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Syntheses of 4,6′-epoxymorphinan derivatives and their pharmacologies

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Abstract—A modification of the message site in the skeleton of naltrexone was carried out to improve the potency and selectivity of the compound for an opioid receptor subtype. In the course of conversion, we synthesized 7-membered ring ether derivatives, which had an inserted OCH_2 group between 4- and 6-positions of morphinan skeleton. One of the 7-membered ring ether derivatives possessed more potent antagonistic activity than naltrexone for the μ opioid receptor. Another compound possessing 17-methyl group derived from noroxycodone may be a μ opioid receptor partial agonist and showed analgesic activity. We are currently examining the subtype selectivity of these compounds.

1. Introduction

Three types of opioid receptors (μ, δ, κ) are now well established not only by pharmacological studies but also by molecular biological characterizations.[1] Although many highly selective and potent ligands for opioid receptor types are presently available,[2–6] the only selective ligands that target opioid receptor subtypes are TAN-67[7,8] (δ_1), BNTX[9] (δ_1), and NTB[10] (δ_2).

Ligands that are specifically directed against the subtypes of opioid receptors (μ_1, μ_2, δ_1, δ_2 and κ_1, κ_2, κ_3) are highly desirable for the investigation of the pharmacological effects attributed to these receptor subtypes. We have been interested in the design of κ and δ opioid receptor subtype (δ_1, δ_2 and κ_1, κ_2, κ_3) selective compounds and have attempted to design and synthesize them using naltrexone (**1**) with the 4,5-epoxymorphinan skeleton. However, we had limited success in obtaining subtype selective ligands synthesized from 4,5-epoxymorphinan derivatives. Therefore, we focused on modifying the 4,5-epoxymorphinan skeleton itself. The 4,5-epoxymorphinan structure of naltrexone is believed to influence the intrinsic activity of the opioid receptor (message site) and the substituents may help distinguish among the opioid receptor types (address site).[11] The 4,5-epoxymorphinan structure has been considered to contribute to three points of association between the drug molecule and the receptor site, which are an ionic interaction, a π–π interaction, and a hydrogen bond.[12,13] After noting that the conformation of the C-ring of naltrexone is flexible and rather half chair in form, we attempted to change the flexible chair form into a fixed form in order to obtain ligands selective for the μ opioid receptor subtype. As a part of this study, we have reported the synthesis of compound **2** (NS13) with a 4,6′-epoxymorphinan skeleton.[14] At that time we also found that compound **2** showed stronger antagonist activity than naltrexone, which led us to design an agonist. Generally speaking, in morphinan derivatives 17-nitrogen substituent play an important role in distinguishing between agonist and antagonist. Especially in μ opioid receptor morphine with 17-methyl group is an agonist and naltrexone with 17-cyclopropylmethyl group shows antagonist activity. On the basis of the rationale, we designed and synthesized compound **3** (NS16). Herein, we report the synthesis of **3** (NS16), also with the 4,6′-epoxymorphinan skeleton, and the pharmacological effects of NS13 and NS16 (Fig. 1).

Keywords: Opioid; Naltrexone; Analgesics; 4,6′-Epoxymorphinan.
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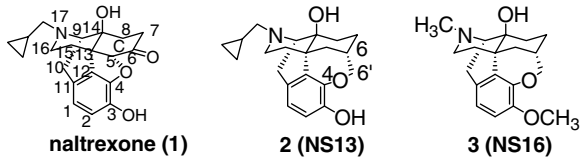


Figure 1. The structures of NS13 and NS16.

2. Results

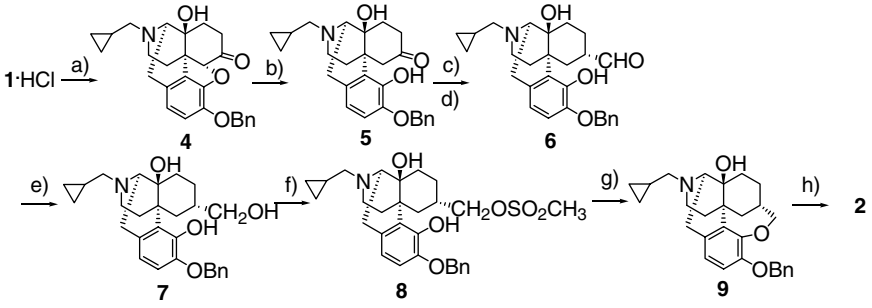
2.1. Chemistry

17-Cyclopropylmethyl 7-membered ring analog **2** was prepared by the method reported before shown in Scheme 1.[14] The phenolic hydroxyl group of naltrexone (**1**) was benzylated with BnBr, and the epoxy ring of the resulting naltrexone benzyl ether **4** was reductively cleaved with zinc in acetic acid and hydrochloric acid to give compound **5**.[15] Ketone **5** was converted to aldehyde **6** by Wittig reaction with methoxymethyltriphenylphosphonium chloride and sodium hydride in DMSO followed by hydrolysis. The reduction of aldehyde **6** gave triol **7**, and the mesylation of the resulting triol **7** with methanesulfonyl chloride in pyridine afforded the desired mono mesylate **8**. The intramolecular cyclization of mesylate **8** gave the objective 7-membered ring ether **9**. Finally, **9** was converted to compound **2** by catalytic hydrogenation with Pd (Scheme 1).

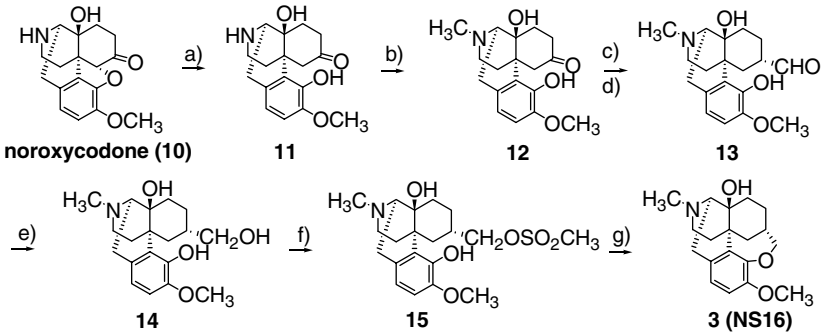
The designed compound, 17-methyl 7-membered ring analog **3** was prepared from noroxycodone (**10**) as shown in Scheme 2. At first, noroxycodone (**10**) was reductively cleaved with zinc in acetic acid and hydrochloric acid to give ketone **11**, which was subjected to reductive methylation reaction with Pd/C, formalin, and sodium acetate in acetic acid to give 17-methyl derivative **12**.[16,17] The ketone **12** was converted to the objective 7-membered ring ether **3** by the same method as Scheme 1.

