

Drug design and synthesis of ϵ opioid receptor agonist: 17-(cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14- endoethenomorphinan-7 α -(*N*-methyl-*N*-phenethyl)carboxamide (TAN-821) inducing antinociception mediated by putative ϵ opioid receptor

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Abstract—Here we report the new drug design and synthesis of a series of 6,14-endoethenomorphinan-7-carboxamide derivatives as a putative ϵ opioid receptor agonist. One of these compounds, 17-(cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-methyl-*N*-phenethyl)carboxamide (TAN-821), showed agonistic activity for a putative ϵ opioid receptor ($IC_{50} = 71.71$ nM) in the rat vas deferens (RVD) preparations. TAN-821 stimulated the binding of the nonhydrolyzable guanosine 5'-triphosphate analog, guanosine 5'-(γ -thio)-triphosphate (GTP γ S), to the mouse pons/medulla membrane via the activation of putative ϵ opioid receptor. Moreover, TAN-821 given intracerebroventricularly (i.c.v.) produced a marked antinociception in the tail-flick test ($ED_{50} = 1.73$ μ g) and the hot-plate test ($ED_{50} = 2.05$ μ g) in a dose-dependent manner. The antinociception induced by TAN-821 administered i.c.v. was blocked by the i.c.v.-pretreatment with a putative ϵ opioid receptor partial agonist β -endorphin [1–27], but not a μ opioid receptor antagonist β -FNA, a δ opioid receptor antagonist NTI, or a κ opioid receptor antagonist nor-BNI. The present results suggest that TAN-821 may be a useful tool for the investigation on the pharmacological properties of the putative ϵ opioid receptor.

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1. Introduction

β -Endorphin is a nonselective endogenous opioid peptide consisted of 31 amino acid residues, and has a moderate affinity for the μ and δ opioid receptors.^{1,2} Until the discovery of endomorphins,³ which were recently identified as endogenous μ opioid peptides,^{4,5} β -endorphin had been proposed to be a prototypical

endogenous ligand for the μ opioid receptor.^{6,7} However, pharmacological effects of β -endorphin seem to be mediated by not only μ but also other opioid receptors. The detailed studies on β -endorphin provided evidence that the antinociceptive effect of β -endorphin is mediated by the β -endorphin-sensitive non- μ , non- δ , and non- κ opioid receptors, so-called putative ϵ opioid receptor.^{8–10} Unlike μ , δ , and κ opioid receptors, which have been more precisely characterized and cloned, the putative ϵ opioid receptor has not yet been cloned, because selective agonists and antagonists for the putative ϵ opioid receptor are not available so far described. Although several compounds, like etorphine¹¹ or bremazocine¹² as well as β -endorphin produce the antinociception mediated through the putative ϵ opioid receptor, those actions can be also regulated by the μ or

Keywords: Putative ϵ opioid receptor; TAN-821; Analgesics; Message-address concept.

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κ opioid receptors. We report here for the first time that the newly synthesized compounds, 6,14-endoethenomorphinan-7 α -carboxamide derivatives are likely to be classified as a selective agonist for the putative ϵ opioid receptor.

2. Design rationale

Several selective opioid ligands such as nor-BNI,¹³ NTI,^{14,15} TRK-820,¹⁶ and (–)-TAN-67^{17–19} were discovered by use of a message–address concept.^{15,20,21} According to the concept, opioid ligands can be viewed to contain two elements: an essential message site that is recognized by the receptor subsite responsible for the signal transduction process and an address site that is recognized by a subsite that is unique to a single receptor type such as μ , δ , κ , or ϵ and functions to enhance binding to the subsite. Since the Tyr¹ amine moiety in opioid peptide structures or the tertiary amine moiety in nonpeptidic opioid structures is known to be important for activity, an identical element in the amine moiety of opioid peptidic or nonpeptidic ligands can be viewed as the message.²¹ The message–address concept is useful for rational drug design of selective opioid receptor ligands.

On the basis of the stereostructure of β -endorphin,²² a binding model of β -endorphin to the putative ϵ opioid receptor has been proposed (Fig. 1).²³ According to the model, the C-terminal part, which has an α -helical structure, and the N-terminal part are in close contact with each other. As a result, the C-terminal part is held above the Gly²-Gly³-Phe⁴-Met⁵ sequence by some, most probably, hydrophobic or hydrogen bonding interactions, and may interact with the putative ϵ opioid receptor. It is considered that the Tyr¹ residue (bold lines in Fig. 1) and the C-terminal part may correspond to the message and the address sites, respectively, in terms of the message–address concept.^{15,20,21} This model suggests that the spatial relationship between the mes-

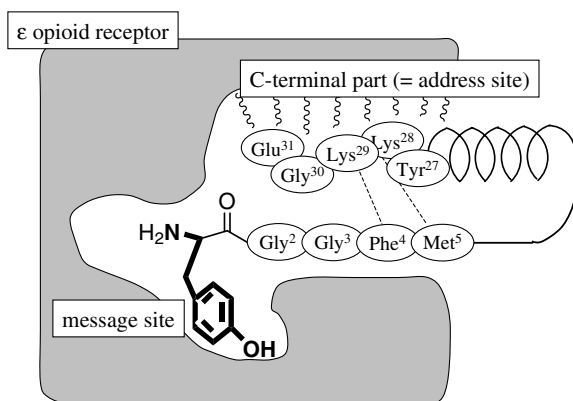


Figure 1. Binding model of β -endorphin to putative ϵ opioid receptor. The model was taken from Ref. 23 and modified. Bold lines and dotted lines express the message site and the hydrophobic or hydrogen bonding interactions between the amino acid residues, respectively. The interactions between the address site and the putative ϵ opioid receptor are indicated by wavy lines.

