-methylindole derivatives **14a–c**. Compounds **14a–c** were treated according to the same procedure described for the preparation of **8a–c** to give the desired indole-4-carbaldehyde derivatives **15a–c**.

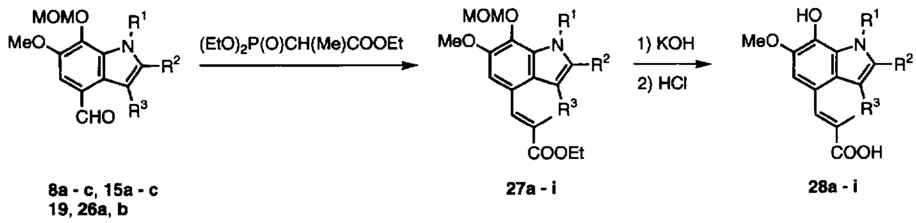
Compound **2** was treated according to the same procedure described for the preparation of **3**, **4b**, **5b** and **6b** but with use of 3-chloro-2-butanone instead of 2-bromopropiophenone to give the 1,2,3-trialkylindole-6-carbaldehyde **16**. Deprotection of the phenolic hydroxy group of **16** with concentrated HCl, followed by bromination with tetrabutylammonium tribromide [19] gave 4-bromo-2,3-dimethyl-1-ethyl-7-hydroxyindole-6-carbaldehyde, which was then treated with sodium hydride and chloromethyl methyl ether to yield the 1,2,3-trialkylindole-6-carbaldehyde derivative **17**. Compound **17** was treated according to the same procedure described for the preparation of **7b** to yield the 6-methoxy-1,2,3-trialkylindole derivative **18**. Formylation of **18** with *n*-BuLi and DMF gave the desired indole-4-carbaldehyde derivative **19**.

Formylation of **9** with titanium (IV) chloride and dichloromethyl methyl ether [20] gave the benzaldehyde derivative **20**, which was treated with concentrated HNO_3 to yield the nitrobenzaldehyde derivative **21**. Protection of the phenolic hydroxy group of **21** with benzyl bromide, followed by oxidation of the formyl group with sodium chlorite gave the benzoic acid derivative, which was then treated with potassium carbonate and iodomethane to yield the methyl benzoate **23**. Condensation of **23** with *N,N*-dimethylformamide dimethylacetal in the presence of pyrrolidine [21], followed by reductive cyclization of the intermediate enamine with zinc powder gave the methyl indole-4-carboxylate derivative **24**. Alkylation of the NH group of **24** using alkyl halides and sodium hydride, followed by debenzylation, gave the 7-hydroxyindole derivatives, which were then protected with the methoxymethoxy group to yield the methyl 1-alkylindole-4-carboxylate derivatives **25a,b**. Reduction of the methyl ester of **25a,b** with diisobutylaluminium hydride (DIBAL) to the corresponding alcohol derivatives, followed by oxidation with MnO_2, gave the desired indole-4-carbaldehyde derivatives **26a,b**.

(*E*)-3-(7-Hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid derivatives were prepared as shown in scheme 2. The Wadsworth–Emmons reaction between the appropriate aldehyde **8a–c**, **15a–c**, **19** or **26a,b** and triethyl 2-phosphonopropionate gave the (*E*)-propenoates **27a–i**, 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-(7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid derivatives **28a–i**.



Scheme 1. Preparation of 6-methoxy-7-(methoxymethoxy)indole-4-carbaldehyde derivatives.



Scheme 2. Preparation of (*E*)-3-(7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid derivatives.

Pharmacology

The inhibitory activities against LPS-induced IL-1 generation were evaluated in an in vitro system using exudate cells from the rat CMC-induced air-pouch. At 24 h after the injection of air (10 mL) into the dorsum of rats, sodium carboxymethyl cellulose (CMC-Na) was injected into the air-pouch. The exudates were harvested from the air-pouch 24 h after the CMC injection, and were cultured in the presence or absence of test drugs along with LPS (final concentration; 1 ng/mL). After cultivation for 4 h, the extra- and intracellular IL-1 activities were determined by the standard lymphocyte-activating factor (LAF) assay [22]. IL-1 activity was mainly detected in the intracellular fraction (70–80%), and was completely inhibited by polyclonal anti-rat-IL-1α antibody prepared in our laboratories. In vivo inhibitory activities were evaluated using the rat CMC-LPS air-pouch model [13] after oral administration (200 mg/kg).

Selected compounds were evaluated with an in vitro system using human monocytes stimulated with LPS (final concentration; 10 ng/mL). After incubation for 18 h with the stimulus and the compound being tested, the extra- and intracellular amounts of IL-1α and IL-1β were determined by using a human IL-1α and IL-1β enzyme immunoassay kit. Intracellular percentages of IL-1α and IL-1β were 95 and 40%, respectively.

The inhibitory activities against leukotriene-B_4 (LTB_4) and prostaglandin-E_2 (PGE_2) generations were evaluated in an in vitro system using rat glycogen-induced peritoneal cells [23]. After incubation for 10 min with A23187 and the compound being tested, the amounts of LTB_4 and PGE_2 were determined using a enzyme immunoassay kit.

Results and discussion

Rat CMC-induced air-pouch model

In vitro inhibitory effects of the various compounds against IL-1 generation were evaluated simultaneously using exudate cells from the rat CMC-induced air-pouch model (table I). At the same time, the cell viabilities were checked by measuring incorporation of ^{3}H-amino acid.

Firstly, the effects of substituents at the 1-position of the indole ring were evaluated. Introduction of an alkyl substituent slightly affected the inhibitory activity with a trend for activity to increase in parallel with the size of substituent. Secondly, the effects of methoxy substituents on the phenyl group at the 2-position of the indole ring were evaluated, and it was found that compounds **28d–f** showed higher inhibitory activities than the prototype compound **28b**. Thirdly, in order to evaluate the necessity of the phenyl group at the 2-position, the inhibitory activities of 2,3-dimethyl-substituted **28g** and 2,3-unsubstituted **28h,i** compounds were evaluated. These compounds also showed inhibitory activities, but they were less active than the 2-phenyl-substituted compounds **28a–f**. Thus the 2-phenyl substituent is necessary for strong inhibition of IL-1 generation. All the compounds in this series inhibited the generation of IL-1, and none affected cell viability at the concentrations at which IL-1 generation was inhibited.

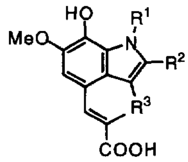
Among the compounds evaluated, the methoxy-substituted 2-phenyl compounds **28d–f** were the most potent inhibitors of IL-1 generation in the in vitro system using exudate cells from the rat CMC-induced air-pouch model.

