Studies on the Synthesis and Opioid Agonistic Activities of Mitragynine-Related Indole Alkaloids: Discovery of Opioid Agonists Structurally Different from Other Opioid Ligands

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Mitragynine (**1**) is a major alkaloidal component in the Thai traditional medicinal herb, *Mitragyna speciosa*, and has been proven to exhibit analgesic activity mediated by opioid receptors. By utilizing this natural product as a lead compound, synthesis of some derivatives, evaluations of the structure−activity relationship, and surveys of the intrinsic activities and potencies on opioid receptors were performed with guinea pig ileum. The affinities of some compounds for μ-, δ-, and κ-receptors were determined in a receptor binding assay. The essential structural moieties in the Corynanthe type indole alkaloids for inducing the opioid agonistic activity were also clarified. The oxidative derivatives of mitragynine, i.e., mitragynine pseudoindoxyl (**2**) and 7-hydroxymitragynine (**12**), were found as opioid agonists with higher potency than morphine in the experiment with guinea pig ileum. In addition, **2** induced an analgesic activity in the tail flick test in mice.

Introduction

In the recent chemical[1−5] and pharmacological[6−10] studies on the Rubiaceous plant, *Mitragyna speciosa*,[11−13] which has been traditionally used in tropical areas as a substitute for opium,[14] we have found that mitragynine (**1**), a major Corynanthe type indole alkaloid of this plant, elicited potent analgesic activity mainly via μ-opioid receptors.[15] In addition, mitragynine pseudoindoxyl (**2**),[16] an oxidative derivative of **1**, was found to show potent opioid agonistic activity in the guinea pig ileum and in mouse vas deferens. The potency of **2** on μ-opioid receptors in the guinea pig ileum was about 100- and 20-fold higher than that of **1** and morphine (**4**), respectively. Additionally, its potency on δ-opioid receptors in mouse vas deferens was about 700- and 35-fold higher than that of **1** and **4**, respectively. These *Mitragyna* alkaloids from Thai herbal medicine have different chemical structures as compared with the morphine skeleton. These findings prompted us to embark on the creation of novel lead compounds based on the mitragynine skeleton, which may be utilized for development of practical analgesics and as a new type of probe for the study of opioid receptor systems. In the present paper, our findings on a survey of the structure−activity relationship using natural and semisynthetic derivatives of **1** as well as potent and unique opioid agonistic and antagonistic activities of the mitragynine derivatives in the guinea pig ileum test will be described. In addition, an analgesic activity of one of the mitragynine derivatives, i.e., **2**, will be reported.

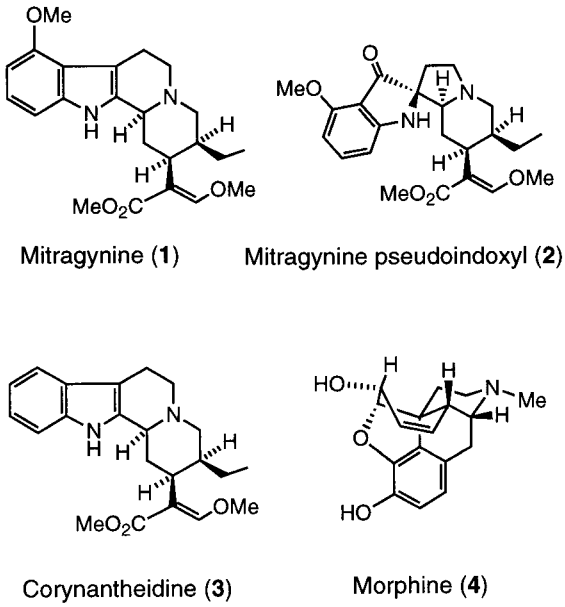

Figure 1.

Chemistry

In investigating the structure−activity relationship, we initially directed our attention to the presence of a methoxyl group at the C9 position on the indole ring in **1**, because it was a structural characteristic of *Mitragyna* alkaloids, as compared with common Corynan-

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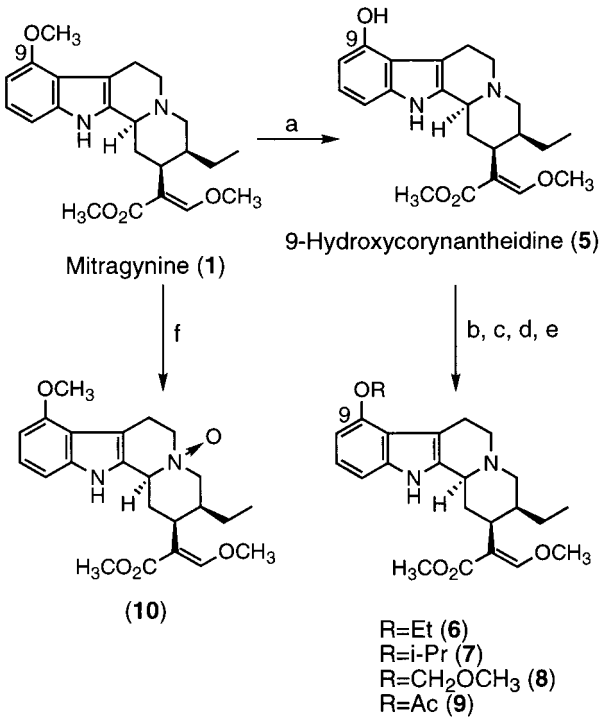
Scheme 1[a]



[a] Reagents: (a) EtSH, $AlCl_3$, CH_2Cl_2; (b) for **6**, EtI, 15% aqueous NaOH, *n*-Bu_4NHSO_4, benzene; (c) for **7**, *i*-PrI, 15% aqueous NaOH, *n*-Bu_4NHSO_4, benzene; (d) for **8**, CH_3OCH_2Cl, 15% NaOH, methyltrialkyl(C_8-C_{10})ammonium chloride, CH_2Cl_2; (e) for **9**, Ac_2O, pyridine; (f) *m*-chloroperbenzoic acid, CH_2Cl_2.

the type indole alkaloids isolated from other plant genus. As described in the Biological Results and Discussion in detail, corynantheidine (**3**), which corresponds to 9-demethoxy-mitragynine, was devoid of agonistic activity in guinea pig ileum preparation. This fact indicated that the methoxy group on C9 in **1** is essential for eliciting the analgesic activity. On the basis of this result, we initially carried out chemical modification of the C9 function in **1**. As shown in Scheme 1, the methyl ether at C9 was selectively cleaved with EtSH and $AlCl_3$ in CH_2Cl_2 to give 9-hydroxycorynantheidine (**5**) in 90% yield. The thus-obtained phenolic function was converted to ethyl, *i*-propyl, and methoxymethyl ether derivatives (**6**−**8**). In addition, acetate (**9**) was also prepared with a view to its relation to morphine and heroin. The *N*b oxide derivative (**10**) was also prepared by treatment of **1** with one equivalent of *m*-chloroperbenzoic acid.