2.2. Pharmacological activity

To evaluate the specific involvement of the opioid receptor types in NS13- or NS16-induced pharmacological actions, we performed the competitive displacement binding assay. At first, we demonstrated the competitive displacement binding of the μ opioid receptor ligand [^{3}H]DAMGO (Fig. 2A), the δ opioid receptor ligand [^{3}H]DPDPE (Fig. 2B) or the κ opioid receptor ligand [^{3}H]U69,593 (Fig. 2C) with graded concentrations (10^{-11}–10^{-6} M) of the unlabeled test compounds in membranes of the mouse whole brain (Fig. 2A or B) and the guinea pig cerebellum, which is relatively rich in κ opioid receptor sites (Fig. 2C). As shown in Table 1, IC_{50} values were determined by the displacement of [^{3}H]DAMGO, [^{3}H]DPDPE, or [^{3}H]U69,593. The [^{3}H]DAMGO binding was completely displaced by either morphine or NS13 in a concentration-dependent



Scheme 1. Reagents and conditions: (a) BnBr, K_2CO_3, DMF, rt; (b) Zn, HCl, CH_3COOH, reflux, 53% (two steps); (c) $Ph_3P^+C^-HOMe$, Wittig reaction; (d) HCl, rt, separation from β isomer, 17% (two steps); (e) $NaBH_4$, MeOH, 0 °C to rt, 63%; (f) CH_3SO_2Cl, Py, 0 °C; (g) K_2CO_3, DMF, rt, 57% (two steps); (h) Pd–C, H_2, MeOH, rt, 80%.



Scheme 2. Reagents and conditions: (a) Zn, HCl, CH_3COOH, reflux, 89%; (b) HCHO, CH_3COONa, Pd/C, H_2, CH_3COOH, rt, 85%; (c) $Ph_3P^+C^-HOMe$, Wittig reaction; (d) HCl, rt, separation from β isomer, 58% (two steps); (e) $NaBH_4$, CH_3OH, 0 °C to rt, 60%; (f) CH_3SO_2Cl, Py, 0 °C; (g) K_2CO_3, DMF, rt, 30% (two steps).

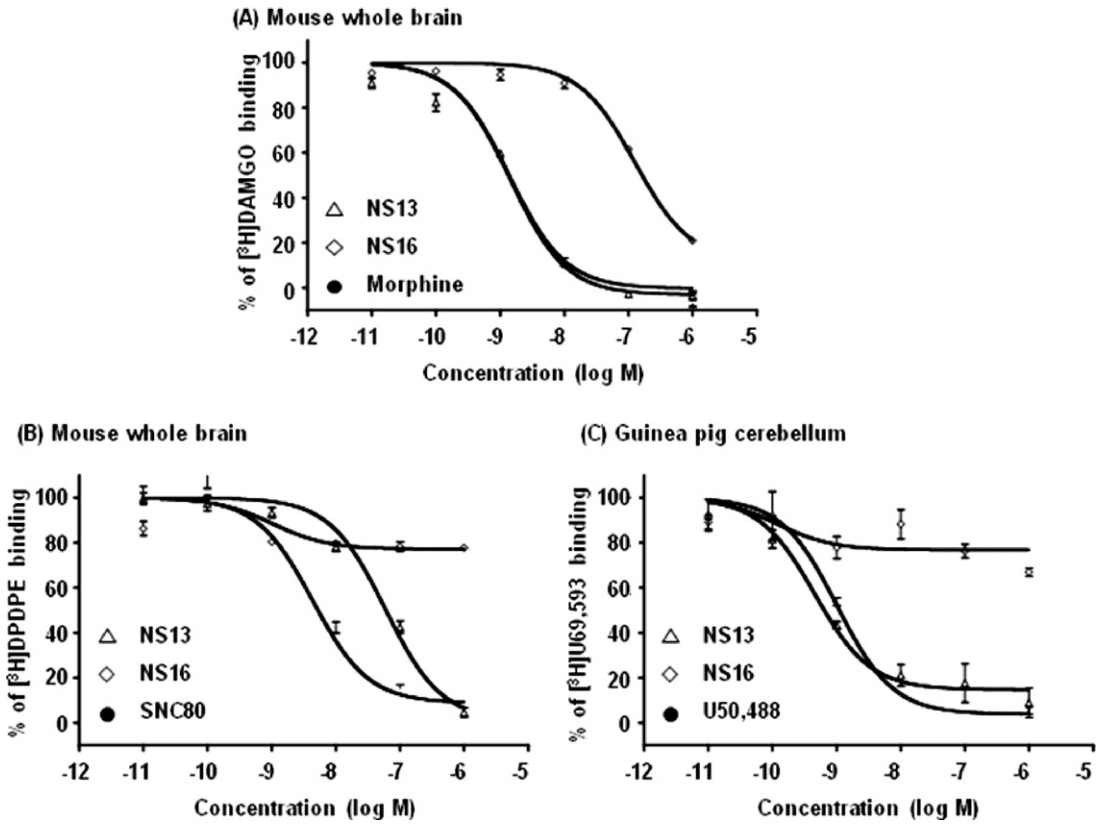


Figure 2. Displacement of the μ opioid receptor ligand [^{3}H]DAMGO (A), δ opioid receptor ligand [^{3}H]DPDPE (B) or κ opioid receptor ligand [^{3}H]U69,593 (C) binding in homogenates of the mouse whole brain without cerebellum (A or B) and the guinea pig cerebellum (C) by NS13, NS16, morphine, SNC80, or U50,488. Experiments were performed in the presence of either [^{3}H]DAMGO (1 nM), [^{3}H]DPDPE (2 nM), or [^{3}H]U69,593 (2 nM) and increasing concentrations of NS13, NS16, morphine, SNC80, or U50,488. The data represent means ± SEM of four samples.