In vivo inhibitory activities of the 2-aryl-substituted compounds **28d–f** were evaluated using the rat CMC-LPS air-pouch model [13] after oral administration. These compounds did not inhibit the generation of IL-1 even at a dose of 200 mg/kg, in spite of their strong inhibition in in vitro. In order to clarify the reason for this difference, the pharmacokinetic profiles of compounds **28e,f** were examined (50 mg/kg, po). However, plasma concentrations of **28e,f** were below the lower detection limit (30 ng/mL) even at 15 min after administration. The lack of inhibition was due to their poor pharmacokinetic profiles.

IL-1 generation using human monocytes

The inhibitory effects of four different types of compounds, 2-phenyl **28b**, 2-(4-methoxyphenyl) **28d**,

Table I. In vitro inhibitory activities against IL-1 generation.



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Compound	R^1	R^2	R^3	Mp[b], °C dec	Formula[c]	IC_{50}[a] (μM) IL-1	IC_{50}[a] (μM) Protein
a	Me	C_6H_5	Me	190–192	$C_{21}H_{21}NO_4{\cdot}0.1H_2O$	2.9	16.2
b	Et	C_6H_5	Me	148–149	$C_{22}H_{23}NO_4$	2.2	25.3
c	Pr	C_6H_5	Me	194–196	$C_{23}H_{25}NO_4$	1.9	8.8
d	Et	4-$MeOC_6H_4$	Me	186–188	$C_{23}H_{25}NO_5{\cdot}0.2H_2O$	0.8	13.0
e	Et	3,4-$(MeO)_2C_6H_3$	Me	221–223	$C_{24}H_{27}NO_6$[d]	1.0	16.5
f	Et	3,4,5-$(MeO)_3C_6H_2$	Me	233–235	$C_{25}H_{29}NO_7$[d]	0.8	12.5
g	Et	Me	Me	181–183	$C_{17}H_{21}NO_4{\cdot}0.2H_2O$	4.2	> 30
h	Et	H	H	186–188	$C_{15}H_{17}NO_4$	7.1	> 30
i	CH_2Ph	H	H	187–189	$C_{20}H_{19}NO_4$	7.1	> 30

[a]Concentration of drug inhibiting IL-1 generation by 50% of control values. IC_{50} values were calculated by the least-squares method using four concentrations of compound. Values are the mean of duplicate samples. [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]Formula was obtained by high-resolution mass spectroscopy.

2,3-dimethyl **28g** and 2,3-unsubstituted **28h**, on IL-1 generation were evaluated simultaneously using human monocytes stimulated with LPS (table II). Similarly to the compound **1** [13], these compounds also showed inhibitory activities on IL-1α and IL-1β generation.

In order to investigate whether the activities depend on the inhibition of protein synthesis, the inhibitory activities of compound **28b** and emetine (a protein synthesis inhibitor used as a positive control), against incorporation of ^{3}H-amino acid were evaluated using human monocytes (table III).

Emetine inhibited protein synthesis in parallel with its inhibition of IL-1 generation. However, similarly to compound **1** (IC_{50} > 30 μM) [13], compound **28b** did not affect 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.

LTB_4 and PGE_2 generation using rat glycogen-induced peritoneal cells

The inhibitory activities of compounds **28b,d,g,h**, against LTB_4 and PGE_2 generation were evaluated in an in vitro system using rat glycogen-induced peritoneal cells. Similarly to their inhibition of IL-1 generation, these compounds also inhibited LTB_4 generation (IC_{50}: **28b**, 0.8 μM; **28d**, 0.9 μM; **28g**, 1.2 μM; **28h**, 5.7 μM). Their inhibitory activity may be due to their phenolic character, because IL-1 generation did not

Table II. In vitro inhibitory effects of compounds **28b,d,g** and **h** on IL-1 generation.

Compound	IC_{50}[a] (μM) IL-1α[b]	IC_{50}[a] (μM) IL-1β[b]
28b	1.4	0.9
28d	2.0	1.1
28g	6.1	6.6
28h	5.8	4.9

[a]Concentration of drug inhibiting IL-1 generation by 50% of control values. IC_{50} values were calculated by the least-squares method using four concentrations of compound. Values are the mean of duplicate samples. [b]The amounts of LPS-treated control were 6.488 (α) and 0.633 ng/mL (β).

Table III. Inhibitory effects of compounds **28b** and emetine against IL-1 generation and protein synthesis.

Compound	*IC_{50}[a] (μM)*		
	IL-1α[b]	*IL-1β*[b]	*Protein*
28b	1.4	0.9	> 30
Emetine	0.10	0.05	0.12

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

occur within the incubation time (10 min) of this assay system. As regards PGE_2 generation, while compounds **28b,d** showed fairly weak inhibitory activities (IC_{50}: **28b**, 6.4 μM; **28d**, 3.7 μM), compounds **28g,h** did not inhibit PGE_2 generation even at a dose of 30 μM.

Conclusion

A series of (*E*)-3-(7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid derivatives was synthesized and the inhibitory activities of its members on LPS-induced IL-1 generation were evaluated by an in vitro system using exudate cells from the rat CMC-induced air-pouch model. All the compounds in this new series were found to be inhibitors of IL-1 generation. A trend for activity to increase in parallel with size was observed for substituents at the 1-position of the indole ring. For substituents at the 2-position, aryl-substituted compounds showed higher inhibitory activities than those of methyl-substituted or unsubstituted compounds. In particular, the methoxy-substituted 2-phenyl compounds **28d–f** were the most potent inhibitors of IL-1 generation.

The compounds in this new series also inhibited IL-1α and IL-1β generations from human monocytes stimulated with LPS. For example, compound **28b** had IC_{50} values of 1.4 μM (α) and 0.9 μM (β). This compound did not affect incorporation of ^{3}H-amino acid using human monocytes. The inhibitory activities of this series on IL-1 generation were not due to general protein synthesis inhibition.

The pharmacological profiles of this series are very similar to those of naphthols [24] and previously reported indoles [13]. These three series have the same moieties, hydroxy, methoxy and propenoic acid, on the ring and are thought to be bioisosteric with each other.

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).

7-Methoxy-3-methyl-2-phenylindole ***3***
A mixture of 2-methoxyaniline **2** (59.3 g, 0.48 mol) and 2-bromopropiophenone (46.9 g, 0.22 mol) in methoxyethanol/*n*-butoxyethanol (30 mL/20 mL) was heated at 80 °C for 30 min, and then stirring was continued at 130 °C for 1 h. Water was added and the mixture was extracted with EtOAc. The organic extract was washed with water, dried, and evaporated. The crude residue was purified by column chromatography on silica gel, eluting with EtOAc/hexane (2:98) to afford **3** (45.3 g, 87%) as a colorless solid: mp 91–92 °C; ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3 H), 3.98 (s, 3 H), 6.67 (dd, *J* = 0.5 Hz, 8.0 Hz, 1 H), 7.07 (t, *J* = 8.0 Hz, 1 H), 7.22 (d, *J* = 8.0 Hz, 1 H), 7.32–7.38 (m, 1 H), 7.44–7.50 (m, 2 H), 7.58–7.63 (m, 2 H), 8.25 (br, s, 1 H).