As described in the Introduction, we have already found that **2**, an oxidative derivative of **1**, has high agonist potency in in vitro experiments.[16] On the basis of this knowledge, we prepared some oxidative derivatives of the indole nucleus in **1**, as shown in Scheme 2. Treatment of **1** with lead tetraacetate gave the 7-acetoxyindolenine derivative (**11**) in 50% yield. By alkaline hydrolysis of **11**, 7-hydroxy-7*H*-mitragynine (**12**), which has been found as a minor constituent in the leaves of *M. speciosa*,[17] was obtained in 95% yield. Treatment of **12** with sodium methoxide in methanol gave the pseudoindoxy derivative (**2**). Compound **1** afforded 7-methoxy or 7-ethoxy indolenine derivatives (**13** and **14**) by treatment with idosobenzene diacetate in MeOH or EtOH, respectively. Treatment of **1** with NaH in dimethyl formamide (DMF) under air gave the 4-quinolone derivative (**15**) in 49% yield.

Biological Results and Discussion

Pharmacological Evaluation of Potencies and Affinities of Compounds for Opioid Receptors Using Electrically Stimulated Contraction in Isolated Guinea Pig Ileum and Receptor Binding Assay. The opioid agonistic activities of the natural analogue of mitragynine and semisynthetic compounds derived from **1** were evaluated using twitch contraction induced by electrical stimulation in guinea pig ileum preparation. Opioid agonistic activities are defined as the inhibition of the twitch contraction, which is reversed by the opioid antagonist naloxone. The affinities for μ-, δ-, and κ-receptors were determined by displacement upon the binding of [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U69593, respectively, to guinea pig brain membranes.

In guinea pig ileum, the main constituent **1** inhibited the twitch contraction induced by electrical stimulation, which was reversed by the addition of naloxone.[10,15] Figure 2 shows that the inhibitory effect of **1** was concentration-dependent. Its potency is one-fourth of that of **4** (Table 1). The first noteworthy result is that a 9-demethoxy analogue of mitragynine, i.e., **3**, did not show any opioid agonistic activity at all (Figure 2) but reversed the morphine-inhibited twitch contraction in guinea pig ileum (Figure 3). Its antagonistic effect was in a concentration-dependent manner (Table 2). Compound **3** did not affect the muscarinic receptor antagonist atropine or the Ca^{2+} channel blocker verapamil-inhibited twitch contraction (Table 2). These results suggest that **3** inhibits the effect of morphine via functional antagonism of opioid receptors. The receptor binding assay substantiated that **3** selectively binds to μ-receptors (Table 3). Taken together, **3** was found to have an opioid antagonistic property on μ-opioid receptors.

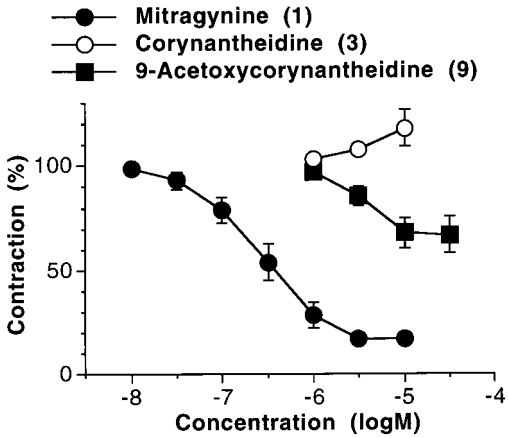
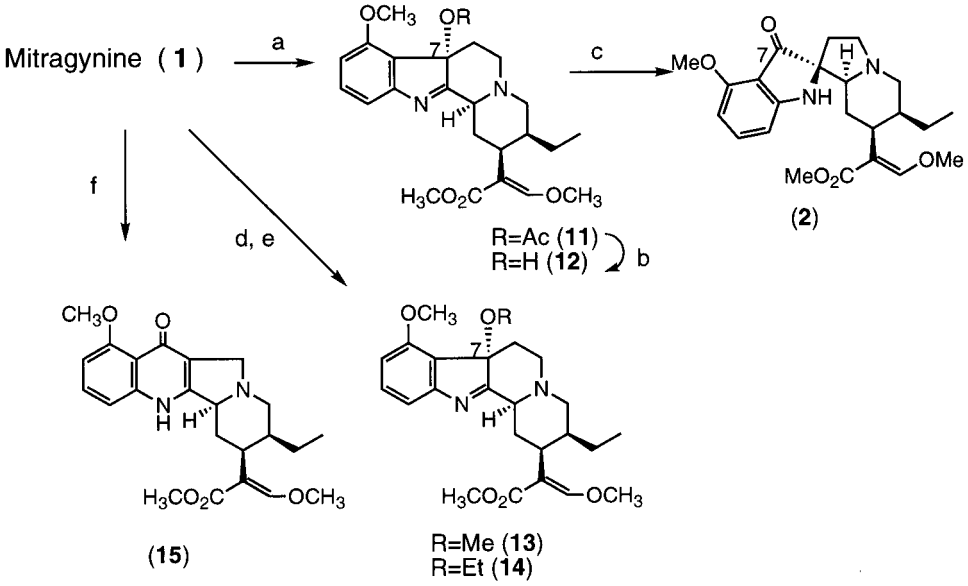


Figure 2. Effects of **1**, **3**, and **9** on twitch contraction induced by electrical stimulation in guinea pig ileum. Each point represents means ± SEM of five animals.

The 9-demethyl analogue of mitragynine, **5**, also inhibited electrically induced twitch contraction in guinea pig ileum, but its maximum inhibition percent is less potent than that of **1** (Table 1). On the other hand, the receptor binding assay clarified that **5** binds to μ-receptors (Table 3). Taken together, it is suggested

Scheme 2[a]



[a] (a) $Pb(OAc)_4$, CH_2Cl_2; (b) 15% aqueous NaOH, MeOH; (c) NaOMe, MeOH; (d) for **13**, idosobenzene diacetate, MeOH; (e) for **14**, idosobenzene diacetate, EtOH; (f) NaH, air, DMF.