Table 1. Binding property of NS13 or NS16 for the μ, δ, or κ opioid receptor determined by displacement of [^{3}H]DAMGO, [^{3}H]DPDPE, or [^{3}H]U69,593[a]

	IC_{50} value (nM)				
	NS13	NS16	Morphine	SNC80	U50,488
[^{3}H]DAMGO	1.21 (0.88–1.70)	183.4 (151.8–221.6)	1.27 (0.91–1.82)	ND	ND
[^{3}H]DPDPE	57.29 (44.55–73.67)	500<	500<	6.41 (4.63–8.87)	ND
[^{3}H]U69,593	0.98 (0.49–1.96)	500<	500<	ND	1.14 (0.97–1.34)

[a] IC_{50} values were determined using the analysis of variance and liner ression techniques. Groups were treated with NS13, NS16, morphine, SNC80, or U50,488. To calculate IC_{50} values, at least 6 drug doses were used and 2–4 samples were used for each dose. Values in parentheses indicate the 95% confidence range.

manner and partially displaced by NS16. Furthermore, the binding of [^{3}H] DPDPE was clealy displaced by increasing concentrations of a selective δ opioid receptor agonist SNC80 and partialy displaced by those of NS13. In addition, the [^{3}H] U69,593 binding was apparently displaced by either NS13 or a κ opioid receptor agonist U50,488 in a concentration-dependent manner. In contrast, the binding of either [^{3}H] DPDPE or [^{3}H] U69,593 was not affected by NS16.

We next investigated the ability of NS13 or NS16 to activate G-proteins in the mouse whole brain or the guinea pig cerebellum membranes. Either DAMGO, SNC80, and U50,488 showed an increase in the binding of [^{35}S]GTPγS to membranes. Co-incubation with NS13 (10^{-7}–10^{-6} M) had significantly attenuated either a selective μ opioid receptor agonist DAMGO (10^{-5} M)-, a selective δ opioid receptor agonist SNC80 (10^{-5} M)-or a selective κ opioid receptor agonist U50,488 (10^{-5} M)-induced G-protein activation ($^{***}p < 0.001$ vs DAMGO, $^{\#\#}p < 0.01$ vs SNC80, $^{+++}p < 0.001$, $^{++}p < 0.01$ vs U50,488; Fig. 3A–C). NS13 (10^{-7}–10^{-5} M) itself had no effect in the binding of [^{35}S]GTPγS to membranes of the mouse whole brain (Fig. 3D). In addition, the increase in the G-protein activation induced by NS16 (10^{-5} M) was significantly suppressed by a selective μ opioid receptor antagonist β-FNA (10^{-6} M, $^{*}p < 0.05$ vs NS16; Fig. 4A). Interestingly, co-incubation with NS16 (10^{-8}–10^{-6} M) significantly attenuated DAMGO-induced G-protein activation ($^{\#\#\#}p < 0.001$ vs DAMGO; Fig. 4B).

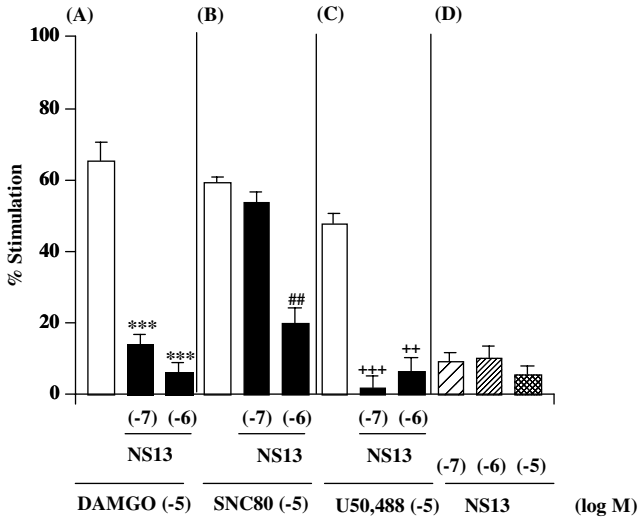


Figure 3. Effect of NS13 on the G-protein activation induced by morphine-, SNC80-, or U50,488-induced G-protein activation in the mouse brain (A or B) or guinea pig cerebellum (C). Membranes were incubated with [^{35}S]GTPγS and GDP with DAMGO, SNC80, or U50,488 in the presence or absence of NS13. The data are shown as the percentage of basal [^{35}S]GTPγS binding measured in the presence of GDP and absence of morphine, SNC80, or U50,488. Each value represents the mean with SEM of six samples. $^{***}p < 0.001$ versus DAMGO, $^{\#\#}p < 0.01$ versus SNC80, $^{+++}p < 0.001$, $^{++}p < 0.01$ versus U50,488.

We next evaluated the antinociceptive effects induced by sc administration of NS16 (10–70 mg/kg) using the hot-plate and tail-flick tests. A single sc administration of NS16 produced a dose-dependent antinociceptive effect ($^{*}p < 0.05$, $^{**}p < 0.01$ or $^{***}p < 0.001$ vs saline group; Fig. 5A and B).

3. Discussion

Compound **2** (NS13) showed antagonistic activity for μ, δ, and κ opioid receptors in the GTPγS binding test (Fig. 3), although the naltrexone, 4,5-epoxymorphinan derivative is well known as the selective μ opioid receptor antagonist. These results gave us a clue to the design of new κ and δ opioid receptor selective ligands.

On the other hand, compound **3** (NS16) showed a partial agonistic activity for the μ opioid receptor in the GTPγS binding test (Fig. 4) and an analgesic activity in the mouse hot-plate and tail-flick tests (Fig. 5). The structure of NS16 is similar to that of codeine,[18] a partial μ opioid receptor agonist, which is metabolized to give morphine. The antinociceptive effects induced by NS16 may include the effects elicited by its possible metabolite, 3-hydroxy compound **16** (Fig. 6).

Conversion of the 4,5-epoxy ring to the 4,6′-epoxy ring resulted in a more rigid morphinan skeleton. This modification may influence some pharmacophoric interactions between the ligands and the opioid receptors resulting in changes in the selectivity of these