1-Ethyl-7-methoxy-3-methyl-2-phenylindole ***4b***
To a solution of **3** (2.37 g, 10 mmol) in DMF (20 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil; 440 mg, 11 mmol), followed by iodoethane (0.96 mL, 12 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 (2:98) to afford **4b** (2.3 g, 87%) as a colorless solid: mp 52–53 °C; ^{1}H NMR ($CDCl_3$) δ 1.11 (t, *J* = 7.0 Hz, 3 H), 2.18 (s, 3H), 3.96 (s, 3H), 4.27 (q, *J* = 7.0 Hz, 2H), 6.68 (dd, *J* = 0.8 Hz, 7.6 Hz, 1 H), 7.05 (t, *J* = 7.6 Hz, 1 H), 7.19 (dd, *J* = 0.8 Hz, 7.6 Hz, 1 H), 7.36–7.50 (m, 5 H).

1-Ethyl-7-(methoxymethoxy)-3-methyl-2-phenylindole ***5b***
To a solution of **4b** (5.0 g, 18.8 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added dropwise a solution of boron tribromide (1.0 M in CH_2Cl_2; 28.2 mL, 28.2 mmol). After being stirred at room temperature for 1 h, the mixture was poured into ice water and extracted with CH_2Cl_2. The organic extract was washed with water, dried, and evaporated. To a solution of this residue in DMF (50 mL) at 0 °C was added sodium hydride (60% dispersion in mineral oil; 830 mg, 20.8 mmol), followed by addition of chloromethyl methyl ether (1.7 mL, 22.6 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 (2:98) to afford **5b** (4.4 g, 79%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.14 (t, *J* = 6.8 Hz, 3H), 2.18 (s, 3H), 3.56 (s, 3H), 4.29 (q, *J* = 6.8 Hz, 2 H), 5.35 (s, 2 H), 6.90 (dd, *J* = 0.8 Hz, 8.0 Hz, 1 H), 7.03 (t, *J* = 8.0 Hz, 1 H), 7.23 (dd, *J* = 0.8 Hz, 8.0 Hz, 1 H), 7.37–7.51 (m, 5 H).

*1-Ethyl-7-(methoxymethoxy)-3-methyl-2-phenylindole-6-carbaldehyde **6b***

To a solution of **5b** (4.4 g, 14.9 mmol) in anhydrous Et_2O (50 mL) at –30 °C under a nitrogen atmosphere was added *n*-BuLi (1.6 M in hexanes; 11.2 mL, 17.9 mmol) over a period of 5 min. The mixture was allowed to warm to room temperature and stirring was continued for 2 h. The mixture was recooled to –40 °C and treated dropwise with DMF (1.73 mL, 22.4 mmol). After stirring of the reaction mixture at room temperature for 30 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 **6b** (3.2 g, 66%) as a pale brown oil: ^1H NMR ($CDCl_3$) δ 1.09 (t, *J* = 7.0 Hz, 3 H), 2.19 (s, 3 H), 3.60 (s, 3H), 4.33 (q, *J* = 7.0 Hz, 2 H), 5.27 (s, 2H), 7.38–7.43 (m, 3 H), 7.45–7.55 (m, 3 H), 7.63 (d, *J* = 8.2 Hz, 1 H), 10.36 (s, 1 H).

*1-Ethyl-6-methoxy-7-(methoxymethoxy)-3-methyl-2-phenylindole **7b***

To a solution of **6b** (3.2 g, 9.9 mmol) in MeOH (100 mL) at room temperature were added H_2O_2 (31%, 1.3 mL, 11.9 mmol) and a catalytic amount of potassium hydrogen sulfate. After being stirred at the same temperature for 2 h, the mixture was poured into water and extracted with EtOAc. The organic extract was washed with a saturated aqueous sodium thiosulfate solution and brine, dried and evaporated to afford crude 1-ethyl-6-hydroxy-7-(methoxymethoxy)-3-methyl-2-phenylindole as a brown oil. This crude indole was treated according to the same procedure described for the preparation of **5b** (in part) with use of iodomethane instead of chloromethyl methyl ether to afford **7b** (2.3 g, 71%) as a colorless solid: mp 70–71 °C; ^1H NMR ($CDCl_3$) δ 1.05 (t, *J* = 7.0 Hz, 3 H), 2.17 (s, 3 H), 3.61 (s, 3 H), 3.93 (s, 3 H), 4.31 (q, *J* = 7.0 Hz, 2 H), 5.27 (s, 2 H), 6.88 (d, *J* = 8.4 Hz, 1 H), 7.24 (d, *J* = 8.4 Hz, 1 H), 7.36–7.51 (m, 5 H).

*1-Ethyl-6-methoxy-7-(methoxymethoxy)-3-methyl-2-phenylindole-4-carbaldehyde **8b***

Phosphorus oxychloride (2.86 mL, 30.7 mmol) was added dropwise to DMF (10 mL) at 0 °C. After stirring of the mixture at the same temperature for 30 min, a solution of **7b** (1.0 g, 3.07 mmol) in DMF (15 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-ethyl-7-hydroxy-6-methoxy-3-methyl-2-phenylindole-4-carbaldehyde, which was used in the next step without further purification.

This aldehyde was treated according to the same procedure described for the preparation of **5b** (in part) to afford **8b** (680 mg, 62%) as a yellow solid: mp 87–88 °C; ^1H NMR ($CDCl_3$) δ 1.09 (t, *J* = 7.0 Hz, 3 H), 2.36 (s, 3 H), 3.58 (s, 3 H), 3.98 (s, 3 H), 4.31 (q, *J* = 7.0 Hz, 2 H), 5.37 (s, 2 H), 7.37–7.41 (m, 2 H), 7.45–7.55 (m, 3 H), 7.61 (s, 1 H), 10.71 (s, 1 H).

*1,3-Dimethyl-6-methoxy-7-(methoxymethoxy)-2-phenylindole-4-carbaldehyde **8a***

Compound **3** was treated according to the same procedure described for the preparation of **4b**–**8b** using iodomethane instead of iodoethane to afford **8a** as a yellow solid: mp 76–77 °C; ^1H NMR ($CDCl_3$) δ 2.41 (s, 3 H), 3.58 (s, 3 H), 3.83 (s, 3 H), 3.97 (s, 3 H), 5.35 (s, 2 H), 7.36–7.40 (m, 2 H), 7.44–7.55 (m, 3 H), 7.60 (s, 1 H), 10.71 (s, 1 H).

*6-Methoxy-7-(methoxymethoxy)-3-methyl-2-phenyl-1-propylindole-4-carbaldehyde **8c***

Compound **3** was treated according to the same procedure described for the preparation of **4b**–**8b** using *n*-propyl bromide instead of iodoethane to afford **8c** as a yellow solid: mp 67–68 °C; ^1H NMR ($CDCl_3$) δ 0.62 (t, *J* = 7.2 Hz, 3 H), 1.46–1.56 (m, 2 H), 2.37 (s, 3 H), 3.59 (s, 3 H), 3.97 (s, 3 H), 4.18–4.25 (m, 2 H), 5.36 (s, 2 H), 7.35–7.40 (m, 2 H), 7.43–7.54 (m, 3 H), 7.60 (s, 1 H), 10.70 (s, 1 H).