Table 1. Opioid Agonistic Activities of Mitragynine-Related Compounds and Morphine in Electrically Stimulated Guinea Pig Ileum Preparation[a]

compds	*n*	pD_2 value	relative potency (%)	maximum inhibition (%)	relative inhibitory activity (%)
4	5	7.17 ± 0.05	100	87.2 ± 1.8	100
1	5	6.59 ± 0.13**	26	83.1 ± 3.7	95
2	6	8.71 ± 0.07***	3467	83.5 ± 3.3	96
3	5			−18.1 ± 8.6***	−21
5	5	6.78 ± 0.23	41	49.4 ± 3.1***	57
6[b]	5	NS	NS	NS	NS
7[b]	5	NS	NS	NS	NS
8[c]	5	NE	NE	NE	NE
9	5	5.39 ± 0.12	2	33.2 ± 8.8***	38
10	5			−123.5 ± 39.2***	−142
11	6	6.50 ± 0.16**	21	13.4 ± 12.7***	15
12	5	8.20 ± 0.14***	1071	86.3 ± 4.8	99
13	5	6.45 ± 0.04***	19	60.9 ± 7.2**	70
14	5	5.29 ± 0.12***	1	22.9 ± 1.1***	26
15	5	6.70 ± 0.04***	34	74.1 ± 5.6	85
16	5	5.40 ± 0.07***	2	85.9 ± 2.7	101

[a] Opioid agonistic activities of the compounds are evaluated by their ability to inhibit the electrically induced twitch contraction, which is reversed by naloxone (300 nM). Relative potency is expressed as a percentage of the pD_2 value of the compound against that of morphine. The pD_2 values of **2** and **12** are significantly high as compared with that of morphine, while the pD_2 values of **1**, **11**, and **13**−**16** are significantly low as compared with that of morphine. Maximum inhibition (%), which is elicited by the compound when the response reached a plateau, was calculated by regarding electrically induced contraction as 100%. The concentration range of tested compounds was from 100 pM to 30 μM. Relative inhibitory activity, which means intrinsic activity on opioid receptors, is expressed as a percentage of the maximum inhibition by compounds against that by morphine. Each value represents the mean ± SEM of the results obtained from five to six animals. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, significantly different from the morphine group. [b] In the case of the naloxone insensitive inhibition, the effect was regarded as "nonspecific (NS)". [c] In the case that significant inhibition was not obtained at 30 μM of the compound, the effect was regarded as "no effect (NE)".

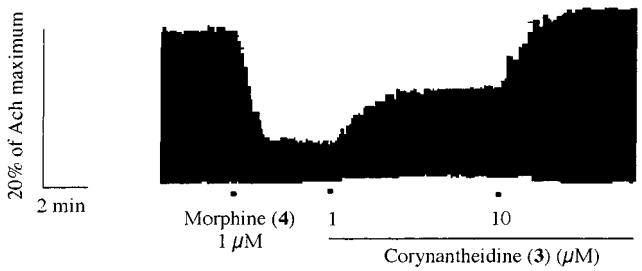


Figure 3. Typical recording of effect of **3** on twitch contraction inhibited by morphine in guinea pig ileum.

Table 2. Effects of **3** on Twitch Contraction Inhibited by **4**, Atropine, and Verapamil in Electrically Stimulated Guinea Pig Ileum Preparation[a]

drugs (concn)	contraction (%) inhibited by drugs (control)	contraction (%) reversed by **3**	
		1 μM	10 μM
4 (1 μM)	27.4 ± 3.5	43.0 ± 4.2*	109.2 ± 6.2***
atropine (0.1 μM)	3.9 ± 1.3	5.7 ± 1.6	6.3 ± 1.4
verapamil (3 μM)	6.8 ± 1.8	7.0 ± 1.9	13.2 ± 3.8

[a] Each value represents the mean ± SEM of the results obtained from five animals. *, $P < 0.05$; ***, $P < 0.001$, significantly different from the control group.

that **5** is a partial agonist of opioid receptors. It is an interesting finding that a fine transformation of the substituent on C9, i.e., from OMe to OH or to H, led to a shift of activity from that of a full agonist via a partial agonist to that of an antagonist on opioid receptors. Thus, it is found that the functional group on C9 of

Table 3. Opioid Receptor Binding Affinities of Mitragynine-Related Compounds at μ-, δ-, and κ-Receptor[a]

	p*K*i			relative affinity (%)		
compds	[^{3}H]DAMGO	[^{3}H]DPDPE	[^{3}H]U69593	μ	δ	κ
4	8.46 ± 0.29	6.38 ± 0.13	6.33 ± 0.22	98.5	0.8	0.7
1	8.14 ± 0.28	7.22 ± 0.21	5.96 ± 0.22	88.7	10.7	0.6
2	10.06 ± 0.39	8.52 ± 0.22	7.10 ± 0.32	97.1	2.8	0.1
3	7.14 ± 0.18	5.54 ± 0.44	5.59 ± 0.04	95.8	1.0	3.2
5	7.92 ± 0.05	4.51 ± 0.15	5.53 ± 0.07	99.6	< 0.1	0.4
12	7.87 ± 0.16	6.81 ± 0.19	6.91 ± 0.07	83.3	6.6	10.1

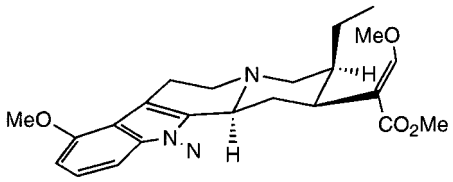
[a] Receptor binding assays were carried out using membranes prepared from guinea pig brain. Specific binding of each radioligand for opioid receptor subtype was saturated, and Scatchard plots were linear. The K_D values of [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U69593 were 0.44 ± 0.04, 0.83± 0.05, and 0.69 ± 0.10 nM, respectively. Radioligand displacement curves were generated from binding data. The binding constants (pK_i) were calculated using the IC_{50} values obtained from the displacement curves. Values are the means ± SEM of 3−5 separate displacement curves, each assayed in triplicate. Relative affinities (%) of compounds were calculated according to the following equations: relative affinity = $K_a x/(K_a\mu + K_a\delta + K_a\kappa)$, ($K_a = 1/K_i$).

mitragynine-related compounds managed the relative inhibitory activity, which means the intrinsic activity on opioid receptors. The affinities of **1**, **3**, and **5** on μ-, δ-, and κ-receptors were determined by displacement of the binding of specific radioligands at each receptor in guinea pig brain membranes. Table 3 shows a close similarity of the pK_i values of **3** and **5** on each receptor. The relative affinities for μ-, δ-, and κ-receptors are also shown in Table 3. The binding assay demonstrated that these compounds have relatively high selectivity for μ-receptors.

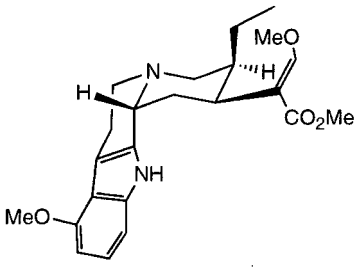
The introduction of the acetoxy group at C9 on the indole ring (compound **9**) led to marked reduction in both intrinsic activity and potency as compared with those of **1** (Figure 2). The compounds **6** and **7** with the elongation of the methyl group of 9-methoxy ether induced naloxone insensitive inhibition on twitch contraction (Table 1), suggesting a inhibitory effect via mechanisms distinct from the stimulation of opioid receptors. The compound **8** did not show any opioid agonistic activities. These results demonstrate that the intrinsic activities of compounds on opioid receptors are determined by their functional groups at the C9 position and that a methoxy group at the C9 position is the most suitable functional group for pharmacophore binding to opioid receptors.