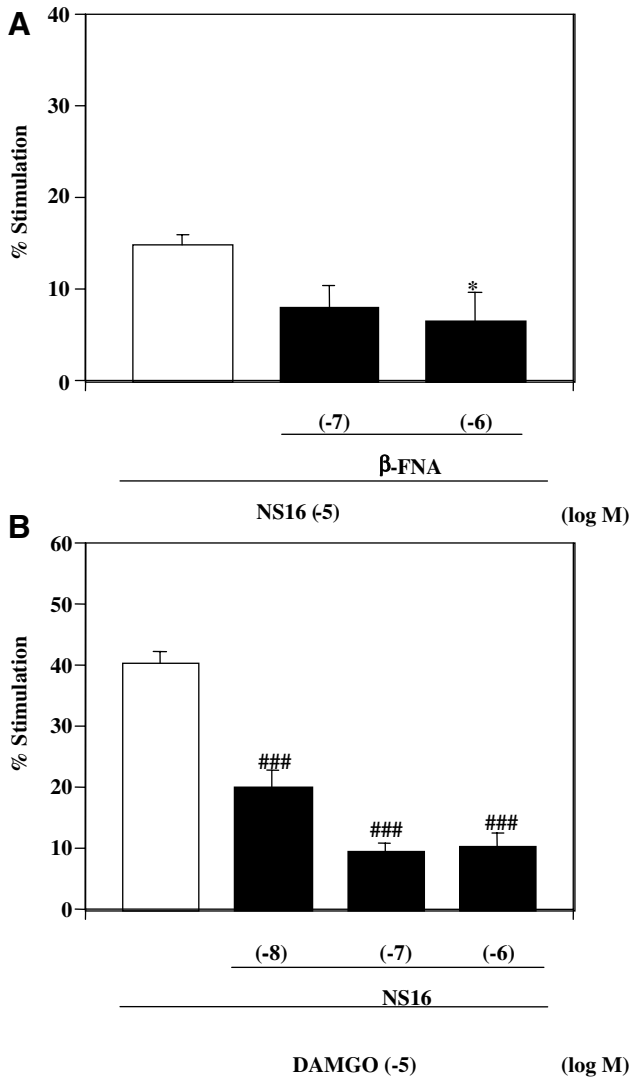


Figure 4. (A) Effect of the selective μ opioid receptor antagonist β-FNA on the G-protein activation induced by NS16 in the mouse brain. Membranes were incubated with [^{35}S]GTPγS and GDP with NS16 in the presence or absence of β-FNA. (B) Effect of NS16 on the G-protein activation induced by DAMGO in the presence or absence of NS16. The data are shown as the percentage of basal [^{35}S]GTPγS binding measured in the presence of GDP and absence of NS16. Each value represents the mean with SEM of six samples. $^{*}p < 0.05$ versus NS16 group, $^{\#\#\#}p<0.001$ vs DAMGO.

compounds for the diverse opioid receptors. The designed 4,6′-epoxymorphinan skeleton could be an alternative message site. Detailed investigations of the structure–activity relationships of the 4,6′-epoxymorphinan derivatives may clarify the influence of receptor subtype selectivities and activities that occur upon conversion of a flexible chair form on the C-ring into a rigid chair form. Introduction of new functional groups to the 4,6′-epoxymorphinan skeleton may modify the recognition of the address site, resulting in compounds that could selectively bind with κ or δ opioid receptors. In addition, we anticipate that new analgesics would be obtained by synthesizing various derivatives with this skeleton. We are currently examining the subtype selectivity of 4,6′-epoxymorphinan derivatives.

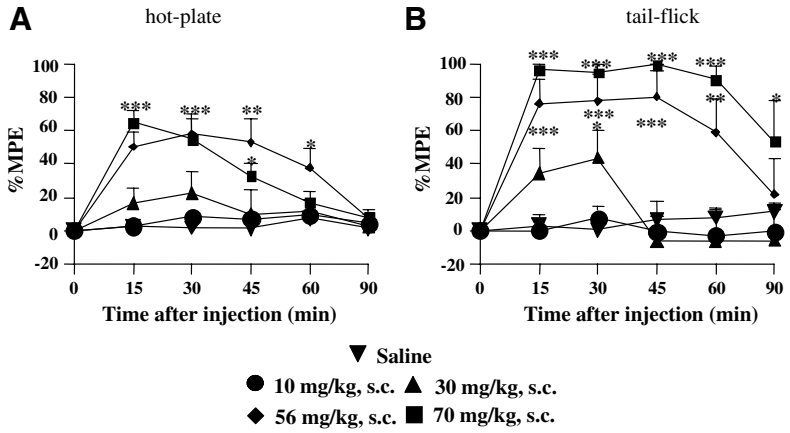


Figure 5. Time courses of antinociceptive effect induced by sc-administered NS16 at 10–70 mg/kg in the mouse hot-plate (A) or tail-flick test (B). Each value represents the mean with SEM of 5–8 mice. $^{***}p < 0.001$, $^{**}p < 0.01$ or $^{*}p < 0.05$ versus saline group.

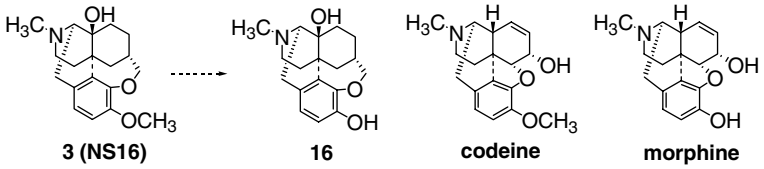


Figure 6. The structure of compound **16**.

4. Conclusion

We succeeded in the syntheses of 4,6′-epoxymorphinan derivatives. One of the 7-membered ring ether derivatives, NS13, showed antagonistic activity for μ, δ, and κ opioid receptors. Another 7-membered ring ether derivative, NS16, induced agonistic activity for μ opioid receptor, and may be a partial agonist like codeine. The 4,6′-epoxymorphinan skeleton could be an alternative message site.

5. Experimental

5.1. Chemistry

Melting points were determined on a Yazawa BY-1 melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded on a JASCO FT/IR-460Plus. Nuclear magnetic resonance (NMR) spectra were recorded on a Valian Mercury-300 or Varian UNITY-400 for ^{1}H NMR. Chemical shifts were reported as δ values (ppm) related to tetramethylsilane (TMS). Mass spectra (MS) were obtained on a JMS-AX505HA or JMS-700 M station instruments by applying a fast atom bombardment (FAB) ionization method. Elemental analyses were determined with a Yanako MT-5 for carbon, hydrogen, and nitrogen. The progress of the reaction was determined on Merck Silica Gel Art. 5715. Column chromatographies were carried out using Kanto Silica Gel 60N (40–100 μm).

Anhydrous dimethoxyethane was prepared by distillation after treatment with lithium aluminum hydride. Anhydrous dimethylsulfoxide was prepared by distillation after treatment with calcium hydride.