*2-(Methoxymethoxy)-4-methylanisole **10***

2-Methoxy-5-methylphenol **9** (13.8 g, 0.1 mol) was treated according to the same procedure described for the preparation of **5b** (in part) to afford **10** (17.3 g, 95%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 2.28 (s, 3 H), 3.52 (s, 3 H), 3.85 (s, 3 H), 5.21 (s, 2 H), 6.76–6.81 (m, 2 H), 6.98 (m, 1 H).

*3-Methoxy-2-(methoxymethoxy)-6-methylaniline **11***

To a solution of **10** (17.3 g, 95 mmol) in THF (300 mL) at 0 °C under a nitrogen atmosphere was added *n*-BuLi (1.6 M in hexane; 89 mL, 142 mmol) over a period of 20 min, and this mixture was stirred at the same temperature for 1.5 h.

To a solution of diphenylphosphoryl azide (39.2 g, 142 mmol) in THF (500 mL) at –78 °C under a nitrogen atmosphere was added the above reaction mixture via a syringe. After stirring at the same temperature for 1 h, sodium bis(2-methoxyethoxy)aluminum hydride (3.4 M in toluene; 168 mL, 520 mmol) was added. The mixture was allowed to warm to 0 °C and stirring was continued at the same temperature for 1 h. Water was carefully added and the mixture was filtered through celite. The filtrate was washed with dilute NaOH solution and brine, dried, and evaporated. The crude residue was purified by column chromatography on silica gel, eluting with EtOAc/hexane (1:4) to afford **11** (9.8 g, 52%) as a pale brown oil: ^1H NMR ($CDCl_3$) δ 2.11 (d, *J* = 0.6 Hz, 3 H), 3.59 (s, 3 H), 3.79 (s, 3 H), 3.90 (br, s, 2 H), 5.10 (s, 2 H), 6.27 (d, *J* = 8.3 Hz, 1 H), 6.75 (dd, *J* = 0.6 Hz, 8.3 Hz, 1 H).

*3′-Methoxy-4-methoxy-2′-(methoxymethoxy)-6′-methylbenzanilide **12a***

To a solution of **11** (5.0 g, 25.4 mmol) in THF (50 mL) at 0 °C were added pyridine (2.9 mL, 35.9 mmol) and 4-methoxybenzoyl chloride (5.2 g, 30.5 mmol). 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 1 N HCl, water and brine, dried, and evaporated. The crude residue was purified by column chromatography on silica gel, eluting with EtOAc/hexane (7:3) to afford **12a** (6.8 g, 81%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 2.24 (s, 3 H), 3.37 (s, 3 H), 3.83 (s, 3 H), 3.87 (s, 3 H), 5.03 (s, 2 H), 6.79 (d, *J* = 8.6 Hz, 1 H), 6.96–7.01 (m, 3 H), 7.92 (m, 2 H), 8.18 (br, s, 1 H).

*6-Methoxy-7-(methoxymethoxy)-2-(4-methoxyphenyl)indole **13a***

To a suspension of **12a** (6.8 g, 20.5 mmol) in anhydrous THF (80 mL) at 0 °C under a nitrogen atmosphere was added dropwise *n*-BuLi (1.6 M in hexane; 38.4 mL, 61.5 mmol) over a period of 15 min. 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 (3:17) to afford **13a** (2.5 g, 39%) as a pale yellow solid: mp 100–101 °C; ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3 H), 3.86 (s, 3 H), 3.93 (s, 3 H), 5.32 (s, 2 H), 6.64 (br, s, 1 H), 6.82 (d, J = 8.4 Hz, 1 H), 6.98 (m, 2 H), 7.25 (d, J = 8.4 Hz, 1 H), 7.57 (m, 2 H), 9.51 (br, s, 1 H).

*1-Ethyl-6-methoxy-7-(methoxymethoxy)-3-methyl-2-(4-methoxyphenyl)indole **14a***

Phosphorus oxychloride (0.9 mL, 9.6 mmol) was added dropwise to DMF (10 mL) at 0 °C. After being stirred at room temperature for 30 min, a solution of **13a** (2.5 g, 8.0 mmol) in DMF (14 mL) was added dropwise and stirring was continued for 1 h. The mixture was poured into ice-cooled 5 N NaOH (30 mL) and extracted with EtOAc. The organic extract was washed successively with water and brine, dried, and evaporated to afford crude 6-methoxy-7-(methoxymethoxy)-2-(4-methoxyphenyl)indole-3-carbaldehyde as a pale brown solid, which was used in the next step without further purification.

To a suspension of $LiAlH_4$ (1.1 g, 32 mmol) in THF (50 mL) at 0 °C was added a solution of the above crude aldehyde in THF (30 mL) and the reaction mixture was refluxed for 2 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 to afford crude 6-methoxy-7-(methoxymethoxy)-3-methyl-2-(4-methoxyphenyl)indole as a brown solid, which was used in the next step without further purification.

This crude indole was treated according to the same procedure described for the preparation of **4b** to afford **14a** (2.38 g, 84%) as a colorless solid: mp 88–89 °C; ^{1}H NMR ($CDCl_3$) δ 1.06 (t, J = 7.0 Hz, 3 H), 2.15 (s, 3 H), 3.60 (s, 3 H), 3.88 (s, 3 H), 3.92 (s, 3 H), 4.29 (q, J = 7.0 Hz, 2 H), 5.26 (s, 2 H), 6.87 (d, J = 8.4 Hz, 1 H),7.01 (m, 2 H), 7.22 (d, J = 8.4 Hz, 1 H), 7.31 (m, 2 H).

*1-Ethyl-6-methoxy-7-(methoxymethoxy)-3-methyl-2-(4-methoxyphenyl)indole-4-carbaldehyde **15a***

Compound **14a** (2.3 g, 6.47 mmol) was treated according to the same procedure described for the preparation of **8b** to afford **15a** (1.06 g, 43%) as a yellow solid: mp 105–106 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (t, J = 7.0 Hz, 3 H), 2.35 (s, 3 H), 3.58 (s, 3 H), 3.90 (s, 3 H), 3.97 (s, 3 H), 4.30 (q, J = 7.0 Hz, 2 H), 5.36 (s, 2 H), 7.04 (m, 2 H), 7.31 (m, 2 H), 7.60 (s, 1 H), 10.70 (s, 1 H).