Speciociliatine (**16**), a minor constituent of this plant, is the C3 stereoisomer of mitragynine, which takes a folded *cis*-quinolizidine conformation in the C/D-ring junction as depicted in Figure 4. The potency of this compound to opioid receptors was 14-fold weaker than that of **1** in ileal preparation (Table 1), indicating that the flat *trans*-quinolizidine form was a more efficient conformation for exhibiting the activity than the folded *cis* form. The *N*b oxide derivative (**10**) showed no opioid agonistic activity in guinea pig ileum. The *N*b lone electron pair was also found to be essential for opioid agonistic activity. These results suggest that the *N*b lone electron pair of this series of compounds constitutes pharmacophore binding to opioid receptors.

Compound **12**, a minor constituent of *M. speciosa*, was found to exhibit high potency to opioid receptors: about 13- and 46-fold higher than those of **4** and **1**, respectively. The intrinsic activity of **12** suggested its full agonistic property on opioid receptors. The introduction of a hydroxy group at the C7 position led to higher potency as compared with that of **1**. As shown in Table 3, **12** tends to show selectivity at μ-receptors. The relative affinity for κ-receptors of **12** is higher than that of **1**.



Mitragynine (**1**)



Speciociliatine (**16**)

Figure 4. Stereostructure of mitragynine and speciociliatine.

In turn, **11** has lower intrinsic activity than **1**, although its potency is nearly equal to that of **1** (Table 1). The introduction of a methoxy or an ethoxy group at the C7 position (compounds **13** and **14**) led to a dramatic reduction in both intrinsic activity and potency for opioid receptors (Table 1). It is conceivable that the hydroxyl group at the C7 position in the mitragynine skeleton is a necessary functional group for the increased potency to opioid receptors. 4-Quinolone derivative (**15**) retained almost the same opioid agonistic activity as that of **1**.

Pharmacological Evaluation of Analgesic Activities of Compounds in Mice. Among the mitragynine derivatives described above, **2** emerged as compounds of potential analgesics in the guinea pig ileum test. Thereupon, the antinociceptive activities of compound **2** having a different structural skeleton from morphine were evaluated using mice tail flick test.

Compound **2**, administered by intracerebroventricular (icv) injections, showed antinociceptive effects in the tail flick test in mice. Its effect reached a maximum at about 15−45 min after the injection (Figure 6). The effect of **2** is less potent than that of morphine (Figure 5). In addition, the effect of **1** is less potent than that of **2** (Figure 5). The analgesic effects of these compounds are dose-dependent. The ED_{50} value estimated for **2** was 6.51 nmol/mouse (95% confidence limits 3.78−11.20 nmol/mouse), while that of **4** was 3.20 nmol/mouse (95% confidence limits 2.11−4.88 nmol/mouse). The ED_{50} value estimated for **1** was 60.22 nmol/mouse (95% confidence limits 39.10−92.75 nmol/mouse). The antinociceptive effects of **1**, **2**, and **4** were completely inhibited by naloxone at 2 mg/kg, s.c. (Figure 6). Therefore, these compounds induce analgesic effect via opioid receptors. Compound **2** exhibited less potent

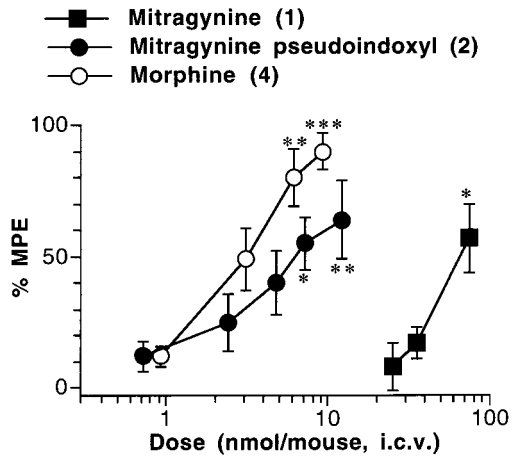


Figure 5. Dose−response curves for analgesic activities induced by intracerebroventricular administration of **1**, **2**, and **4** in tail flick test in mice. Tail flick latencies are presented as % MPE. Values are mean ± SEM of values obtained from 9 to 12 animals. Each point represents % MPE at 30 min after the administration of each drug. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, significantly different from the control (vehicle) group.

analgesic activity than **4**, despite very high opioid activity in isolated guinea pig ileum test. We speculate that the low analgesic activity of **2** results from the instability of the compound in the brain.

Computational Superposition of Morphine and Mitragynines. Complementarily, we tried to look for any structural similarity between morphine and mitragynine or its derivative using molecular modeling techniques. A theory that spatial arrangement of the three functional groups, i.e., a nitrogen atom, a benzene residue, and a phenol function, in the structure of morphine plays a significant role in exhibiting analgesic activity has been proposed by Beckett and Casy.[18] After that, Portoghese has suggested a new concept "different binding modes for different opioid skeletons".[19] These models have since been refined by researchers and now include the receptor-based models to interpret the interactions between the opioid receptors and the agonists including the nonmorphinan skeletons so far developed.[20] At the outset, we examined the respective superposition between the nitrogen atom, benzene ring, and oxygen function on the benzene ring in **4** and those in **1** or **2**. However, as shown in Figure 7, we have not been able to superimpose all three functions of the two molecules. Therefore, we concluded that *Mitragyna* alkaloids are structurally different from the morphine skeleton.

Conclusion

A series of novel Corynanthe type ligands derived from a natural indole alkaloid, **1**, was prepared and evaluated for pharmacological activities on opioid receptors. Consequently, we found that some mitragynine derivatives, whose basic skeleton is completely different from that of **4**, exhibit potent agonistic properties on opioid receptors in guinea pig ileum. It was also found that the methoxy group on the indole ring at the C9 position as well as the *N*b lone electron pair in the fundamental structure of Corynanthe type indole alkaloid are essential structural functions for opioid agonistic activity. With altering the functional group at the C9 position, i.e., OMe → OH → H, of mitragynine, the activities of compounds dramatically shifted from that of full agonists through partial agonists to that of antagonists on opioid receptors. Among the mitragynine derivatives prepared in the present study, **2**, which is an oxidized derivative of **1**, and **12**, which was also isolated as a minor constituent of the Thai medicinal herb, *M. speciosa*, were found as opioid agonists with a higher potency than **4** in the experiment with guinea pig ileum. In addition, **2** induced an analgesic activity in the tail flick test in mice. Detailed studies on their analgesic effects are under way to investigate the potential abilities of mitragynine derivatives for clinical use.