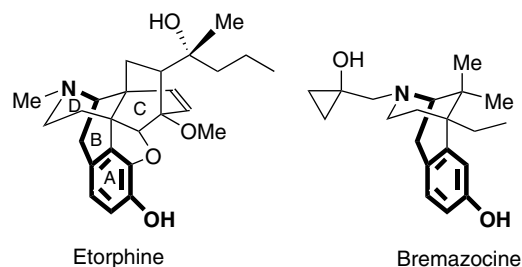


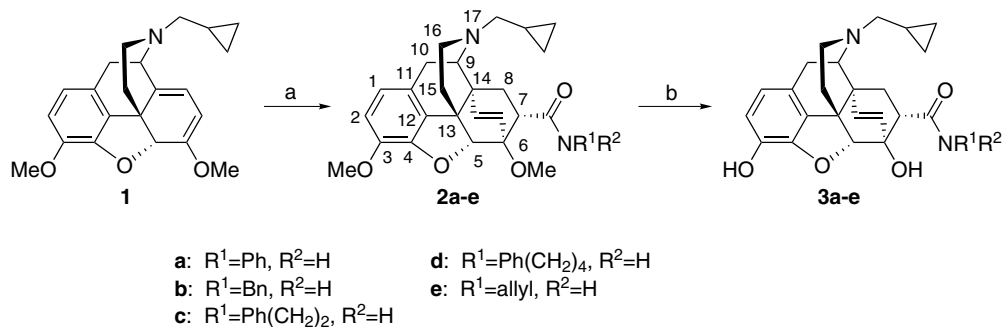
Figure 2. Stereo model of etorphine and bremazocine. Ring letters in 6,14-endoethenomorphinan are shown in etorphine structure.

sage and address sites may play a crucial role to elicit agonistic activity for the putative ϵ opioid receptor. According to this hypothesis, location of the address site is important in order to design the putative ϵ opioid receptor agonist.

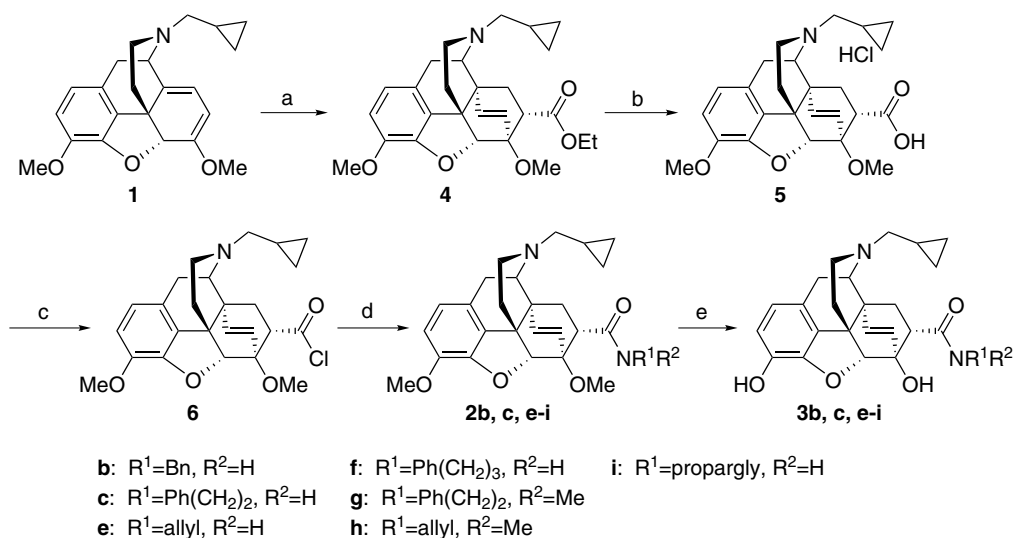
With respect to nonpeptidic ligands, it has been reported that etorphine¹¹ and bremazocine¹² showed antinociceptive effect partially mediated by the putative ϵ opioid receptor. A stereo model of these compounds is shown in Figure 2. The substituent of etorphine, the 1-hydroxyalkyl moiety, is held above the C-ring of the morphinan skeleton by a bicyclo[2.2.2]octene structure and seems to extend in a direction toward the α -helical C-terminal region of β -endorphin, that is, the address site. On the contrary, bremazocine has no substituent located in the spatial area. Therefore, etorphine's skeleton would be more suitable than bremazocine's skeleton for the design of a selective ϵ opioid receptor agonist. On the other hand, many 7 α -(1-hydroxyalkyl)-6,14-endoethenomorphinan derivatives such as etorphine have already been synthesized and their antinociceptive effects have been evaluated.^{24–26} Consequently, 6,14-endoethenomorphina-7 α -carboxamide derivatives **3** were selected because the derivatives **3** have a 7 α -carboxamide substituent as a possible address site and there are few examples synthesizing the 7-carboxamide derivatives.

3. Chemistry

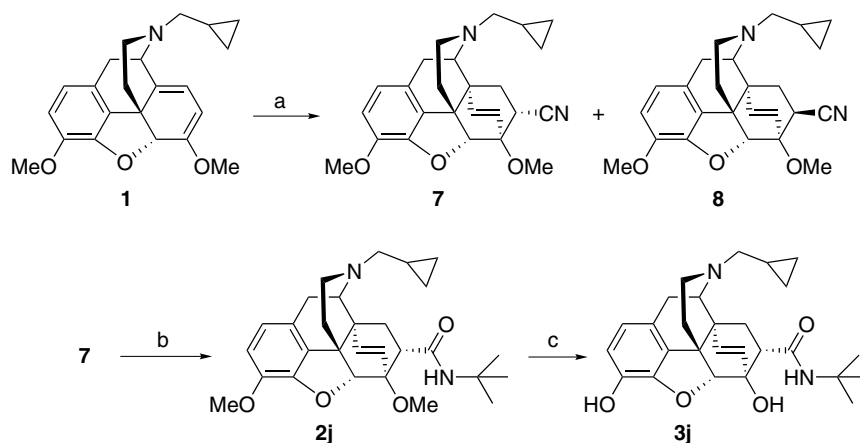
The target compounds **3a–j** were prepared (Schemes 1–3) from 17-(cyclopropylmethyl)northebaïne (**1**)^{27–29} by Diels–Alder reaction with acrylamide derivatives, ethyl acrylate,³⁰ or acrylonitrile. The carboxylic acid **5** was obtained by hydrolysis of the Diels–Alder adduct **4**. The carboxylic acid **5** was converted to the intermediates **2b,c**, or **2e–i** via the acid chloride **6**, which was obtained by treatment of the carboxylic acid **5** with oxalyl chloride, followed by amidation with the corresponding amines. The intermediate **2j** was prepared (Scheme 3) from the 7 α -carbonitrile **7** by Ritter reaction with *t*-BuOH. The *O*-methyl groups of the intermediates **2a–j** were removed by treatment with BBr₃ to afford the target compounds **3a–j**. The ethanomorphinan derivatives **10**, 7 β -carboxamide **9**, and 7 α -acylamino derivative **15** were also synthesized in order to study the role of



Scheme 1. Reagents and conditions: (a) N - R^1 - N - R^2 -acrylamide, xylene, reflux; (b) $\text{BBr}_3/\text{CH}_2\text{Cl}_2$, CH_2Cl_2 , rt. Numbering of atoms in 6,14-endo-ethenomorphinan is shown in formula **2**.