*2-(3,4-Dimethoxyphenyl)-1-ethyl-6-methoxy-7-(methoxymethoxy)-3-methylindole-4-carbaldehyde **15b***

Compound **11** was treated according to the same procedure described for the preparation of **12a–15a** using 3,4-dimethoxybenzoyl chloride instead of 4-methoxybenzoyl chloride to afford **15b** as a yellow solid: mp 142–143 °C; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, J = 7.0 Hz, 3 H), 2.37 (s, 3 H), 3.59 (s, 3 H), 3.91 (s, 3 H), 3.97 (s, 3 H), 4.31 (q, J = 7.0 Hz, 2 H), 5.37 (s, 2 H), 6.88 (d, J = 2.0 Hz, 1 H), 6.95 (dd, J = 2.0 Hz, 8.0 Hz, 1 H), 7.01 (d, J = 8.0 Hz, 1 H), 7.60 (s, 1 H), 10.70 (s, 1 H).

*1-Ethyl-6-methoxy-7-(methoxymethoxy)-3-methyl-2-(3,4,5-trimethoxyphenyl)indole-4-carbaldehyde **15c***

Compound **11** was treated according to the same procedure described for the preparation of **12a–15a** using 3,4,5-trimethoxybenzoyl chloride instead of 4-methoxybenzoyl chloride to afford **15c** as a yellow solid: mp 130–131 °C; ^{1}H NMR ($CDCl_3$) δ 1.16 (t, J = 7.0 Hz, 3 H), 2.39 (s, 3 H), 3.60 (s, 3 H), 3.89 (s, 6 H), 3.95 (s, 3 H), 3.98 (s, 3 H), 4.32 (q, J = 7.0 Hz, 2 H), 5.37 (s, 2 H), 6.59 (s, 2 H), 7.60 (s, 1 H), 10.70 (s, 1 H).

*2,3-Dimethyl-1-ethyl-7-(methoxymethoxy)indole-6-carbaldehyde **16***

2-Methoxyaniline **2** was treated according to the same procedure described for the preparation of **3, 4b–6b** using 3-chloro-2-butanone instead of 2-bromopropiophenone to afford **16** as a brown-yellowish solid: mp 74–75 °C; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J = 7.2 Hz, 3 H), 2.22 (s, 3 H), 2.37 (s, 3 H), 3.57 (s, 3 H), 4.44 (q, J = 7.2 Hz, 2 H), 5.23 (s, 2 H), 7.29 (d, J = 8.4 Hz, 1 H), 7.53 (d, J = 8.4 Hz, 1 H), 10.28 (s, 1 H).

*4-Bromo-2,3-dimethyl-1-ethyl-7-(methoxymethoxy)indole-6-carbaldehyde **17***

To a solution of **16** (1.5 g, 5.7 mmol) in acetone (20 mL) was added concentrated HCl (0.5 mL) and this mixture was stirred at room temperature for 1 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 2,3-dimethyl-1-ethyl-7-hydroxyindole-6-carbaldehyde (1.16 g, 93%) as a yellow solid: mp 68–69 °C; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, J = 7.2 Hz, 3 H), 2.21 (s, 3 H), 2.35 (s, 3 H), 4.46 (q, J = 7.2 Hz, 2 H), 7.02 (d, J = 8.2 Hz, 1 H), 7.08 (d, J = 8.2 Hz, 1 H), 9.80 (s, 1 H), 12.54 (s, 1 H).

To a solution of this indole (1.16 g, 5.3 mmol) in chloroform (50 mL) at room temperature was added tetrabutylammonium tribromide (2.7 g, 5.6 mmol). After stirring of the mixture at the same temperature for 30 min, water was added and the mixture was extracted with chloroform. The organic extract was washed successively with water and brine, dried, and evaporated to afford crude 4-bromo-2,3-dimethyl-1-ethyl-7-hydroxyindole-6-carbaldehyde as a brown-yellow solid, which was used in the next step without further purification.

This aldehyde was treated according to the same procedure described for the preparation of **5b** (in part) to afford **17** (1.6 g, 88%) as a pale brown-yellow solid: mp 75–76 °C; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J = 7.2 Hz, 3 H), 2.37 (s, 3 H), 2.50 (s, 3 H), 3.56 (s, 3 H), 4.45 (q, J = 7.2 Hz, 2 H), 5.19 (s, 2 H), 7.67 (s, 1 H), 10.18 (s, 1 H).

*4-Bromo-2,3-dimethyl-1-ethyl-6-methoxy-7-(methoxymethoxy)-indole **18***

Compound **17** (2.5 g, 7.3 mmol) was treated according to the same procedure described for the preparation of **7b** to afford **18** (690 mg, 27%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J = 7.0 Hz, 3 H), 2.28 (s, 3 H), 2.45 (s, 3 H), 3.55 (s, 3 H), 3.86 (s, 3 H), 4.39 (q, J = 7.0 Hz, 2 H), 5.20 (s, 2 H), 6.91 (s, 1 H).

*2,3-Dimethyl-1-ethyl-6-methoxy-7-(methoxymethoxy)indole-4-carbaldehyde **19***

To a solution of **18** (690 mg, 2.0 mmol) in anhydrous THF (5.0 mL) at –70 °C under a nitrogen atmosphere was added *n*-BuLi (1.6 M in hexane; 1.9 mL, 3.0 mmol). After stirring at –70 to –40 °C for 1.5 h, the mixture was then treated with DMF (0.62 mL, 8.0 mmol). After warming of the reaction mixture to room temperature, 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 (3:17) to afford **19** (440 mg, 75%) as a yellow solid: mp 119–120 °C; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J = 7.1 Hz, 3 H), 2.37 (s, 3 H), 2.45 (s, 3 H), 3.56 (s, 3 H), 3.94 (s, 3 H), 4.46 (q, J = 7.1 Hz, 2 H), 5.34 (s, 2 H), 7.52 (s, 1 H), 10.65 (s, 1 H).

4-Hydroxy-5-methoxy-2-methylbenzaldehyde **20**
To a solution of **9** (345 g, 2.5 mol) in CH_2Cl_2 (1500 mL) at 0 °C were added titanium (IV) chloride (548 mL, 5.0 mol) and dichloromethyl methyl ether (365 mL, 4.1 mol). After being stirred at room temperature for 1 h, the mixture was poured into ice water. The resulting precipitate was collected by filtration and then washed with EtOAc and Et_2O to afford **20** (158.2 g, 38%) as a pale yellow solid. The filtrate was washed with water, dried, and evaporated. The resulting solid residue was washed with EtOAc and Et_2O to afford **20** (78.2 g, 19%) as a pale yellow solid: total yield 236.4 g, 57%; mp 168–169 °C; ^{1}H NMR ($CDCl_3$) δ 2.59 (s, 3 H), 3.93 (s, 3 H), 6.12 (s, 1 H), 6.77 (s, 1 H), 7.35 (s, 1 H), 10.19 (s, 1 H).