Experimental Section

General Methods. UV spectra were recorded in MeOH. Hitachi U 3400. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded at 500 and 125.65 MHz, respectively (ppm, *J* in Hz with tetramethylsilane (TMS) as internal standard). JEOL JNM A-500. Electron ionization mass spectrometry (EI-MS): direct probe insertion at 70 eV. JEOL JMS-AM20. Fast atom bombardment (FAB)-MS: JEOL JMS-HX110. Thin-layer chromatography (TLC): precoated Kieselgel 60 F_{254} plates (Merck, 0.25 mm thick). Column chromatography: Kieselgel 60 [Merck, 70−230 (for open chromatography) and 230−400 mesh (for flash chromatography)], Sephadex LH-20 [Pharmacia Biotech]. Performance liquid chromatography (MPLC): silica gel prepacked column Kusano CPS-HS-221-05. Preparative TLC: silica gel 60 GF_{254} (Merck 7730, 0.5 mm thick). Male albino Dunkin−Hartley guinea pigs (300−400 g) and male ddY mice (30−35 g) were purchased from Takasugi Laboratory Animals Co. (Saitama, Japan) and Japan SLC Inc. (Shizuoka, Japan), respectively. They were housed in 12 h/12 h light/dark cycles, at 25 °C ± 1 for at least 1 week before the experiments with free access to food and water. Each animal was used only once. The experiments were carried out in strict accordance with "Guiding Principles for the Care and Use of Laboratory Animals" approved by The Japanese Pharmacological Society and the guideline approved by the Ethical Committee on Animal Care and Animal Experiment of our Faculty. Chemicals for pharmacological assay were obtained from the following sources: acetylcholine chloride (Dai-ichi Seiyaku Co., Ltd., Tokyo, Japan); morphine hydrochloride (Takeda Chemical Industries, Japan); [^{3}H][D-Ala^2, MePhe^4, Glyol^5] enkephalin ([^{3}H]DAMGO), [^{3}H][D-Pen^{2,5}] enkephalin ([^{3}H]DPDPE), and [^{3}H]U-69593 (Du Pont NEN Life Science Products, Boston, MA); [D-Ala^2, Met-Phe^4, Glyol^5] enkephalin (DAMGO), naloxone hydrochloride, U-69593 (Sigma Chemical Co., St. Louis, MO); [D-Pen^{2,5}] enkephalin (DPDPE) (BACHEM Feinchemikalien, Switzerland).

Chemistry. Conversion of 1 to 5. To a stirred solution of **1** (300 mg, 0.75 mmol) in dry CH_2Cl_2 (6 mL) was successively added $AlCl_3$ (302 mg, 2.26 mmol) and then EtSH (1.0 mL, 13.5 mmol) at 0 °C under argon atmosphere. After the reaction mixture was stirred for 3 h at room temperature, the volatile material was thoroughly removed under reduced pressure. The residue was separated by Al_2O_3 column chromatography (*n*-hexane/AcOEt 1:1) to give (−)-**5** (258 mg, 90%) as an amorphous powder. UV (MeOH) λ_{max}: 294, 226 nm. IR ν_{max} ($CHCl_3$): 3213, 1695, 1636 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.78 (1H, br.s, *Na*-H), 7.43 (1H, s, H-17), 6.89 (1H, dd, J = 7.8, 7.8, H-11), 6.84 (1H, d, J = 7.3, H-12), 6.37 (1H, d, J = 7.3, H-10), 3.71 (6H, s, 17-OCH_3 and 22-OCH_3), 3.19 (1H, m, H-6), 3.13 (1H, br.d, J = 11.6, H-3), 3.04 (2H, m, H-15 and H-21), 2.94 (2H, m, H-5 and H-6), 2.56 (1H, m, H-5), 2.08 (1H, m, H-14), 2.44 (1H, m, H-21), 1.76 (2H, m, H-14 and H-19), 1.63 (1H, m, H-20), 1.22 (1H, m, H-19), 0.86 (3H, dd, J = 7.3, 7.3, H-18). ^{13}C NMR ($CDCl_3$): δ 169.3 (C-22), 160.6(C-17), 150.0 (C-9), 137.9 (C-13), 134.0 (C-2), 121.9 (C-11), 116.6 (C-7), 111.4 (C-16), 106.7 (C-8), 104.3 (C-10), 103.8 (C-12), 61.5 (17-O*CH*$_3$), 61.2 (C-3), 57.6 (C-21), 53.5 (C-5), 51.4 (22-OCH_3), 40.6 (C-20), 39.8

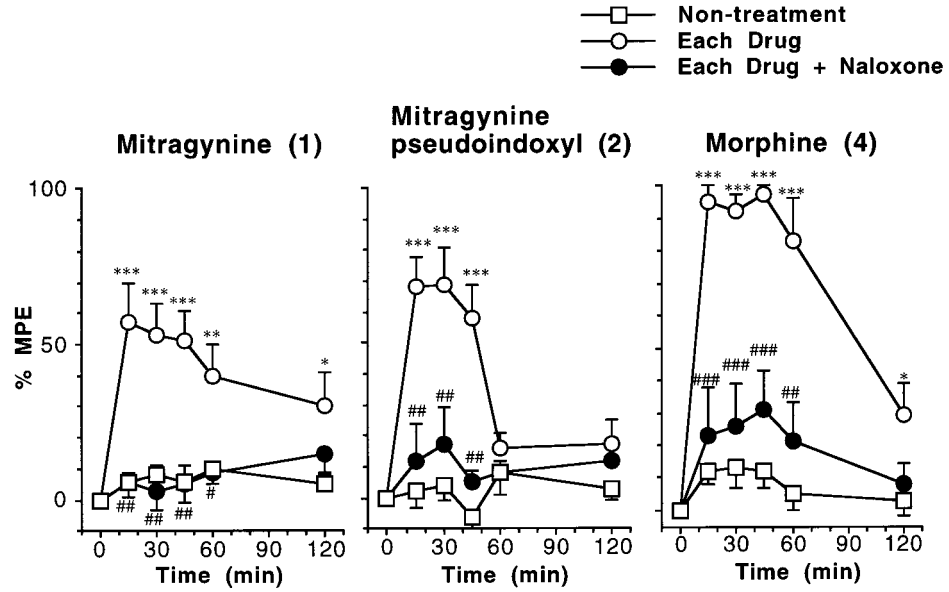


Figure 6. Effects of naloxone on analgesic activities of **1** (75 nmol/mouse, icv), **2** (12 nmol/mouse, icv), and **4** (9 nmol/mouse, icv) in tail flick test in mice. Naloxone (2 mg/kg, s.c.) was administered 15 min before injection of each drug. Tail flick latencies are presented as a percentage of MPE. Values are mean ± SEM of values obtained from 7 to 12 animals. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, significant difference in comparison of each nontreated group. #, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$, significant difference in comparison of each drug group.