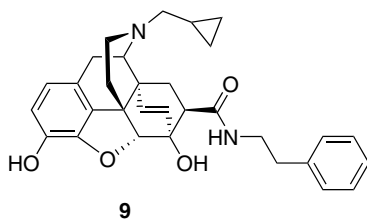
Scheme 2. Reagents and conditions: (a) ethyl acrylate, reflux; (b) 6 M HCl , reflux; (c) $(\text{COCl})_2$, CHCl_3 , reflux; (d) HNR^1R^2 , Et_3N , CH_2Cl_2 , 0°C ; (e) $\text{BBr}_3/\text{CH}_2\text{Cl}_2$, CH_2Cl_2 , rt.



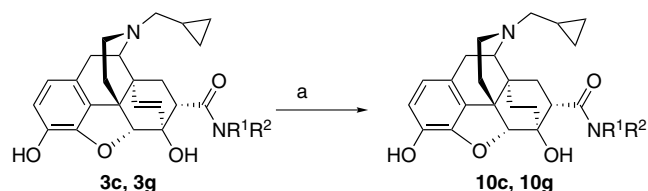
Scheme 3. Reagents and conditions: (a) acrylonitrile, reflux; (b) t - BuOH , 85% H_2SO_4 , AcOH , rt; (c) $\text{BBr}_3/\text{CH}_2\text{Cl}_2$, CH_2Cl_2 , rt.

the etheno-bridge or the 7α -carbamoyl group for the putative ϵ opioid receptor activity. The epimer **9** (Fig. 3) of 7α -carboxamide **3c** was obtained by Diels–Alder reaction with N -phenethylacrylamide as a by-product followed by demethylation.

The target compounds **10c** and **10g** were prepared (Scheme 4) from compounds **3c** and **3g** by the catalytic hydrogenation, respectively. The target compound **15** was prepared by Curtius rearrangement as a key reaction (Scheme 5).^{31,32} Amine **13** was obtained from



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Figure 3. Structure of compound **9**.

c: $R^1 = \text{Ph}(\text{CH}_2)_2$, $R^2 = \text{H}$, **g:** $R^1 = \text{Ph}(\text{CH}_2)_2$, $R^2 = \text{Me}$

Scheme 4. Reagents and conditions: (a) H_2 , Pd/C, $\text{CH}_3\text{SO}_3\text{H}$, MeOH, rt.

hydrazide **11** by Curtius rearrangement. Compound **15** was prepared by amidation of amine **13** with 3-phenylpropionyl chloride followed by demethylation.

4. Inhibition of smooth muscle contraction

It is postulated that the putative ϵ opioid receptor, but not μ , δ , and κ opioid receptors, is present in the rat vas deferens (RVD). This contention is supported by the evidence that β -endorphin exhibits agonistic activity (inhibition of the electrically-stimulated RVD contractions) in the RVD preparation, while the μ opioid receptor agonist morphine, the endogenous δ opioid peptide enkephalin, or the endogenous κ opioid peptide dynorphin does not show agonistic activity in the same preparation.^{33,34} The agonistic activities of compounds **3a–j**, **9**, **10c,g**, and **15** for putative ϵ opioid receptor were evaluated on the electrically-stimulated RVD contrac-

tions (Table 1). In the present study, compounds **3b,c,g**, **10c**, and **10g** showed agonistic activity in the RVD preparation. Compound **3g** (TAN-821) was the most potent agonist among them and its potency ($\text{IC}_{50} = 71.71 \text{ nM}$) is comparable to that of β -endorphin ($\text{IC}_{50} = 73.91 \text{ nM}$). All these compounds possess a phenyl group in their 7-substituents.

Compounds **3e** and **3h–j**, which have no phenyl group in their 7-substituents, exhibited no agonistic activity in this preparation. Compounds **3a,d**, and **3f** also did not show agonistic activity, though they possess a phenyl group in their 7-substituents. The length between the carbamoyl group and the phenyl group in these compounds is longer or shorter than that of the compounds showing the agonistic activity. Both conversions of the 7 α -carboxamide **3c** into the 7 β -carboxamide **9** and the 7 α -acylamino derivative **15** led to no agonistic activity.

Table 1. Effects of compounds **3a–j**, **9**, **10c,g**, and **15** on the electrically-stimulated RVD contractions

Compound	IC_{50} (nM) or %Max response
β -Endorphin	73.91 nM (38.35–109.46) ^a
Morphine ^b	No inhibition at 0.1 mM
Etorphine ^b	50 nM
3a	— ^c
3b	45.8% (28.7–62.8) ^a at 10 μM
3c	34.4% (22.6–46.2) ^a at 8 μM
3d	— ^c
3e	— ^c
3f	— ^c
3g (TAN-821)	71.71 nM (51.21–100.41) ^a
3h	— ^c
3i	— ^c
3j	— ^c
9	— ^c
10c	44.2% (18.4–70.1) ^a at 1 μM
10g	60.9% (45.1–76.7) ^a at 2 μM
15	— ^c

^a Numbers in parentheses are 95% confidence intervals.

^b Ref. 35.

^c The inhibition of electrically-stimulated contraction was not observed.

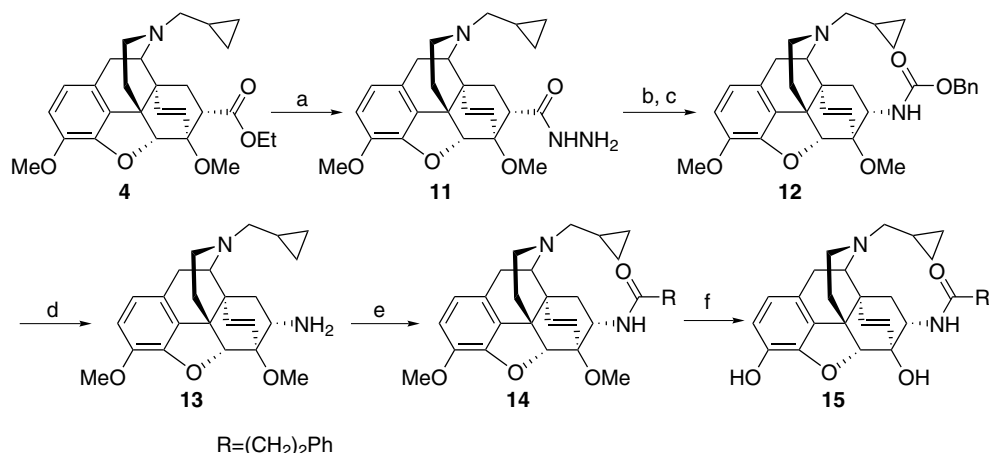
**Scheme 5.** Reagents and conditions: (a) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$, 2-ethoxyethanol, reflux; (b) NaNO_2 aq, 6 M HCl; (c) BnOH, reflux, (d) AcOH, concd HCl, 80 °C; (e) RCOCl , Et_3N , CH_2Cl_2 , 0 °C; (f) $\text{BBr}_3/\text{CH}_2\text{Cl}_2$, CH_2Cl_2 , rt.