4-Hydroxy-5-methoxy-2-methyl-3-nitrobenzaldehyde **21**
To a suspension of **20** (158 g, 0.95 mol) in THF/AcOH (700 mL/700 mL) at 0 °C was added concentrated HNO_3 (78.4 mL, 1.05 mol) over a period of 1 h. After being stirred at room temperature for 1 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 resulting solid was collected by filtration and then washed with diisopropyl ether to afford **21** (122 g, 61%) as a pale yellow solid: mp 189–190 °C; ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3 H), 3.91 (s, 3 H), 7.55 (s, 1 H), 10.11 (s, 1 H), 11.56 (br, s, 1 H).

4-Benzyloxy-5-methoxy-2-methyl-3-nitrobenzaldehyde **22**
To a solution of **21** (71 g, 336 mmol) in DMF (500 mL) at 0 °C was added in portions sodium hydride (55% dispersion in mineral oil; 15.4 g, 353 mmol) over a period of 15 min, followed by benzyl bromide (43.9 mL, 369 mmol) at the same temperature. After being stirred at 60 °C for 1 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 **22** (82.3 g, 81%) as a colorless solid: mp 91–92 °C; ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3 H), 3.98 (s, 3 H), 5.26 (s, 2 H), 7.31–7.41 (m, 5 H), 7.52 (s, 1 H), 10.23 (s, 1 H).

Methyl 4-benzyloxy-5-methoxy-2-methyl-3-nitrobenzoate **23**
To a solution of **22** (82.3 g, 273 mmol) in DMSO (1500 mL) at 0 °C were added a solution of sodium dihydrogenphosphate (11.4 g, 73 mmol) in H_2O (100 mL) and a solution of sodium chlorite (80%; 43.2 g, 382 mmol) in H_2O (360 mL). 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 resulting solid was collected by filtration and washed with diisopropyl ether to afford 4-benzyloxy-5-methoxy-2-methyl-3-nitrobenzoic acid (80.2 g, 94%) as a colorless solid: mp 197–199 °C; ^{1}H NMR ($CDCl_3$) δ 2.47 (s, 3 H), 3.97 (s, 3 H), 5.24 (s, 2 H), 7.31–7.42 (m, 5 H), 7.74 (s, 1 H).

To a solution of this acid (119.2 g, 0.38 mol) in DMF (500 mL) were added potassium carbonate (52.5 g, 0.38 mol) and iodomethane (35.5 mL, 0.57 mol). After being stirred at room temperature for 12 h, the mixture was poured into water and extracted with EtOAc. The organic extract was washed 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 **23** (110 g, 88%) as a colorless solid: mp 109–110 °C; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3 H), 3.91 (s, 3 H), 3.95 (s, 3 H), 5.20 (s, 2 H), 7.30–7.41 (m, 5 H), 7.59 (s, 1 H).

Methyl 7-benzyloxy-6-methoxyindole-4-carboxylate **24**
To a solution of **23** (73.2 g, 224 mmol) in DMF (200 mL) were added *N,N*-dimethylformamide dimethyl acetal (89 mL, 670 mmol) and pyrrolidine (37.3 mL, 447 mmol). The mixture was refluxed for 6 h under a nitrogen atmosphere. Water was added and the mixture was extracted with EtOAc. The organic extract was washed with water and brine, dried, and evaporated. To a solution of this crude residue (53.4 g) in 80% aqueous AcOH (400 mL) at 85 °C was added in portions zinc powder (53.4 g) over a period of 30 min. After being stirred at the same temperature for 1 h, the zinc powder was filtered off. The filtrate 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 (1:19) to afford **24** (13.5 g, 19%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3 H), 3.99 (s, 3 H), 5.29 (s, 2 H), 6.99–7.01 (m, 1 H), 7.14–7.16 (m, 1 H), 7.26–7.44 (m, 5 H), 7.68 (s, 1 H), 8.17 (br, s, 1 H).

Methyl 1-ethyl-6-methoxy-7-(methoxymethoxy)indole-4-carboxylate **25a**
Compound **24** (4.2 g, 13.5 mmol) was treated according to the same procedure described for the preparation of **4b** to afford methyl 7-benzyloxy-1-ethyl-6-methoxyindole-4-carboxylate (3.0 g, 65%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J = 7.2 Hz, 3 H), 3.97 (s, 3 H), 3.98 (s, 3 H), 4.26 (q, J = 7.2 Hz, 2 H), 5.25 (s, 2 H), 7.00 (d, J = 3.2 Hz, 1 H), 7.06 (d, J = 3.2 Hz, 1 H), 7.30–7.42 (m, 3 H), 7.44–7.50 (m, 2 H), 7.67 (s, 1 H).

A solution of this methyl indole-4-carboxylate (3.0 g, 8.8 mmol) in EtOAc (50 mL) was hydrogenated over 10% palladium on carbon (water content ~ 50%; 0.15 g) at 1 atm for 5 h. The catalyst was filtered off and the filtrate was evaporated to afford methyl 1-ethyl-7-hydroxy-6-methoxyindole-4-carboxylate, which was used in the next step without further purification.

To a solution of this crude indole in CH_2Cl_2 (100 mL) at 0 °C was added *N,N*-diisopropylethylamine (1.69 mL, 9.7 mmol), followed by addition of chloromethyl methyl ether (0.74 mL, 9.7 mmol) at the same temperature. After being stirred at room temperature for 1 h, the mixture was poured into water and extracted with CH_2Cl_2. The organic extract was washed 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 **25a** (2.09 g, 81%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.42 (t, J = 7.2 Hz, 3 H), 3.54 (s, 3 H), 3.94 (s, 3 H), 3.96 (s, 3 H), 4.48 (q, J = 7.2 Hz, 2 H), 5.33 (s, 2 H), 7.01 (d, J = 3.2 Hz, 1 H), 7.11 (d, J = 3.2 Hz, 1 H), 7.63 (s, 1 H).

1-Ethyl-6-methoxy-7-(methoxymethoxy)indole-4-carbaldehyde **26a**
To a solution of **25a** (2.09 g, 7.1 mmol) in CH_2Cl_2 (20 mL) at −78 °C under a nitrogen atmosphere was added DIBAL (1.0 M in toluene; 10.7 mL, 10.7 mmol). After warming the reaction mixture to room temperature, water was added and the mixture was extracted with CH_2Cl_2. The organic extract was washed with water, dried, and evaporated. To a solution of this residue in CH_2Cl_2 (30 mL) was added MnO_2 (10 g), and this mixture was then stirred at room temperature for 6 h. The oxidizing agent was filtered off and the filtrate was evaporated to give the crude product, which was purified by column chromatography on silica gel, eluting with EtOAc/hexane (1:4) to afford **26a** (1.80 g, 96%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.45 (t, J =

7.2 Hz, 3 H), 3.56 (s, 3 H), 3.96 (s, 3 H), 4.50 (q, J = 7.2 Hz, 2 H), 5.38 (s, 2 H), 7.15 (d, J = 3.2 Hz, 1 H), 7.19 (d, J = 3.2 Hz, 1 H), 7.35 (s, 1 H), 10.16 (s, 1 H).