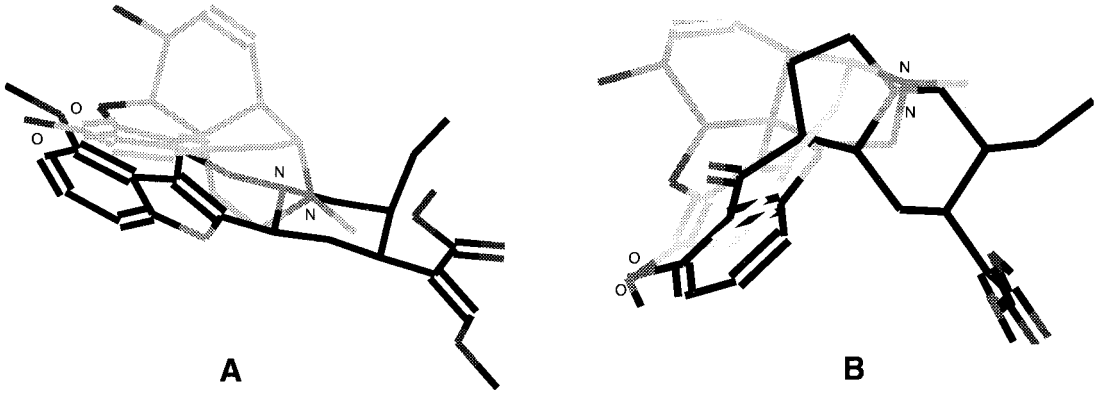


Figure 7. Overlays of the low-energy conformation of morphine (gray)/mitragynine (black) (A) and morphine (gray)/mitragynine pseudoindoxyl (black) (B). Hydrogen atoms are omitted.

(C-15), 29.8 (C-14), 23.6 (C-6), 19.1 (C-19), 12.8 (C-18). EI-MS *m*/*z* (%): 384 (M^+, 100), 369 (35), 255 (30), 200 (76). HR-FABMS: calcd for $C_{22}H_{29}O_4N_2$ [MH^+], 385.2127; found, 385.2123.

Preparation of 9. A mixture of **5** (11 mg, 0.028 mmol) and acetic anhydride (250 μL, 0.265 mmol) in dry pyridine (0.5 mL) was stirred at room temperature under argon atmosphere for 1 h. After the volatile material was removed under reduced pressure, the residue was diluted with 5% aqueous $NaHCO_3$ solution. The whole mixture was extracted with $CHCl_3$ three times. The combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was separated by SiO_2 column chromatography (3% MeOH−$CHCl_3$) to give **9** (9 mg, 76%) as an amorphous powder. UV (MeOH) λ_{max}: 279 (sh), 227 nm. ^{1}H NMR ($CDCl_3$): δ 7.95 (1H, br.s, *Na*-H), 7.43 (1H, s, H-17), 7.15 (1H, dd, J = 8.1, 0.8, H-12), 7.05 (1H, dd, J = 7.8, 7.8, H-11), 6.75 (1H, dd, J = 7.7, 0.8, H-10), 3.72 (3H, s, 17-OCH_3), 3.71 (3H, s, 22-OCH_3), 3.15 (1H, br.d, J = 11.3, H-3), 3.04 (3H, m, H-6 and H-15 and H-21), 2.92 (1H, m, H-5), 2.71 (1H, br.d, J = 10.4, H-6), 2.55 (2H, m, H-5 and H-14), 2.46 (1H, dd, J = 11.5, 2.5, H-21), 2.35 (3H, s, 9-OCOCH_3), 1.80 (1H, br.d, J = 12.6, H-14) 1.75 (1H, m, H-19), 1.63 (1H, br.d, J = 10.9, H-20), 1.21 (1H, m, H-19), 0.86 (3H, dd, J = 7.4, 7.4, H-18).^{13}C NMR ($CDCl_3$): δ 170.1 (9-O*C*OCH$_3$), 169.3 (C-22), 160.7 (C-17), 143.7 (C-9), 137.9 (C-13), 136.2 (C-2), 121.3 (C-11), 120.4 (C-7), 112.0 (C-10), 111.4 (C-16), 108.9 (C-12), 106.3 (C-8), 61.6 (17-OCH_3), 61.1 (C-3), 57.7 (C-21), 53.4 (C-5), 51.5 (22-OCH_3), 40.7 (C-20), 40.0 (C-15), 29.9 (C-14), 22.9 (C-6), 21.1 (9-OCOCH_3), 19.1 (C-19), 12.9 (C-18). EI-MS *m*/*z* (%): 426 (M^+, 85), 411 (35), 297 (35), 242 (100). HR-FABMS: calcd for $C_{24}H_{31}O_5N_2$ [MH^+], 427.2233; found, 427.2233.

Preparation of 10. To a stirred solution of **1** (52 mg, 0.13 mmol) in dry CH_2Cl_2 (1 mL) was added a solution of *m*-chloroperbenzoic acid (25 mg, 0.16 mmol) in dry CH_2Cl_2 (0.3 mL) at −50 °C under argon atmosphere. After the reaction mixture was stirred for 4 h, the reaction mixture was poured into the chilled 5% $NaHCO_3$ solution and was extracted with $CHCl_3$. The combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was separated by SiO_2 column chromatography (5% MeOH−$CHCl_3$) to give **10** (19 mg, 36%) as an amorphous powder. UV (MeOH) λ_{max}: 291, 283 (sh), 247 (sh), 224 nm. ^{1}H NMR ($CDCl_3$): δ 7.87 (1H, br.s, *N*a−H), 7.52 (1H, s, H-17), 7.00 (1H, dd, J = 7.9, 7.9, H-11), 6.87 (1H, d, J = 8.1, H-12), 6.45 (1H, d, J = 7.8, H-10), 4.31 (1H, br.d, J = 11.9, H-3), 3.86 (3H, s, 9-OCH_3), 3.79 (3H, s, 17-OCH_3), 3.74 (3H, s, 22-OCH_3), 3.61∼3.86 (4H, m, H-5 and H-21), 3.38∼3.48 (1H, m, H-14), 3.31 (1H, dd, J = 12.2, 4.6, H-6), 3.19 (1H, br.d, J = 13.4, H-15), 3.06 (1H, dd, J = 16.1, 3.9, H-6), 2.65∼2.80 (1H, m, H-19), 1.80∼1.95 (2H, m, H-14 and H-20), 1.30∼1.45 (1H, m, H-19), 0.99 (3H, dd, J = 7.4, 7.4, H-18). ^{13}C NMR ($CDCl_3$): δ 168.86 (C-22), 161.27 (C-17), 154.68 (C-9), 137.65 (C-13), 127.20 (C-2), 122.57 (C-11), 117.45 (C-7), 109.92 (C-16), 107.17 (C-8), 104.34 (C-12), 100.08

(C-10), 77.21 (C-5), 71.47 (C-3), 66.85 (C-21), 61.79 (17-OCH_3), 55.41 (9-OCH_3), 51.50 (22-OCH_3), 40.27 (C-20), 39.42 (C-15), 29.95 (C-6), 24.15 (C-14), 20.82 (C-19), 13.31 (C-18). EI-MS *m*/*z* (%): 414 (M^+, 1.7), 398 (16), 397 (18), 383 (10), 200 (100). HR-FABMS: calcd for $C_{23}H_{31}O_5N_2$, 415.2233; found, 415.2243.