5.2. 3-Benzyloxy-17-(cyclopropylmethyl)-4,14β-dihydroxymorphinan-6-one (5)

We modified a method reported before[15] to prepare compound **5**. Zinc dust (8.34 g, 0.128 mol) was added in portions over 10 min to a solution of **4** (prepared by the reported method[15]) (10.0 g, 23.2 mmol) in HCl (37%, 9.1 mL) and AcOH (91 mL) at reflux.[14] After refluxing for an additional 1 h, the solution was filtrated and basified (pH 9) with NH_4OH. The aqueous layer was extracted with $CHCl_3$ three times. The combined organic extracts were washed with brine twice, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (180 g; $CHCl_3$/MeOH = 33:1 to 20:1) to give **5** (5.53 g, 53%, two steps) as a yellow solid: IR (film) cm^{-1}: 3399, 1710; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.06–0.20 (2H, m), 0.48–0.59 (2H, m), 0.86 (1H, m), 1.58 (1H, m), 1.82 (1H, ddd, J = 2.0, 7.5, 13.5 Hz), 1.95 (1H, dd, J = 5.5, 13.5 Hz), 1.98–2.20 (3H, m), 2.35 (1H, dd, J = 6.0, 13.0 Hz), 2.39 (1H, dd, J = 6.0, 13.0 Hz), 2.61 (1H, m), 2.76 (1H, dd, J = 7.5, 13.5 Hz), 2.84 (1H, dd, J = 6.0, 18.0 Hz), 2.95 (1H, d, J = 13.5 Hz), 3.00 (1H, d, J = 18.0 Hz), 3.10 (1H, d, J = 6.0 Hz), 3.91 (1H, dd, J = 2.0, 13.5 Hz), 4.72 (1H, s), 4.96–5.08 (2H, m), 6.19 (1H, s), 6.55 (1H, d, J = 8.5 Hz), 6.73 (1H, d, J = 8.5 Hz), 7.32–7.42 (5H, m). MS (FAB) m/z = 434 $[M+H]^+$. HRMS (FAB) Calcd for $C_{27}H_{32}NO_4$ $[M+H]^+$: 434.2331. Found: 434.2344.

5.3. 4,14β-Dihydroxy-3-methoxynormorphinan-6-one (11)

We modified a method reported before[16] to prepare compound **11**. Compound **11** was prepared from noroxycodone (**10**) according to the procedure used to prepare **5**. Compound **11** as an oily product was used for

the next reaction (Yield, 89%). Crude compound **11** in small amounts (300 mg) was crystallized from MeOH to give a white solid (81 mg): mp 220–223 °C; IR (KBr) cm^{-1}: 3403 1705; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.56 (1H, m), 1.81 (1H, ddd, J = 2.0, 7.5, 13.5 Hz), 1.93 (1H, dt, J = 5.5, 13.5 Hz), 2.04 (1H, m), 2.14 (1H, ddd, J = 2.0, 4.5, 14.5 Hz), 2.54–2.68 (2H, m), 2.78 (1H, ddd, J = 1.0, 7.0, 13.5 Hz), 2.93 (1H, d, J = 18.0 Hz), 2.95 (1H, d, J = 13.0 Hz), 3.09 (1H, d, J = 5.5 Hz), 3.30 (1H, ddd, J = 1.0, 5.5, 18.0 Hz), 3.83 (3H, s), 3.89 (1H, dd, J = 2.0, 13.0 Hz), 4.42 (1H, s), 6.57 (1H, dd, J = 1.0, 8.5 Hz), 6.67 (1H, d, J = 8.5 Hz). MS (FAB) m/z = 304 $[M+H]^+$. HRMS (FAB) Calcd for $C_{17}H_{22}NO_4$ $[M+H]^+$: 304.1549. Found: 304.1548.

5.4. 4,14β-Dihydroxy-3-methoxy-17-methylmorphinan-6-one (12)

To a stirred solution of **11** (1.50 g, 4.95 mmol) in 2 M AcOH (188 mL) were added anhydrous sodium acetate (2.03 g, 24.7 mmol) and 37% formaldehyde solution (1.50 mL) and 10% Pd–C (375 mg) at rt under a H_2 atmosphere. After 22 h with stirring, the reaction mixture was filtered and evaporated in vacuo. The residue was basified (pH 9) with NH_4OH and the aqueous layer was extracted with $CHCl_3$ three times. The combined organic extracts were washed with brine twice, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (60 g; $CHCl_3$/MeOH = 20:1–10:1) to give **12** (1.34 g, 85%) as an oil. Compound **12** was crystallized from AcOEt to give a white solid (1.05 g, 67%): mp 146–148 °C; IR (KBr) cm^{-1}: 3415, 1712; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.57 (1H, ddd, J = 2.0, 12.0, 13.0 Hz), 1.80 (1H, ddd, J = 2.0, 7.0, 13.0 Hz), 1.93 (1H, dt, J = 5.0, 13.0 Hz), 1.98–2.10 (2H, m), 2.13 (1H, ddt, J = 2.0, 5.0, 13.0 Hz), 2.35 (1H, m), 2.36 (3H, s), 2.70–2.88 (3H, m), 2.92 (1H, d, J = 13.5 Hz), 3.08 (1H, m), 3.82 (3H, s), 3.92 (1H, dd, J = 2.0, 13.5 Hz), 4.57 (1H, s), 6.12 (1H, s), 6.57 (1H, d, J = 8.5 Hz), 6.67 (1H, d, J = 8.5 Hz). MS (FAB) m/z = 317 $[M+H]^+$. HRMS (FAB) Calcd for $C_{18}H_{24}NO_4$ $[M+H]^+$: 318.1705. Found: 318.1691.

5.5. 3-Benzyloxy-17-(cyclopropylmethyl)-4,14β-dihydroxymorphinan-6α-calbaldehyde (6)

Methylsulfinyl carbanion was prepared from NaH (55% mineral oil dispersion, 2.00 g, 45.8 mmol) and dry DMSO (18 mL) at 60 °C under an Ar atmosphere for 1.5 h. A solution of the phosphonium ylide was prepared by the reaction of the methylsulfinyl carbanion (34.6 mmol) and methoxymethyltriphenylphosphonium chloride (12.7 g, 41.5 mmol) in DME (100 mL) at rt for 10 min under an Ar atmosphere. To a stirred solution of the phosphonium ylide was added dropwise **5** (3.00 g, 6.92 mmol) in dry DMSO (18 mL) at rt under an Ar atmosphere. After 1.5 h with stirring, water was added to the solution, and the aqueous layer was extracted with benzene three times. The combined organic extracts were extracted with 2 M HCl three times. After the combined aqueous layer was basified (pH 9) with $NaHCO_3$, the aqueous layer was extracted with $CHCl_3$ three times. The combined organic extracts were washed with brine twice, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (100 g; $CHCl_3$/MeOH = 50:1–20:1) to give **6** (526 mg, 17%) as a white amorphous residue: IR (film) cm^{-1}: 3415, 1717; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.03–0.17 (2H, m), 0.42–0.58 (2H, m), 0.82 (1H, m), 1.34 (1H, ddd, J = 2.0, 4.0, 13.0 Hz), 1.56 (1H, ddd, J = 2.5, 4.0, 12.0 Hz), 1.70 (1H, dt, J = 4.5, 13.0 Hz), 1.80–2.06 (4H, m), 2.25 (1H, ddd, J = 1.0, 6.5, 12.0 Hz), 2.28 (1H, dd, J = 6.5, 14.5 Hz), 2.34 (1H, dd, J = 6.5, 12.0 Hz), 2.44 (1H, m), 2.59 (1H, ddd, J = 3.0, 12.0, 19.0 Hz), 2.76–2.92 (3H, m), 3.77 (1H, dt, J = 2.0, 14.5 Hz), 5.00 (1H, d, J = 11.5 Hz), 5.06 (1H, d, J = 11.5 Hz), 6.14 (1H, s), 6.52 (1H, d, J = 8.0 Hz), 6.74 (1H, d, J = 8.0 Hz), 7.32–7.43 (5H, m), 9.57 (1H, d, J = 1.0 Hz), one proton (OH) was not observed. MS (FAB) m/z = 448 $[M+H]^+$. HRMS (FAB) Calcd for $C_{28}H_{34}NO_4$ $[M+H]^+$: 448.2488. Found: 448.2504.