*1-Benzyl-6-methoxy-7-(methoxymethoxy)indole-4-carbaldehyde **26b***

Compound **24** was treated according to the same procedure described for the preparation of **25a** and **26a** using benzyl bromide instead of iodoethane to afford **26b** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 3 H), 3.94 (s, 3 H), 5.23 (s, 2 H), 5.71 (s, 2 H), 7.05–7.10 (m, 2 H), 7.18 (d, J = 3.2 Hz, 1 H), 7.21 (d, J = 3.2 Hz, 1 H), 7.21–7.31 (m, 3 H), 7.37 (s, 1 H), 10.17 (s, 1 H).

*Ethyl (E)-3-[1-ethyl-6-methoxy-7-(methoxymethoxy)-3-methyl-2-phenylindole-4-yl]-2-methylpropenoate **27b***

To a suspension of sodium hydride (60% dispersion in mineral oil; 70 mg, 1.75 mmol) in DMF (10 mL) at 0 °C was added a solution of triethyl 2-phosphonopropionate (453 mg, 1.90 mmol) in DMF (5 mL). After stirring of the mixture at the same temperature for 10 min, a solution of **8b** (560 mg, 1.58 mmol) in DMF (5 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 **27b** (640 mg, 92%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.07 (t, J = 7.0 Hz, 3 H), 1.34 (t, J = 7.0 Hz, 3 H), 2.12 (d, J = 1.2 Hz, 3 H), 2.25 (s, 3 H), 3.61 (s, 3 H), 3.91 (s, 3 H), 4.27 (q, J = 7.0 Hz, 2 H), 4.29 (q, J = 7.0 Hz, 2 H), 5.29 (s, 2 H), 6.77 (s, 1 H), 7.33–7.51 (m, 5 H), 8.33 (s, 1 H).

*(E)-3-(1-Ethyl-7-hydroxy-6-methoxy-3-methyl-2-phenylindole-4-yl)-2-methylpropenoic acid **28b***

To a solution of **27b** (640 mg, 1.46 mmol) in EtOH (15 mL) was added a solution of KOH (164 mg, 2.92 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-ethyl-6-methoxy-7-(methoxymethoxy)-3-methyl-2-phenylindole-4-yl]-2-methylpropenoic acid (575 mg, 96%) as a yellow solid: mp 181–183 °C; ^{1}H NMR ($CDCl_3$) δ 1.07 (t, J = 7.2 Hz, 3 H), 2.15 (d, J = 1.6 Hz, 3 H), 2.26 (s, 3 H), 3.61 (s, 3 H), 3.92 (s, 3 H), 4.29 (q, J = 7.2 Hz, 2 H), 5.30 (s, 2 H), 6.81 (s, 1 H), 7.34–7.38 (m, 2 H), 7.40–7.52 (m, 3 H), 8.49 (s, 1 H).

To a solution of this acid (200 mg, 0.49 mmol) in acetone (15 mL) at room temperature was added concentrated HCl (1.0 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 **28b** (150 mg, 84%) as a yellow solid: mp 148–149 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, J = 7.0 Hz, 3 H), 2.18 (d, J = 1.2 Hz, 3 H), 2.28 (s, 3 H), 3.96 (s, 3 H), 4.25 (q, J = 7.0 Hz, 2 H), 5.95 (br, s, 1 H), 6.86 (s, 1 H), 7.34–7.52 (m, 5 H), 8.53 (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 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 LPS (10 ng/mL, Sigma). 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 IL-1β were determined by using a human IL-1α and IL-1β enzyme immunoassay kit (Cayman). The potencies were expressed as the IC_{50} value determined by duplicate samples. The amounts for LPS-treated control were 6.488 ng/mL (α) and 0.633 ng/mL (β), while the amounts for LPS-untreated control were negligible. Intracellular percentages of IL-1α and IL-1β were 95 and 40%, respectively.

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 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.

In vitro IL-1 generation using exudate cells from the rat CMC-induced air-pouch

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. The exudates were harvested from the air-pouch at 24 h after the CMC injection and 200 μL of the exudates was applied to sample tubes; 25 μL of the drug solution dissolved in 1% DMSO–RPMI 1640 medium containing 10% heat-inactivated autologous serum was applied to the tubes, and this suspension was cultured for 4 h along with 25 μL of LPS solution (10 ng/mL of saline). After cultivation, RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum (500 μL) was added to the tubes and this suspension was centrifuged. The supernatants were collected for extracellular assay. The remaining cells were suspended in RPMI 1640 medium containing 5% heat-inactivated fetal bovine serum (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 [22]. 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 IL-1 levels of LPS treated and untreated control were 287.3 and 4.8 ng/mL, respectively. The potencies were expressed as the IC_{50} value determined by duplicate samples using four concentrations of compound.

Incorporation of ^{3}H-amino acid using exudate cells from the rat CMC-induced air-pouch

The exudates from the air-pouch containing the test compound were prepared according to the same procedure described for the in vitro IL-1 generation above, and were cultured along with 25 μL of ^3H-amino acid mixtures (40 μCi/mL). After cultivation, chloroacetic acid precipitable radiolabeled materials were prepared from the supernatants and cell lysates and determined in a liquid scintillation counter. Tests were run in duplicate using four concentrations of compound and the mean of the control levels was 27 968 cpm.

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 suspended in 0.5% methyl cellulose solution were administered orally. Four animals were used in each group. Administration was performed at 2 h 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) containing 5% heat-inactivated fetal bovine serum 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) containing 5% heat-inactivated fetal bovine serum 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 [22]. 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.

LTB_4 and PGE_2 generations using rat glycogen-induced peritoneal cells
Mixed peritoneal leukocytes containing polymorphonuclear leukocytes (PMNs) and mononuclear leukocytes were elicited from male F_{344} rats by an ip injection of 10 mL of 6% glycogen solution (type II, Sigma), according to Moroney et al [23]. The cells were suspended in Hank's balanced salt solution (HBSS) containing Ca^{2+} and Mg^{2+} at a concentration of 5×10^6 cells/mL. Aliquots (135 μL) of the cell suspensions were preincubated for 10 min at 37 °C with test compound or vehicle (0.1% DMSO/0.1% BSA) in 96-well plates (Coaster). The reaction was initiated by adding A23187 (4 μM, Calibiochem, CA). After incubation for 10 min at 37 °C, the mixtures were centrifuged (200 *g*, 10 min), and aliquots of the supernatants were analyzed for LTB_4 and PGE_2 using an enzyme immunoassay kit (Amersham). The potencies were expressed as the IC_{50} value determined by duplicate samples using six concentrations of compound.

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).