Preparation of 11. To a stirred solution of **1** (105 mg, 0.26 mmol) in dry CH_2Cl_2 (14 mL) was added $Pb(OAc)_4$ (259 mg, 91% purity, 0.53 mmol) at 0 °C under argon atmosphere. After the reaction mixture was stirred for 1.5 h, the reaction mixture was poured onto the chilled water and was extracted with CH_2Cl_2 five times. The combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was separated by Al_2O_3 column chromatography (Woelm N, grade III, CH_2Cl_2) to give **11** (60 mg, 50%) as a yellowish amorphous powder. UV (MeOH) λ_{max}: 222, 246 (sh), 310 nm. ^{1}H NMR ($CDCl_3$): δ 7.44 (1H, br.s, H-17), 7.28 (1H, dd, J = 8.4, 8.4, H-11), 7.25 (1H, dd, J = 7.6, 0.9, H-12), 6.72 (1H, d, J = 7.6, H-10), 3.83 (3H, s, 9-OCH_3), 3.81 (3H, s, 17-OCH_3), 3.70 (3H, s, 22-OCH_3), 3.03 (1H, dd, J = 11.4, 2.5, H-3), 2.05 (3H, s, 7-OAc), 0.82 (3H, dd, J = 7.4, 7.4, H-18), 2.40∼3.00 (7H), 1.23∼1.92 (5H). ^{13}C NMR ($CDCl_3$): δ 180.92 (C-2), 169.29 (C-22), 168.54 (7-O*C*OCH$_3$), 160.78 (C-17), 155.72 (C-9), 155.46 (C-13), 130.78 (C-11), 122.87 (C-8), 114.49 (C-12), 111.09 (C-16), 108.88 (C-10), 84.76 (C-7), 61.95 (17-OCH_3), 61.79 (C-3), 58.09 (C-21), 55.41 (9-OCH_3), 51.29 (22-OCH_3), 50.03 (C-5), 40.53 (C-20), 39.38 (C-15), 35.24 (C-6), 25.79 (C-14), 20.77 (7-OCO*C*H$_3$), 18.97 (C-19), 12.82 (C-18). EI-MS *m*/*z* (%): 456 (M^+, 0.26), 397 ([M − OAc]$^+$, 87), 396 (100). HR-FABMS: calcd for $C_{25}H_{33}O_6N_2$, 457.2339; found, 457.2324.

Preparation of 12. A mixture of **11** (70 mg, 0.16 mmol) and aqueous 15% NaOH (0.3 mL) in MeOH (2 mL) was stirred at 0 °C under argon atmosphere for 2 h. The reaction mixture was poured onto the chilled water and was extracted with $CHCl_3$ five times. The combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was separated by Al_2O_3 column chromatography (*n*-hexane/AcOEt 6:4) to give **12** (62 mg, 95%) as an amorphous powder. UV (EtOH) λ_{max}(logϵ): 305 (sh, 3.43), 245 (sh, 4.06), 221 (4.36) nm. IR ν_{max} ($CHCl_3$): 3590, 2850, 2820, 2750, 1700, 1645, 1630, 1600, 1490, 1465, 1440 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.43 (1H, s, H-17), 7.27 (1H, dd, J = 8.0, 8.0, H-11), 7.19 (1H, d, J = 7.6, H-12), 6.71 (1H, d, J = 8.0, H-10), 3.85 (3H, s, 9-OCH_3), 3.80 (3H, s, 17-OCH_3), 3.68 (3H, s, 22-OCH_3), 3.12 (1H, dd, J = 11.0, 2.5, H-3), 3.03 (1H, dd, J = 11.4, 2.2, H-21), 3.00 (1H, ddd, J = 13.8, 3.6, 3.6, H-15), 2.75∼2.84 (1H, m, H-14), 2.75∼2.84 and 2.61∼2.66 (2H, each m, H-5), 2.61∼2.66 and 1.60∼1.73 (2H, each m, H-6), 2.48 (1H, dd, J = 11.2, 2.7, H-21), 1.87 (1H, br.d, J = 13.7, H-14), 1.60∼1.73 and 1.23 (2H, each m, H-19), 1.57∼1.61 (1H, m, H-20), 0.82 (3H, dd, J = 7.3, 7.3, H-18). ^{13}C NMR ($CDCl_3$): δ 184.33 (C-2), 169.22 (C-22), 160.65 (C-17), 155.85 (C-9), 154.98 (C-13), 130.55 (C-11), 126.51 (C-8), 114.10 (C-12), 111.18 (C-16), 108.79 (C-10), 80.88 (C-7), 61.69 (17-OCH3), 61.41 (C-3), 58.10 (C-21), 55.37 (9-OCH_3), 51.19 (22-OCH_3), 49.98 (C-5), 40.44 (C-20), 39.25 (C-15), 35.64 (C-6), 25.99 (C-14), 18.87 (C-19), 12.75 (C-18). EI-MS *m*/*z* (%): 414 (M^+, 91), 397 (100), 383 (38), 367 (39). HR-FABMS: calcd for $C_{23}H_{31}O_5N_2$, 415.2233; found, 415.2235.

Preparation of 2 from 12. A solution of **12** (66 mg, 0.16 mmol) and NaOMe (19 mg, 0.35 mmol) in dry MeOH (6 mL) was heated under reflux for 12 h under argon atmosphere. The reaction mixture was cooled, poured onto the chilled water, and then extracted with $CHCl_3$ three times. The combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was separated by SiO_2 column chromatography (*n*-hexane/AcOEt 1:2) to give **2** (32 mg, 48%) as an amorphous powder. UV (MeOH) λ_{max}: 395, 289, 238, 216, 202 nm. ^{1}H NMR ($CDCl_3$): δ 7.32 (1H, dd, J = 8.2, 8.2, H-11), 7.28 (1H, s, H-17), 6.40 (1H, d, J = 7.8, H-12), 6.14 (1H, d, J = 7.8, H-10), 5.17 (1H, br.s, Na−H), 3.90 (3H, s, 9-OCH_3), 3.66 (3H, s, 17-OCH_3), 3.62 (3H, s, 22-OCH_3), 3.09∼3.15 (2H, m, H-5 and H-21), 2.77 (1H, m, H-15), 2.30∼2.36 (2H, m, H-5 and H-6α), 2.15∼2.26 (2H, m, H-14β and H-3), 2.14 (1H, br.dd, J = 11.4, 3.1, H-21), 1.87∼1.94 (1H, m, H-6β), 1.60∼1.68 (1H, m, H-19), 1.50 (1H, br.d, J = 11.2, H-20), 1.14∼1.24 (1H, m, H-19), 1.10∼1.14 (1H, m, H-14α), 0.85 (3H, dd, J = 7.4, 7.4, H-18). ^{13}C NMR ($CDCl_3$): δ 199.46 (C-7), 168.85 (C-22), 162.12 (C-9), 160.16 (C-17), 158.61 (C-13), 138.62 (C-11), 111.76 (C-16), 109.83 (C-8), 103.76 (C-12), 99.07 (C-10), 75.16 (C-2), 73.20 (C-3), 61.38 (17-OCH_3), 55.65 (9-OCH_3), 54.82 (C-21), 53.15 (C-5), 51.13 (22-OCH3), 40.12 (C-20), 38.41 (C-15), 35.06 (C-6), 23.79 (C-14), 19.31 (C-19), 12.90 (C-18). EI-MS *m*/*z* (%): 414 (M^+, 24), 239 (43), 238 (100). HR-FABMS: calcd for $C_{23}H_{31}O_5N_2$, 415.2233; found, 415.2207.