Table 2. Effects of compounds **3b,c,g**, **10c**, and **10g** on the electrically-stimulated MVD contractions

Compound	IC ₅₀ (nM)	IC ₅₀ ratio ^a (μ) ^b	IC ₅₀ ratio ^a (δ) ^c	IC ₅₀ ratio ^a (κ) ^d
β-Endorphin ^e	89	— ^f	— ^f	— ^f
Morphine ^e	890	— ^f	— ^f	— ^f
Etorphine ^e	0.25	— ^f	— ^f	— ^f
3b	0.36 (0.26–0.52) ^g	2.0	2.6	2.0
3c	0.038 (0.034–0.043) ^g	1.8	2.8	0.94
TAN-821 (3g)	0.0029 (0.0017–0.0048) ^g	1.3	2.6	1.6
10c	0.0015 (0.0011–0.0019) ^g	1.8	1.7	2.4
10g	0.059 (0.0038–0.0090) ^g	1.0	1.4	5.8

^a IC₅₀ ratio = (IC₅₀ value of the agonist in the presence of the antagonist)/(IC₅₀ value of the agonist in the absence of the antagonist).

^b Naloxone (30 nM).

^c NTI (10 nM).

^d nor-BNI (10 nM).

^e Ref. 35.

^f Not evaluated.

^g Numbers in parentheses are 95% confidence intervals.

However, the difference between the etheno-bridge (compounds **3c** and **3g**) and the ethano-bridge (compounds **10c** and **10g**) had little or no effect on their agonistic activities. These results suggest that the spatial position of the phenyl group in the 7-substituent may play an important role to exhibit the agonistic activity for putative ε opioid receptor.

The agonistic activity of compounds **3b,c,g**, **10c**, and **10g**, which are agonists in the RVD preparation, for other opioid receptors was evaluated on the electrically-stimulated mouse vas deferens (MVD) contractions. It is generally accepted that the MVD contains the μ, δ, and κ opioid receptors and their agonists inhibit the electrically stimulated MVD contraction.³³ In the present study, these compounds exhibited the potent agonistic activity in MVD preparation (Table 2). However, their agonistic activities were not well attenuated by the μ opioid receptor antagonist naloxone (30 nM), the δ opioid receptor antagonist NTI (10 nM), or the κ opioid receptor antagonist nor-BNI (10 nM). At present, it is not clear by what receptor the potent agonistic activity of these compounds in the MVD preparation is mediated.

5. [³⁵S]GTPγS binding

The intrinsic activity of TAN-821, the most potent agonist in the RVD preparation, was evaluated by the [³⁵S]GTPγS binding in the mouse pons/medulla membranes.^{9,36} The stimulation of the [³⁵S]GTPγS binding reflects the activation of G-protein coupled with a receptor such as opioid receptors. β-Endorphin has been reported to stimulate the [³⁵S]GTPγS binding in the mouse pons/medulla membranes via the activation of both μ and putative ε opioid receptors. In the present study, TAN-821 stimulated the [³⁵S]GTPγS binding to the mouse pons/medulla membranes in a concentration-dependent manner. Neither the μ opioid receptor antagonist β-FNA (10 μM), NTI (0.03 μM), nor nor-BNI (0.1 μM) at the concentration, which completely attenuated the stimulation of [³⁵S]GTPγS binding in-

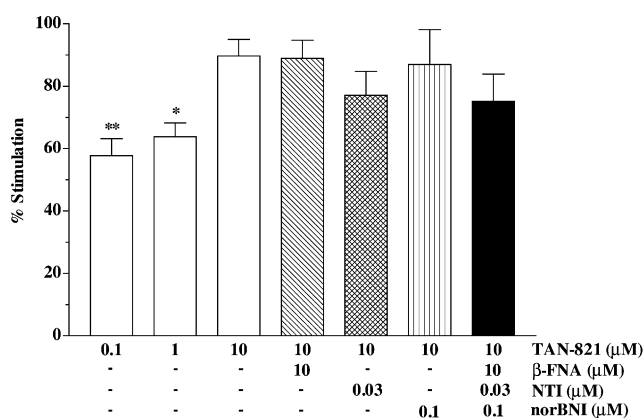


Figure 4. [³⁵S]GTPγS binding by TAN-821 and the effects of opioid receptor antagonists on TAN-821-stimulated [³⁵S]GTPγS binding in the mouse pons/medulla. The statistical significance of differences between the groups was assessed with one-way ANOVA followed by Newman–Keuls's test. **p* < 0.05 and ***p* < 0.01 versus 10 μM TAN-821 alone.

duced by selective μ, δ, and κ opioid receptor agonists, respectively, attenuated the TAN-821-stimulated [³⁵S]GTPγS binding. In the presence of all antagonists, β-FNA, NTI, and nor-BNI, the stimulation of [³⁵S]GTPγS binding induced by TAN-821 still remained (Fig. 4). Unlike TAN-821, the β-endorphin-stimulated [³⁵S]GTPγS binding was partially attenuated by β-FNA, however, no further attenuation of TAN-821-stimulated [³⁵S]GTPγS binding was observed in the presence of all β-FNA, NTI, and nor-BNI. Taken together, the present results suggest that TAN-821-stimulated [³⁵S]GTPγS binding, rather than β-endorphin-stimulated [³⁵S]GTPγS binding, may be more selectively mediated by the putative ε opioid receptor.

6. Antinociception

The antinociceptive effect of TAN-821 was evaluated using the tail-flick³⁷ and the hot-plate³⁸ tests. Groups of mice were injected i.c.v. with 1, 2.5, or 5.0 μg of

TAN-821 and the tail-flick and the hot-plate responses were measured at various times after the injection. TAN-821 given i.c.v. produced a dose-dependent inhibition of the tail-flick response. The inhibition of the tail-flick response reached a peak at 2 h after the injection, and lasted more than 24 h (Fig. 5A). Similarly, TAN-821 administered i.c.v. inhibited the hot-plate response in a dose-dependent manner. The inhibition of the hot-plate response reached at 2 h after the injection, and lasted more than 24 h (Fig. 5B). The ED₅₀ value of TAN-821 for the antinociception using the tail-flick and the hot-plate tests was 1.73 and 2.05 μ g, respectively.