5.6. 4,14β-Dihydroxy-3-methoxy-17-methylmorphinan-6α-calbaldehyde (13)

Compound **13** was prepared from **12** according to the procedure used to prepare **6**. Yield, 58%. IR (film) cm^{-1}: 3410, 1720; ^1H NMR (300 MHz, $CDCl_3$): δ 1.34 (1H, ddd, J = 2.0, 4.5, 13.0 Hz), 1.56 (1H, ddd, J = 1.5, 4.5, 14.0 Hz), 1.70 (1H, dt, J = 4.5, 13.0 Hz), 1.86 (1H, dddt, J = 1.0, 4.5, 5.0, 13.0 Hz), 1.88–2.04 (3H, m), 2.26 (1H, dd, J = 6.0, 14.0 Hz), 2.31 (3H, s), 2.36 (1H, m), 2.44 (1H, m), 2.60 (1H, d, J = 5.5 Hz), 2.82 (1H, ddd, J = 1.0, 5.5, 18.0 Hz), 2.94 (1H, d, J = 18.0 Hz), 3.80 (1H, dt, J = 2.0, 14.0 Hz), 3.80 (3H, s), 4.32 (1H, s), 6.08 (1H, s), 6.56 (1H, dd, J = 1.0, 8.0 Hz), 6.66 (1H, d, J = 8.0 Hz), 9.59 (1H, d, J = 1.0 Hz). MS (FAB) m/z = 332 $[M+H]^+$. HRMS (FAB) Calcd for $C_{19}H_{26}NO_4$ $[M+H]^+$: 332.1862. Found: 332.1853.

5.7. 3-Benzyloxy-17-(cyclopropylmethyl)-6α-hydroxymethylmorphinan-4,14β-diol (7)

To a stirred solution of **6** (580 mg, 1.30 mmol) in MeOH (10 mL) was added $NaBH_4$ (500 mg, 13.2 mmol) at 0 °C under an Ar atmosphere. After 10 min, the reaction mixture was stirred at rt under an Ar atmosphere for 2 h. After adding water and acetone, evaporated, the aqueous layer was extracted with $CHCl_3$ three times. The combined organic extracts were washed with brine twice, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel (40 g; $CHCl_3$/MeOH = 50:1–14:1) to give **7** (365 mg, 63%) as a white amorphous residue: IR (film) cm^{-1}: 3410; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.02–0.17 (2H, m), 0.45–0.55 (2H, m), 0.82 (1H, m), 1.32 (1H, ddd, J = 3.0, 4.0, 13.5 Hz), 1.42 (1H, m), 1.52 (1H, m), 1.66 (1H, dt, J = 4.0, 13.5 Hz), 1.90–1.98 (2H, m), 2.02 (1H, m), 2.06 (1H, dd, J = 5.0, 12.5 Hz), 2.15 (1H, m), 2.27 (1H, dd, J = 6.5, 12.0 Hz), 2.35 (1H, dd, J = 6.0, 12.0 Hz), 2.56 (1H, m), 2.82–2.96 (3H, m), 3.13 (1H, dd, J = 7.0, 11.0 Hz), 3.17 (1H, dd, J = 1.5, 12.5 Hz), 3.38 (1H, dd, J = 9.0, 11.0 Hz), 5.04 (1H, d,

J = 12.0 Hz), 5.09 (1H, d, J = 12.0 Hz), 6.24 (1H, s), 6.58 (1H, d, J = 8.0 Hz), 6.76 (1H, d, J = 8.0 Hz), 7.32–7.45 (5H, m), two protons (OH) were not observed. MS (FAB) m/z = 450 $[M+H]^+$. HRMS (FAB) Calcd for $C_{28}H_{36}NO_4$ $[M+H]^+$: 450.2644. Found: 450.2647.

5.8. 6α-Hydroxymethyl-3-methoxy-17-methylmorphinan-4,14β-diol (14)

Compound **14** was prepared from **13** according to the procedure used to prepare **7**. Yield, 60%. mp 180–184 °C; IR (KBr) cm^{-1}: 3337; ^1H NMR (300 MHz, $CDCl_3$): δ 1.33 (1H, ddd, J = 3.0, 4.0, 13.5 Hz), 1.40 (1H, dddd, J = 1.5, 3.0, 4.0, 13.0 Hz), 1.52 (1H, m), 1.68 (1H, dt, J = 4.0, 13.5 Hz), 1.90–2.10 (4H, m), 2.15 (1H, ddt, J = 4.0, 5.5, 13.5 Hz), 2.33 (3H, s), 2.37 (1H, m), 2.65 (1H, d, J = 5.5 Hz), 2.89 (1H, dd, J = 5.5, 18.0 Hz), 2.99 (1H, d, J = 18.0 Hz), 3.12 (1H, dd, J = 7.0, 11.0 Hz), 3.18 (1H, dd, J = 1.5, 12.5 Hz), 3.39 (1H, dd, J = 9.0, 11.0 Hz), 3.86 (3H, s), 4.38 (1H, s), 6.20 (1H, s), 6.63 (1H, d, J = 8.0 Hz), 6.69 (1H, d, J = 8.0 Hz), one proton (OH) was not observed. MS (FAB) m/z = 334 $[M+H]^+$. HRMS (FAB) Calcd for $C_{19}H_{28}NO_4$ $[M+H]^+$: 334.2018. Found: 334.2028.