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Appendix

(E)-3-(1,3-Dimethyl-7-hydroxy-6-methoxy-2-phenylindole-4-yl)-2-methylpropenoic acid ***28a***
^1H NMR ($CDCl_3$) δ 2.17 (d, *J* = 1.2 Hz, 3 H), 2.31 (s, 3 H), 3.82 (s, 3 H), 3.96 (s, 3 H), 5.89 (br, s, 1 H), 6.84 (s, 1 H), 7.33–7.52 (m, 5 H), 8.52 (s, 1 H). Anal calc for $C_{21}H_{21}NO_4 \cdot 0.1H_2O$: C, 71.41; H, 6.05; N, 3.97. Found: C, 71.29; H, 6.21; N, 3.95.

(E)-3-(1-Ethyl-7-hydroxy-6-methoxy-3-methyl-2-phenylindole-4-yl)-2-methylpropenoic acid ***28b***
^1H NMR ($CDCl_3$) δ 1.15 (t, *J* = 7.0 Hz, 3 H), 2.18 (d, *J* = 1.2 Hz, 3 H), 2.28 (s, 3 H), 3.96 (s, 3 H), 4.25 (q, *J* = 7.0 Hz, 2 H), 5.95 (br, s, 1 H), 6.86 (s, 1 H), 7.34–7.52 (m, 5 H), 8.53 (s, 1 H). Anal calc for $C_{22}H_{23}NO_4$: C, 72.31; H, 6.34; N, 3.83. Found: C, 71.93; H, 6.43; N, 3.76.

(E)-3-(7-Hydroxy-6-methoxy-3-methyl-2-phenyl-1-propylindole-4-yl)-2-methylpropenoic acid ***28c***
^1H NMR ($CDCl_3$) δ 0.67 (t, *J* = 7.2 Hz, 3 H), 1.53–1.63 (m, 2 H), 2.18 (d, *J* = 1.6 Hz, 3 H), 2.28 (s, 3 H), 3.96 (s, 3 H),

4.13–4.20 (m, 2 H), 5.94 (br, s, 1 H), 6.85 (s, 1 H), 7.33–7.37 (m, 2 H), 7.40–7.51 (m, 3 H), 8.54 (s, 1 H). Anal calc for $C_{23}H_{25}NO_4$: C, 72.80; H, 6.64; N, 3.69. Found: C, 72.57; H, 6.66; N, 3.67.

(E)-3[1-Ethyl-7-hydroxy-6-methoxy-2-(4-methoxylphenyl)-3-methylindole-4-yl]-2-methylpropenoic acid ***28d***
^{1}H NMR ($CDCl_3$) δ 1.15 (t, *J* = 7.0 Hz, 3 H), 2.18 (d, *J* = 1.2 Hz, 3 H), 2.27 (s, 3 H), 3.88 (s, 3 H), 3.96 (s, 3 H), 4.23 (q, *J* = 7.0 Hz, 2 H), 5.94 (br, s, 1 H), 6.85 (s, 1 H), 7.01 (m, 2 H), 7.28 (m, 2 H), 8.53 (s, 1 H). Anal calc for $C_{22}H_{23}NO_5{\cdot}0.2H_2O$: C, 69.22; H, 6.42; N, 3.51. Found: C, 69.31; H, 6.55; N, 3.47.

(E)-3-[2-(3,4-Dimethoxylphenyl)-1-ethyl-7-hydroxy-6-methoxy-3-methylindole-4-yl]-2-methylpropenoic acid ***28e***
^{1}H NMR ($CDCl_3$) δ 1.18 (t, *J* = 7.2 Hz, 3 H), 2.17 (d, *J* = 1.2 Hz, 3 H), 2.29 (s, 3 H), 3.90 (s, 3 H), 3.96 (s, 6 H), 4.25 (q, *J* = 7.2 Hz, 2 H), 5.94 (br, s, 1 H), 6.85 (s, 1 H), 6.87 (d, *J* = 2.0 Hz, 1 H), 6.93 (dd, *J* = 2.0 Hz, 8.0 Hz, 1 H), 6.99 (d, *J* = 8.0 Hz, 1 H), 8.52 (s, 1 H). HRMS (M^+) calc for $C_{24}H_{27}NO_6$: 425.1838. Found: 425.1843.

(E)-3-[1-Ethyl-7-hydroxy-6-methoxy-3-methyl-2-(3,4,5-trimethoxyphenyl)indole-4-yl]-2-methylpropenoic acid ***28f***
^{1}H NMR (DMSO-d_6) δ 1.05 (t, *J* = 7.2 Hz, 3 H), 2.02 (d, *J* = 1.2 Hz, 3 H), 2.18 (s, 3 H), 3.72 (s, 3 H), 3.76 (s, 6 H), 3.81 (s, 3 H), 4.22 (q, *J* = 7.2 Hz, 2 H), 6.63 (s, 2 H), 6.79 (s, 1 H), 8.21 (s, 1 H), 9.11 (s, 1 H). HRMS (M^+) calc for $C_{25}H_{29}NO_7$: 455.1944. Found: 455.1972.

(E)-3-(2,3-Dimethyl-1-ethyl-7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid ***28g***
^{1}H NMR ($CDCl_3$) δ 1.32 (t, *J* = 7.2 Hz, 3 H), 2.15 (d, *J* = 1.2 Hz, 3 H), 2.30 (s, 3 H), 2.36 (s, 3 H), 3.93 (s, 3 H), 4.38 (q, *J* = 7.2 Hz, 2 H), 5.89 (br, s, 1 H), 6.77 (s, 1 H), 8.50 (s, 1 H). Anal calc for $C_{17}H_{21}NO_4{\cdot}0.2H_2O$: C, 66.52; H, 7.03; N, 4.56. Found: C, 66.62; H, 7.10; N, 4.45.

(E)-3-(1-Ethyl-7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid ***28h***
^{1}H NMR ($CDCl_3$) δ 1.46 (t, *J* = 7.2 Hz, 3 H), 2.22 (d, *J* = 1.6 Hz, 3 H), 3.96 (s, 3H), 4.43 (q, *J* = 7.2 Hz, 2 H), 5.97 (br, s, 1 H), 6.49 (d, *J* = 3.2 Hz, 1 H), 7.02 (s, 1 H), 7.05 (d, *J* = 3.2 Hz, 1 H), 8.19 (s, 1 H). Anal calc for $C_{15}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09. Found: C, 65.07; H, 6.30; N, 5.00.

(E)-3-(1-Benzyl-7-hydroxy-6-methoxyindole-4-yl)-2-methylpropenoic acid ***28i***
^{1}H NMR ($CDCl_3$) δ 2.22 (d, *J* = 1.6 Hz, 3 H), 3.94 (s, 3 H), 5.63 (s, 2 H), 5.95 (br, s, 1 H), 6.55 (d, *J* = 3.2 Hz, 1 H), 7.02 (s, 1 H), 7.07 (d, *J* = 3.2 Hz, 1 H), 7.12–7.18 (m, 2 H), 7.22–7.32 (m, 3 H), 8.18 (s, 1 H). Anal calc for $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15. Found: C, 70.87; H, 5.78; N, 4.10.