The antinociception induced by the i.c.v.-administered TAN-821 was significantly blocked by the i.c.v.-pretreatment with the putative ϵ opioid receptor partial agonist β -endorphin [1–27],^{39,40} while the i.c.v.-pretreatment with β -FNA (1 μ g), NTI (5 μ g), nor nor-BNI (5 μ g) failed to attenuate the antinociception induced by TAN-821 given i.c.v. (Fig. 6). These results clearly suggest that the antinociception induced by TAN-821 at the supraspinal site may be mediated by the putative ϵ

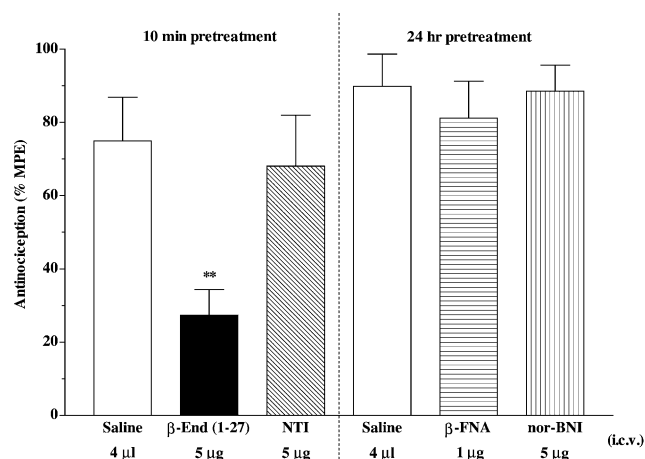


Figure 6. Effects of opioid receptor antagonists on the antinociception induced by TAN-821 in the mouse tail-flick test. The statistical significance of differences between the groups was assessed with one-way ANOVA followed by Newman–Kenls's test. ** $p < 0.01$ versus 10 min pretreatment with saline.

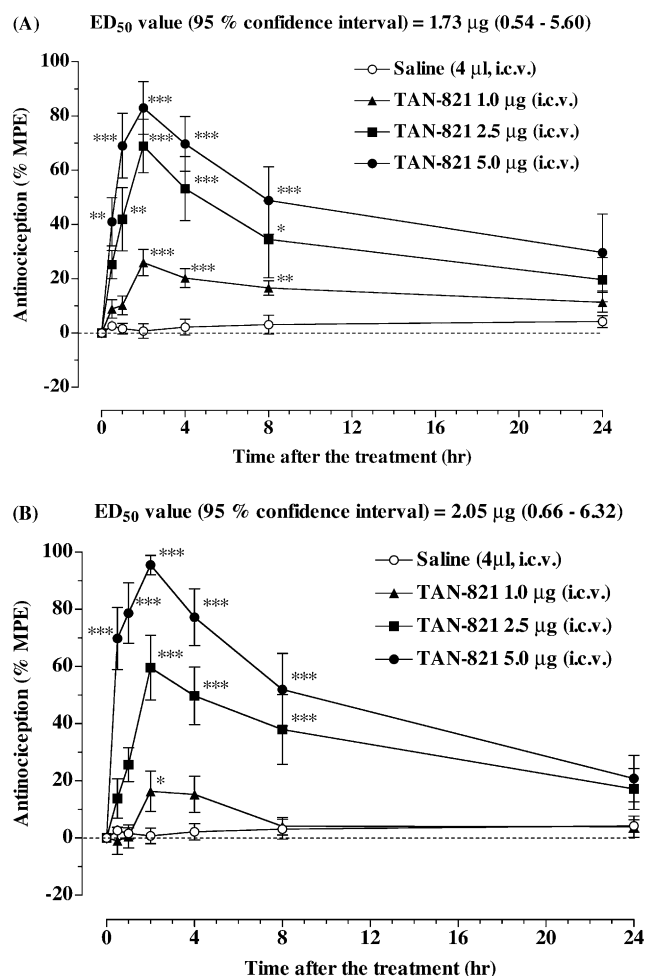


Figure 5. Antinociceptive effect of TAN-821 in the mouse tail-flick test (A) and hot-plate test (B). The statistical significance of differences between the groups was assessed with two-way ANOVA followed by Bonferroni's test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ versus saline-treated mice. Numbers in parentheses are 95% confidence intervals.

opioid receptor. Although it has been already reported that β -endorphin, etorphine,¹¹ and bremazocine¹² show the antinociception mediated by the putative ϵ opioid receptor, these actions are also partially mediated by the μ or κ opioid receptors. TAN-821 is the first compound to produce the antinociception selectively mediated by the putative ϵ opioid receptor. TAN-821 may be a useful tool for the investigation on the pharmacological properties of the putative ϵ opioid receptor.

7. Discussion

We designed and synthesized a selective agonist for the putative ϵ opioid receptor on the basis of the message–address concept. The results of evaluation of the synthesized compounds using RVD preparation suggested that the 7-substituent may play a critical role to produce the agonistic activity for the putative ϵ opioid receptor and indicated the structure–activity relationship as follows: (1) a phenyl group in the 7 α -carbamoyl substituent would be essential. (2) The suitable length between the carbamoyl and phenyl groups would be an ethylene length. One of the synthesized compounds, TAN-821, produced the potent antinociceptive effect selectively mediated by the putative ϵ opioid receptor. Taken together, it is reasonable that the 7 α -(*N*-methyl-*N*-phenethyl)carbamoyl moiety could be viewed as the accessory site. The accessory site, the 7 α -substituent, would interact with the receptor as shown in a binding model of TAN-821 to the putative ϵ opioid receptor (Fig. 7). The morphinan skeleton can generally be viewed as the message site (bold lines in Fig. 7). The address site is held above the message site by a rigid bicyclo[2.2.2]octene structure. On the other hand, the C-terminal part of β -endorphin, the address site, is also held above the message site, the N-terminal part by some, most probably, hydrophobic or hydrogen bonding interactions (Fig. 1). Comparison between binding

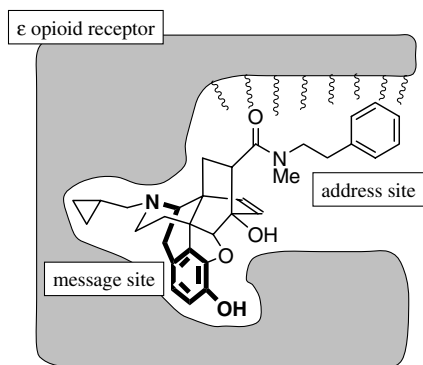


Figure 7. Binding model of TAN-821 to putative ϵ opioid receptor. Bold lines express the message site. The interactions between the address site and the putative ϵ opioid receptor are indicated by wavy lines.

models of β -endorphin and TAN-821 suggests that the relative location between the message and the address sites in TAN-821 seems to be very similar to that in β -endorphin. As mentioned in the design rationale section, the spatial relationship between the message and the address sites may be important to exhibit agonistic activity for the putative ϵ opioid receptor.