5.9. 3-Benzyloxy-17-(cyclopropylmethyl)-6α-methanesulfonyloxymethylmorphinan-4,14β-diol (8)

To a stirred solution of **7** (320 mg, 0.712 mmol) in pyridine (3 mL) was added methanesulfonyl chloride (0.166 mL, 2.13 mmol) at 0 °C. After 3 h, to the reaction mixture was added water and the mixture was evaporated in vacuo. The resulting mixture was basified (pH 9) with a saturated $NaHCO_3$ aqueous solution and extracted with $CHCl_3$ three times. The combined organic extracts were washed with brine twice, dried over Na_2SO_4. Evaporation of the solvent gave a white amorphous residue **8** (365 mg) which was used for the next reaction without further purification. IR (film) cm^{-1}: 3432; MS (FAB) m/z = 528 $[M+H]^+$. HRMS (FAB) Calcd for $C_{29}H_{38}NO_6S$ $[M+H]^+$: 528.2420. Found: 528.2432.

5.10. 6α-Methanesulfonyloxymethyl-3-methoxy-17-methylmorphinan-4,14β-diol (15)

Compound **15** was prepared from **14** according to the procedure used to prepare **8**. Crude compound **15** was used for the next reaction without further purification.

5.11. 3-Benzyloxy-17-(cyclopropylmethyl)-4,6α-epoxymethanomorphinan-14β-ol (9)

To a stirred solution of the above residue **8** (330 mg) in DMF (3 mL) was added potassium carbonate (300 mg, 2.17 mmol) at rt under an Ar atmosphere. After 15 h, the reaction mixture was evaporated in vacuo. The resulting mixture was extracted with $CHCl_3$ three times. The combined organic extracts were washed with brine twice, and dried over Na_2SO_4. The residue was chromatographed on silica gel (50 g; $CHCl_3$/MeOH = 100:0–25:1) to give **9** (155 mg, 57%, two steps) as a yellow oil: IR (film) cm^{-1}: 3381; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.06–0.19 (2H, m), 0.49–0.60 (2H, m), 0.86 (1H, m), 1.18 (1H, ddd, J = 1.0, 4.0, 13.0 Hz), 1.32 (1H, ddt, J = 1.5, 5.5, 14.0 Hz), 1.36 (1H, ddd, J = 1.5, 5.5, 13.5 Hz), 1.66 (1H, dt, J = 5.5, 13.5 Hz), 1.81 (1H, ddd, J = 6.0, 12.5, 13.0 Hz), 2.14 (1H, dd, J = 5.0, 13.0 Hz), 2.20 (1H, m), 2.38 (1 H, dt, J = 4.0, 12.5 Hz), 2.42–2.62 (6H, m), 2.92 (1H, d, J = 6.0 Hz), 3.12 (1H, d, J = 18.0 Hz), 3.41 (1H, dd, J = 6.0, 12.5 Hz), 4.48 (1H, dd, J = 11.0, 12.5 Hz), 5.05 (1H, d, J = 12.0 Hz), 5.13 (1H, d, J = 12.0 Hz), 6.64 (1H, d, J = 8.0 Hz), 6.71 (1H, d, J = 8.0 Hz), 7.27–7.48 (5H, m), one proton (OH) was not observed. MS (FAB) m/z = 432 $[M+H]^+$. HRMS (FAB) Calcd for $C_{28}H_{34}NO_3$ $[M+H]^+$: 432.2539. Found: 432.2556.

5.12. 4,6α-Epoxymethano-3-methoxy-17-methylmorphinan-14β-ol (3)

Compound **3** was prepared from **15** according to the procedure used to prepare **9**. Yield, 30% (two steps). mp 190–193 °C; IR (KBr) cm^{-1}: 3362; ^1H NMR (300 MHz, $CDCl_3$): δ 1.16 (1H, ddd, J = 1.5, 4.5, 14.0 Hz), 1.31 (1H, ddt, J = 1.5, 5.5, 13.5 Hz), 1.36 (1H, m), 1.65 (1H, dt, J = 5.5, 13.5 Hz), 1.80 (1H, ddd, J = 6.0, 12.5, 14.0 Hz), 2.12 (1H, dd, J = 5.5, 14.0 Hz), 2.18 (1H, dt, J = 5.5, 13.5 Hz), 2.30–2.58 (5H, m), 2.36 (3 H, s), 2.62 (1H, d, J = 6.0 Hz), 3.22 (1H, d, J = 18.0 Hz), 3.42 (1H, dd, J = 6.0, 12.0 Hz), 3.81 (3H, s), 4.46 (1H, dd, J = 10.5, 12.0 Hz), 6.69 (1H, d, J = 8.0 Hz), 6.73 (1H, d, J = 8.0 Hz), one proton (OH) was not observed. MS (FAB) m/z = 316 $[M+H]^+$. HRMS (FAB) Calcd for $C_{19}H_{26}NO_3$ $[M+H]^+$: 316.1913. Found: 316.1913.

5.13. 4,6α-Epoxymethano-3-methoxy-17-methylmorphinan-14β-ol hydrochloride (3·HCl)

To a solution of **3** (53 mg, 0.168 mmol) in MeOH (1 mL) was added dropwise HCl–MeOH (1 mL). After evaporation, to the residue was added diethyl ether to give a white solid. Filtration followed by drying the solid gave **3**·HCl salt (51 mg, 86%) as a white solid: mp 230–233 °C (dec); Anal. Calcd for $C_{19}H_{25}NO_3$·HCl·0.67 H_2O: C, 62.71; H, 7.57; N, 3.85. Found: C, 62.87; H, 7.45; N, 3.82.

5.14. 17-(Cyclopropylmethyl)-4,6α-epoxymethanomorphinan-3,14β-diol (2)

To a stirred solution of **9** (160 mg, 0.371 mmol) in MeOH (6 mL) was added 5% Pd–C (140 mg) at rt under a H_2 atmosphere. After 14 h with stirring, the reaction mixture was filtrated and evaporated in vacuo. The residue was chromatographed on silica gel (20 g; $CHCl_3$/MeOH = 50:1–20:1) to give **2** (101 mg, 80%) as an oil, which was crystalized from AcOEt to give compound **2** (55 mg, 44%) as a white solid: mp 150–153 °C; IR (KBr) cm^{-1}: 3375; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.09–0.16 (2H, m), 0.46–0.58 (2H, m), 0.86 (1H, m), 1.20 (1H, ddd, J = 1.5, 4.0, 13.0 Hz), 1.28–1.36 (2H, m), 1.60 (1H, dt, J = 5.0, 13.5 Hz), 1.81 (1H, dt, J = 6.0, 13.0 Hz), 2.12–2.24 (2H, m), 2.37 (1H, dt, J = 4.0, 13.0 Hz), 2.36–2.45 (3H, m), 2.52 (1H, m),

2.54 (1H, dd, J = 6.5, 18.0 Hz), 2.58 (1H, ddd, J = 1.5, 6.0, 13.0 Hz), 2.92 (1H, d, J = 6.5 Hz), 3.13 (1H, d, J = 18.0 Hz), 3.42 (1H, dd, J = 6.0, 12.5 Hz), 4.45 (1H, dd, J = 11.0, 12.5 Hz), 5.75 (1H, s), 6.68 (1H, d, J = 8.0 Hz), 6.73 (1H, d, J = 8.0 Hz), one proton (OH) was not observed. MS (FAB) m/z = 342 $[M+H]^+$. HRMS (FAB) Calcd for $C_{21}H_{28}NO_3$ $[M+H]^+$: 342.2069. Found: 342.2078. Anal. Calcd for $C_{21}H_{27}NO_3$: C, 73.87; H, 7.97; N, 4.10. Found: C, 73.76; H, 7.98; N, 4.17.