Molecular Modeling. Compounds **4**, **1**, and **2** were subjected to an energy minimization using the semiempirical quantum mechanics method AM1 as implemented in the MOPAC 5.0 programs. The superimposed ensembles of the compounds, **4**/**1** and **4**/**2**, were subjected to the overlay program implemented in Chem 3D 6.0.

Magnus Assays Using Guinea Pig Ileum Preparations. Male, albino guinea pigs (Dunkin−Hartley) weighing 300−400 g purchased from Takasugi Laboratory Animals Co. Ltd. were stunned by a blow on the head and exsanguinated. The ileum was removed and placed in Krebs−Henseleit solution (mM): NaCl, 112.1; KCl, 5.9; $CaCl_2$, 2.0; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25.0; and glucose, 11.5. The solution was prepared as reported by Cox and Weinstock.[21] The ileum was set up under 1 g of tension in a 5 mL organ bath containing the nutrient solution. The bath was maintained at 37 °C and continuously bubbled with a gas mixture of 95% O_2 and 5% CO_2. At the start of each experiment, a maximum response to acetylcholine (3 μM) was obtained in each tissue to check its suitability. Tissues were stimulated through platinum needle-ring (a ring was placed 20 mm above the base of the needle 5 mm in length) electrodes using square wave pulses of supramaximal voltage. The ileum was transmurally stimulated with monophasic pulses (0.2 Hz) and 0.3 ms duration by a stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan). Contractions were isotonically recorded by using a displacement transducer (NEC, San-ei Instruments Ltd., Type 45347), DC strain amplifier (San-ei 6M92), and a DC recorder (Hitachi, Mod 056, Tokyo, Japan). All concentration−response curves were constructed in a cumulative manner. The height of the twitch response to transmural stimulation was measured before and after drug challenge. The percentage of the inhibition response remaining after the agonist was determined by dividing the contractile height after each agonist addition by the twitch height before agonist administration multiplied by 100. To obtain the percentage inhibition of the twitch height, this value was subtracted from 100. Agonist activity was expressed as a pD_2 value, which is the negative logarithm of the molar concentration required to produce 50% of the maximum responses to the drug (EC_{50}). To investigate the involvement of opioid receptors in the inhibitory effect of the samples, antagonistic effect of naloxone on the inhibited contraction was examined. The inhibitory effects of samples on electrically stimulated contraction can be regarded as opioid activities when the antagonistic effect of naloxone is observed.

Radioligand Binding Assays for μ-, δ-, and κ-Receptors. Male Dunkin−Hartley guinea pigs were killed by cervical dislocation and exsanguinated. The whole brain (excluding cerebellum) was quickly removed and placed in ice-cold 0.05 M Tris-HCl buffer, pH 7.4, at 25 °C, weighed, and immediately frozen in dry ice containing acetone. For each experiment, frozen brains from two animals were thawed and homogenized with a homogenizer (Kinematica GmbH LITTAU, Polytron, PT 10-35, Switzerland) for 60 s in 40 volumes of 50 mM Tris HCl (pH 7.4) and centrifuged at 49 000*g* for 10 min. The pellet was rehomogenized and centrifuged again. For the binding assays, membrane fractions were suspended in assay buffer at a protein concentration of 50 mg/mL. Saturation binding isotherms were produced by incubating each labeled compound at nine or ten different concentrations (10−2000 pM) with 2 mg of membrane protein. To start the reaction, 0.1 mL aliquots of protein were added to 0.9 mL of 50 mM Tris-HCl (pH 7.4) assay buffer containing 1 nM [^{3}H]DAMGO, [^{3}H]DPDPE, or [^{3}H]U-69593 and appropriate concentrations of competing unlabeled ligands in a total volume of 1 mL. The incubation

times were 1.5, 2, and 1 h for [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U-69593, respectively, at 25 °C. The reaction was terminated by rapid filtration under reduced pressure through glass microfiber filters (Whatman GF/B, presoaked in Tris-HCl buffer) followed by the addition of 4 mL of ice-cold Tris-HCl buffer. Filters were further washed with 4 mL of ice cold buffer and left to dry for 12 h. Radioactivity bound to the filters was quantitated by liquid scintillation spectrometry (ALOKA LSC-5100, Japan). Nonspecific binding for [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U-69593 was determined in the presence of 1 μM unlabeled DAMGO, naltrindole, and U-69593, respectively. The apparent dissociation constant (K_D) and maximum binding site density (B_{max}) for radioligands were estimated by Scatchard analysis of the saturation data over a concentration range of 0.01−2 nM. The ability of unlabeled drugs to inhibit specific radioligand binding was expressed as the IC_{50} value, which was the molar concentration of unlabeled drug necessary to displace 50% of the specific binding. Competitive inhibition studies were carried out in the presence of 6−9 different inhibitor concentrations. Inhibition constant values (K_i) of unlabeled compounds were calculated as described by Cheng and Prusoff.[22] Their negative logarithm values were shown as pK_i values.

Antinociceptive Assays Using Mouse Tail Flick Test. The tail flick test described by D'Amour and Smith[23] employing a tail flick apparatus (Ugo Basile, Verese, Italy) was used. Before injections, three drug-free trials were used to establish basal reaction times. The ventral surface of the tail (2−5 cm from the tip) was exposed to radiant heat, and the latency of the tail movement was recorded. The radiant heat source was set such that basal latencies were 2−4 s. Only mice with a control reaction between the basal latencies were used. The test latency after drug treatment was assessed at appropriate times, and a 10 s maximum cutoff time was used to prevent damage to the tail. Antinociception was quantified as the maximum possible effect (% MPE) using the following formula: % MPE = 100 × [(test − control)/(10 − control)]. The % MPE was calculated for each mouse with the use of at least five mice per dose. Intracerebroventricular injection of test drugs was performed as described previously.[24] The test drugs were injected in total volumes of 5 μL. The median antinociceptive dose (ED_{50}) values and 95% confidence limits were calculated by log-probit analysis according to the method of Litchfield and Wilcoxon.[25] ED_{50} was defined as the dose of the compound required to induce 50% of analgesia.

Data Analysis. The difference between the mean of any two groups was estimated using Student's *t*-test. The difference between two values was considered significant when P was less than 0.05.

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Supporting Information Available: Experimental procedures for the preparation of compounds **6**−**8** and **13**−**15**, HPLC analyses data for compounds **1**−**3**, **5**, and **12**, and ^{13}C NMR spectra of compounds **6**−**11** and **13**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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