8. Conclusion

We have designed and synthesized a series of 6,14-endoethenomorphinan-7 α -carboxamide derivatives for the putative ϵ opioid receptor agonist. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-methyl-*N*-phenethyl)carboxamide, TAN-821 (**3g**), one of the synthesized compounds, showed agonistic activity for the putative ϵ opioid receptor on the electrically-stimulated RVD contractions and the [³⁵S]GTP γ S binding to the mouse pons/medulla membrane. Furthermore, TAN-821 given i.c.v. produced the β -endorphin-like antinociceptive effect. These findings support the idea that TAN-821 may be a useful tool for investigation on the pharmacological properties of the putative ϵ opioid receptor.

9. Experimental

Melting points were determined on a Yanaco MP-500D melting point apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian GEMINI 300 (300 MHz), JEOL GX-400 (400 MHz), Varian UNITY plus 500 (500 MHz), or Varian UNITY INOVA 600 (600 MHz) spectrometers and the chemical shifts are reported as δ values (ppm) related to tetramethylsilane (TMS). Infrared (IR) spectra were obtained using a JASCO FT/IR-5000 as KBr pellets or neat. Mass spectra (MS) were obtained on a JEOL JMS-D300, JEOL JMS-DX303, VG ZAB-HF, or micromass LCT (HP1100) (A-MS-4) instruments by applying an electric ionization (EI) method, a fast atom bombardment (FAB) ionization method, or an electrospray ionization (ESI) method. Elemental analyses were

determined with a Heraeus CHN-ORAPID for carbon, hydrogen, and nitrogen, Yokogawa IC-7000 for sulfur. Elemental analyses were within 0.4% of the theoretical values. The progress of the reaction was determined on Merck Silica Gel Art.5715 or Fuji silysia NH Silica Gel. All the column chromatographies were carried out using Merck Silica Gel Art.9385, Merck Silica Gel Art.7734, Fuji Silysia Silica Gel DM-2010, or Fuji Silysia Silica Gel DM-2035. All the experiments were carried out under an argon atmosphere except for the catalytic hydrogenation.

9.1. Ethyl 17-(cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dimethoxy-6,14-endoethenomorphinan-7 α -carboxylate (**4**)

A stirred solution of 17-(cyclopropylmethyl)northebaïne (**1**) (6.66 g, 19.0 mmol) in ethyl acrylate (20 mL) was refluxed for 15 h. After cooling to rt, the reaction mixture was concentrated in vacuo. The residue was crystallized from AcOEt to give 5.88 g (69%) of **4**: mp 139–142 °C. IR (KBr) cm^{-1} : 3078, 2998, 2938, 2836, 2816, 1721, 1630, 1603, 1499, 1454, 1441, 1377, 1350, 1284, 1261, 1209, 1166, 1100, 1060, 1017, 901, 774, 700. ¹H NMR (CDCl₃, 300 MHz) δ : 0.10–0.20 (2H, m), 0.46–0.59 (2H, m), 0.78–0.90 (1H, m), 1.25 (3H, t, J = 7.2 Hz), 1.47 (1H, dd, J = 6.3, 12.6 Hz), 1.84–1.90 (1H, m), 1.97 (1H, dt, J = 5.7, 12.6 Hz), 2.32–2.51 (4H, m), 2.70 (1H, dd, J = 4.8, 11.7 Hz), 2.85 (1H, dd, J = 6.9, 9.0 Hz), 3.07–3.17 (2H, m), 3.55 (1H, d, J = 6.6 Hz), 3.62 (3H, s), 3.82 (3H, s), 4.12 (1H, dq, J = 7.2, 10.8 Hz), 4.16 (1H, dq, J = 7.2, 10.8 Hz), 4.61 (1H, s), 5.56 (1H, d, J = 8.7 Hz), 5.85 (1H, d, J = 8.4 Hz), 6.52 (1H, d, J = 8.1 Hz), 6.62 (1H, d, J = 8.1 Hz). HREI-MS [M]⁺ m/z calcd for C₂₇H₃₃NO₅: 451.2359. Found: 451.2358.

9.2. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dimethoxy-6,14-endoethenomorphinan-7 α -carboxylic acid-hydrochloride (**5**·HCl)

A stirred solution of **4** (2.02 g, 4.47 mmol) in 6 M HCl (30 mL) was refluxed for 7 h. After cooling to rt, the reaction mixture was cooled in an ice-bath. Cold water (20 mL) was added to the solution to give a precipitate. The precipitate was recrystallized from MeOH to give 1.56 g (76%) of **5**·HCl: mp 190–195 °C (dec.). IR (KBr) cm^{-1} : 3596, 3320, 2932, 2666, 2634, 1736, 1702, 1632, 1508, 1475, 1458, 1437, 1292, 1270, 1214, 1195, 1172, 1127, 1104, 1054, 948, 816. ¹H NMR (DMSO-*d*₆, 300 MHz) δ : 0.36–0.48 (1H, m), 0.54–0.78 (3H, m), 1.07–1.22 (1H, m), 1.44 (1H, dd, J = 6.0, 12.6 Hz), 1.97 (1H, d, J = 12.5 Hz), 2.32 (1H, dt, J = 3.7, 13.9 Hz), 2.79–3.26 (5H, m), 3.32–3.54 (3H, m), 3.46 (3H, s), 3.72 (3H, s), 4.43 (1H, d, J = 6.2 Hz), 4.94 (1H, s), 5.59 (1H, d, J = 8.8 Hz), 5.62 (1H, d, J = 8.8 Hz), 6.60 (1H, d, J = 8.4 Hz), 6.73 (1H, d, J = 8.1 Hz), 9.76 (1H, br s), 12.27 (1H, br s). HRESI-MS [M +H]⁺ m/z calcd for C₂₅H₂₉NO₅: 424.2124. Found: 424.2122.