5.15. 17-(Cyclopropylmethyl)-4,6α-epoxymethanomorphinan-3,14β-diol hydrochloride (2·HCl)

Compound **2**·HCl was prepared from **2** according to the procedure used to prepare **3**·HCl. Yield, 86%. mp 166–170 °C (dec). Anal. Calcd for $C_{21}H_{28}NO_3{\cdot}HCl{\cdot}1.33H_2O$: C, 62.75; H, 7.69; N, 3.48. Found: C, 62.80; H, 7.44; N, 3.69.

5.16. Pharmacology

5.16.1. Animals. Male ICR mice (20–25 g) and male guinea pig (250–300 g) (Tokyo Laboratory Animals Science Co., Ltd, Tokyo) were used in the present study. Animals were housed in a room maintained at 22 ± 1 °C with a 12 h light–dark cycle. Food and water were available ad libitum. Each animal was used only once.

5.16.2. Opioid receptor binding assay. For membrane preparation, the mouse whole brain without cerebellum and the guinea pig cerebellum were quickly removed after decapitation, and rapidly transferred to a tube filled with an ice-cold buffer. The homogenate was centrifuged at 4 °C for 10 min at 1000g and the surpernatant was centrifuged at 4 °C for 20 min at 48,000g. The pellet was homogenated and centrifuged at 4 °C for 20 min at 48,000g. The resulting pellet was resuspended and retained as membrane fraction. The μ, δ or κ opioid receptor binding assays were performed in duplicate with [tylosil-3,5-(3)H(N)]-[D-Ala(2),N-MePhe(4),Gly-ol(5)]enkephalin ([^{3}H]DAMGO) (specific activity, 59.0 Ci/mmol; Amersham Biosciences, Arlington Heights, IL) at 1 nM, (2-D-penicillamine, 5-D-penicillamine)enkephalin ([^{3}H]DPDPE) (specific activity, 45.0 Ci/mmol; PerkinElmer Life science, Arlington Heights, IL) at 2 nM or (+)-(5α,7α,8β)-*N*-methyl-*N*-7-(1-pyrrolidinyl)-1-oxaspiro-[4,5]dec-8-yl]benzeneacetamide ([^{3}H] U69,593) (specific activity, 41.7 Ci/mmol; PerkinElmer Life science, Arlington Heights, IL) at 2 nM in a final volume of 1.0 mL that contained 50 mM Tris–HCl buffer, pH 7.4, and 0.1 mL of the homogenated membrane fraction. The amount of membrane proteins used in each assay was in the range of 90–140 μg, as determined by the method of Narita et al.[19] The test tubes were incubated for 1 h at 25 °C. Specific binding was defined as the difference in bindings observed in the absence and presence of 1 μM unlabeled DAMGO, DPDPE, or U50,488. Incubation was terminated by collecting membranes on Whatman GF/B filters using a Brandel cell harvester. The filters were then washed three times with 5 mL Tris–HCl buffer, pH 7.4, at 4 °C and transferred to scintillation vials. Then, 4 mL of clear-sol 2 (Nacalaitesque, Inc., Kyoto) was added to the vials. After a 12 h equilibration period, radioactivity in the samples was determined in a liquid scintillation analyzer.

Guanosine-5′-o-(3-thio) triphosphate ([^{35}S]GTPγS) binding assay: for membrane preparation, the mouse whole brain without cerebellum or the guinea pig cerebellum was quickly removed after decapitation, and rapidly transferred to a tube filled with ice-cold buffer. The membrane homogenate (3–8 μg protein/assay) was prepared as described previously[20] and incubated at 25 °C for 2 h in 1 mL of assay buffer with various concentrations of each agonist, 30 μM guanosine-5′-diphosphate (GDP) and 50 pM [^{35}S]GTPγS (specific activity, 1000 Ci/mmol; Amersham, Arlington Heights, IL, USA). The reaction was terminated by filtration using Whatman GF/B glass filters (Brandel, Gaithersburg, MD, USA) that had been presoaked in 50 μM Tris–HCl, pH 7.4, and 5 μM $MgCl_2$ at 4 °C for 2 h. The filters were washed three times with 5 mL of ice-cold Tris–HCl buffer, pH 7.4, and then transferred to scintillation-counting vials containing 0.5 mL of Soluene-350 (Packard Instrument Co., Meriden, CT, USA) and 4 mL of Hionic Fluor (Packard Instrument Co.) and equilibrated for 12 h. The radioactivity in the samples was determined with a liquid scintillation analyzer. Nonspecific binding was measured in the presence of 10 μM unlabeled GTPγS.

5.16.3. Assessment of antinociception. Antinociception induced by NS13 or NS16 was determined by the hot-plate test (51 ± 0.5 °C, Muromachi Kikai Co., Ltd, Tokyo) or the tail-flick test (Tail Flick Analgesia Meter Model MK 330B, Muromachi Kikai Co., Ltd, Tokyo). To prevent tissue damage, we established a 30 s (hot-plate test) or 10 s (tail-flick test) cut-off time. Each animal served as its own control, and the latency to response was measured both before and after drug administration. Antinociception was calculated as percentage of the maximum possible effect (%MPE) according to the following formula; %MPE = (test latency − pre-drug latency)/(cut-off time − pre-drug latency) × 100. Antinociceptiive response represents as the mean ± SEM of %MPE.

5.16.4. Statistical analysis. The data for antinociceptive response were shown as means ± SEM of % MPE. The data for [^{35}S]GTPγS binding assay were expressed as means ± SEM of % stimulation. Receptor binding curves were fitted using the GraphPad Prism 4.0 program. The statistical significance of differences between the groups was assessed with a two-way ANOVA followed by Bonferroni/Dunn multiple comparison test or Student's t-test.

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