9.3. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-phenethyl)carboxamide-methane sulfonate (**3c**·MeSO₃H)

Method A: To a solution of 17-(cyclopropylmethyl)northebaïne (**1**) (354 mg, 0.99 mmol) in dichlorobenzene

(10 mL) was added *N*-phenethylacrylamide (365 mg, 2.1 mmol) and the mixture was then refluxed for 20 h. After cooling to rt, the reaction solution was poured into distilled water, then extracted with AcOEt. The combined organic layer was washed with brine and dried over MgSO_4 . After removing the solvent in vacuo, the residue was chromatographed on silica gel to give a mixture of **2c** and *N*-phenethylacrylamide. To the stirred solution of the mixture in dry CH_2Cl_2 (20 mL) was added dropwise a 1.0 M solution of BBr_3 in dry CH_2Cl_2 (6.0 mL, 6.0 mmol) at 0 °C. After stirring at rt for 4 h, the reaction mixture was cooled to 0 °C. To the reaction mixture was added 6% aqueous ammonia (20 mL), then stirred vigorously at rt. The resulting mixture was separated and extracted with CHCl_3 . The combined organic layers were dried over MgSO_4 and evaporated in vacuo. The residue was chromatographed on silica gel to give 130 mg (26% two-step yield) of **3c** and 16 mg (3% two-step yield) of **9**. To the stirred suspension of **3c** or **9** in MeOH was added dropwise MeSO_3H until the solution became acidic or a maleic acid (3.7 mg, 31.9 μmol) solution in MeOH at 0 °C, respectively. Ethyl acetate was then added to the solution. The precipitated amorphous salt was filtered to give **3c**· MeSO_3H or **9**· $\text{HOOCCH}=\text{CHCOOH}$. Compound **3c**· MeSO_3H : IR (KBr) cm^{-1} : 3422, 1657, 1649, 1638, 1562, 1543, 1460, 1323, 1199, 1048, 785, 555. ^1H NMR ($\text{DMSO}-d_6$, 600 MHz) δ : 0.40–0.45 (1H, m), 0.47–0.42 (1H, m), 0.61–0.67 (1H, m), 0.70–0.76 (1H, m), 1.07–1.15 (1H, m), 1.52 (1H, dd, $J = 6.4, 13.5$ Hz), 2.01–2.13 (2H, m), 2.33 (3.3H, s), 2.50–2.56 (1H, m), 2.66–2.78 (3H, m), 2.91–3.06 (3H, m), 3.23 (1H, dt, $J = 6.7, 12.8$ Hz), 3.27–3.40 (3H, m), 3.48 (1H, quintet, $J = 6.7$ Hz), 4.27 (1H, s), 4.36 (1H, d, $J = 7.0$ Hz), 5.46 (1H, d, $J = 8.9$ Hz), 5.62 (1H, d, $J = 8.9$ Hz), 5.72 (1H, br s), 6.45 (1H, d, $J = 7.9$ Hz), 6.53 (1H, d, $J = 7.9$ Hz), 7.18–7.24 (3H, m), 7.26–7.52 (2H, m), 7.74 (1H, br t, $J = 5.0$ Hz), 8.36 (1H, br s), 9.10 (1H, br s). MS (FAB) m/z 499 $[\text{M}+\text{H}]^+$. Anal. Calcd for $\text{C}_{31}\text{H}_{34}\text{N}_2\text{O}_4 \cdot 0.2\text{H}_2\text{O} \cdot 1.3\text{MeSO}_3\text{H}$: C, 61.86; H, 6.36; N, 4.47; S, 6.65. Found: C, 61.88; H, 6.56; N, 4.44; S, 6.51. Compound **9**· $\text{HOOCCH}=\text{CHCOOH}$: IR (free base, KBr) cm^{-1} : 3418, 2946, 1642, 1547, 1502, 1471, 1461, 1321, 1204, 1177, 1048, 784. ^1H NMR (free base, CDCl_3 , 300 MHz) δ : 0.05–0.20 (2H, m), 0.41–0.59 (2H, m), 0.74–0.92 (1H, m), 1.48 (1H, t, $J = 12.4$ Hz), 1.62 (1H, d, $J = 10.7$ Hz), 2.27–2.57 (6H, m), 2.63–2.76 (1H, m), 2.86 (2H, t, $J = 7.0$ Hz), 3.09 (2H, d, $J = 18.1$ Hz), 3.42–3.68 (3H, m), 4.68 (1H, br s), 4.93 (1H, s), 5.37 (1H, d, $J = 8.5$ Hz), 5.70 (1H, dd, $J = 1.2, 8.5$ Hz), 6.32 (1H, br s), 6.46 (1H, d, $J = 8.2$ Hz), 6.62 (1H, d, $J = 8.2$ Hz), 7.12–7.35 (5H, m). MS (EI) m/z 498 $[\text{M}]^+$. Anal. calcd for $\text{C}_{31}\text{H}_{34}\text{N}_2\text{O}_4 \cdot 0.2\text{H}_2\text{O} \cdot \text{C}_4\text{H}_4\text{O}_4$: C, 67.99; H, 6.26; N, 4.53. Found: C, 67.63; H, 6.51; N, 4.92.

Method B: To a stirred suspension of **5**·HCl (445 mg, 0.97 mmol) in dry CHCl_3 (20 mL) was added dropwise oxalyl dichloride (0.3 mL, 3.4 mmol) at 0 °C. The reaction mixture was refluxed gently for 2 h. After cooling to rt, the mixture was concentrated in vacuo to give crude acid chloride **6**. To the stirred solution of phenethylamine (0.50 g, 4.1 mmol) and Et_3N (0.28 mL, 2.0 mmol) in dry CHCl_3 (10 mL) was added dropwise a solution of

prepared **6** in dry CHCl_3 (10 mL) at 0 °C. After stirring at rt for 1 h, the reaction mixture was poured into a saturated NaHCO_3 solution, and then extracted with AcOEt. The combined organic layer was washed with brine and dried over MgSO_4 . After removing the solvent in vacuo, the residue was chromatographed on silica gel to give 509 mg (quantitative yield) of **2c**. To the stirred solution of **2c** (509 mg, 0.97 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise a 1.0 M solution of BBr_3 in dry CH_2Cl_2 (6.0 mL, 6.0 mmol) at 0 °C. After stirring at rt for 1 h, the reaction mixture was cooled to 0 °C. To the reaction mixture was added 6% aqueous ammonia (20 mL), then stirred vigorously at rt. The resulting mixture was separated and extracted with CHCl_3 . The combined organic layers were dried over MgSO_4 and evaporated in vacuo. The residue was chromatographed on silica gel to give 331 mg (69%) of **3c**. To the stirred suspension of **3c** in MeOH was added dropwise MeSO_3H at 0 °C until the solution became acidic. Ethyl acetate was then added to the solution. The precipitated amorphous salt was filtered to give **6c**· MeSO_3H .

The following compounds were synthesized by method A:

9.4. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-phenyl)carboxamide-methane sulfonate (3a**· MeSO_3H)**

Two-step yield (29%). IR (KBr) cm^{-1} : 3400, 1678, 1671, 1657, 1601, 1545, 1499, 1446, 1321, 1209, 1195, 1048. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ : 0.39–0.52 (2H, m), 0.60–0.78 (2H, m), 1.07–1.17 (1H, m), 1.64 (1H, dd, $J = 8.5, 17.3$ Hz), 2.11 (2H, br d, $J = 5.9$ Hz), 2.33 (3.6H, s), 2.76–2.90 (2H, m), 2.92–3.11 (3H, m), 3.28–3.58 (3H, m), 4.36 (1H, s), 4.43 (1H, d, $J = 6.8$ Hz), 5.54 (1H, d, $J = 8.8$ Hz), 5.65 (1H, d, $J = 8.8$ Hz), 5.92 (1H, br s), 6.50 (1H, d, $J = 8.3$ Hz), 6.57 (1H, d, $J = 8.3$ Hz), 7.05 (1H, t, $J = 7.3$ Hz), 7.30 (2H, t, $J = 7.8$ Hz), 7.59 (2H, d, $J = 8.3$ Hz), 8.41 (1H, br s), 9.13 (1H, br s), 9.84 (1H, s). MS (FAB) m/z 471 $[\text{M}+\text{H}]^+$. Anal. Calcd for $\text{C}_{29}\text{H}_{30}\text{N}_2\text{O}_4 \cdot 0.4\text{H}_2\text{O} \cdot 1.2\text{MeSO}_3\text{H}$: C, 61.16; H, 6.05; N, 4.72; S, 6.49. Found: C, 61.05; H, 6.13; N, 4.84; S, 6.45.

9.5. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -[*N*-(4-phenylbutyl)]carboxamide-methane sulfonate (3d**· MeSO_3H)**

Two-step yield (39%). IR (KBr) cm^{-1} : 3450, 2938, 2368, 1649, 1638, 1562, 1460, 1323, 1209, 1195, 1046, 783, 555. ^1H NMR ($\text{DMSO}-d_6$, 300 MHz) δ : 0.37–0.53 (2H, m), 0.57–0.78 (2H, m), 1.04–1.17 (1H, m), 1.33–1.46 (2H, m), 1.47–1.64 (3H, m), 1.97–2.12 (2H, m), 2.35 (4.5H, s), 2.50–2.61 (3H, m), 2.75 (1H, dd, $J = 8.5, 12.6$ Hz), 2.87–3.19 (5H, m), 3.26–3.36 (1H, m), 3.36 (1H, d, $J = 19.2$ Hz), 3.40–3.54 (1H, m), 4.26 (1H, s), 4.36 (1H, d, $J = 6.6$ Hz), 5.46 (1H, d, $J = 8.8$ Hz), 5.60 (1H, d, $J = 8.8$ Hz), 6.45 (1H, d, $J = 8.2$ Hz), 6.53 (1H, d, $J = 8.0$ Hz), 7.12–7.23 (3H, m), 7.23–7.33 (2H, m), 7.66 (1H, br t, $J = 5.4$ Hz), 8.36 (1H, br s). MS (FAB) m/z 527 $[\text{M}+\text{H}]^+$. Anal. Calcd for $\text{C}_{33}\text{H}_{38}\text{N}_2$

$O_4 \cdot 0.6H_2O \cdot 1.5MeSO_3H$: C, 60.79; H, 6.68; N, 4.11; S, 7.06. Found: C, 60.61; H, 6.61; N, 4.41; S, 7.06.

The following compounds were synthesized by both methods A and B:

9.6. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-benzyl)carboxamide-methane sulfonate (3b·MeSO₃H)

Two-step yield (58%, method A), 92% (the first step in method B), 49% (the second step in method B). IR (KBr) cm^{-1} : 3400, 3058, 1640, 1551, 1502, 1473, 1458, 1437, 1361, 1323, 1195, 1120, 1046, 702, 555. ¹H NMR (DMSO-*d*₆, 300 MHz) δ : 0.37–0.54 (2H, m), 0.57–0.77 (2H, m), 1.04–1.18 (1H, m), 1.56 (1H, dd, $J = 6.2$, 12.6 Hz), 1.98–2.18 (2H, m), 2.33 (3H, s), 2.60–2.69 (1H, m), 2.76–2.87 (1H, m), 2.88–3.10 (3H, m), 3.21–3.49 (3H, m), 4.19–4.40 (4H, m), 5.48 (1H, d, $J = 8.7$ Hz), 5.65 (1H, d, $J = 8.7$ Hz), 6.45 (1H, d, $J = 8.1$ Hz), 6.54 (1H, d, $J = 8.1$ Hz), 7.17–7.37 (5H, m), 8.19 (1H, br t, $J = 5.8$ Hz), 8.40 (1H, br s), 9.13 (1H, br s). MS (FAB) m/z 485 [M+H]⁺. Anal. Calcd for C₃₀H₃₂N₂O₄·0.4H₂O·MeSO₃H: C, 63.33; H, 6.31; N, 4.76; S, 5.45. Found: C, 63.18; H, 6.39; N, 4.68; S, 5.75.

9.7. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-allyl)carboxamide-methane sulfonate (3c·MeSO₃H)

Two-step yield (44%, method A), quantitatively (the first step in method B), 87% (the second step in method B). IR (KBr) cm^{-1} : 3398, 1657, 1640, 1543, 1502, 1468, 1421, 1323, 1210, 1195, 1060, 1052, 785. ¹H NMR (DMSO-*d*₆, 300 MHz) δ : 0.36–0.564 (2H, m), 0.56–0.77 (2H, m), 1.03–1.18 (1H, m), 1.53 (1H, dd, $J = 6.1$, 12.3 Hz), 1.98–2.18 (2H, m), 2.34 (3.3H, s), 2.55–2.64 (1H, m), 2.71–2.85 (1H, m), 2.87–3.11 (3H, m), 3.20–3.49 (3H, m), 3.53–3.80 (2H, m), 4.28 (1H, s), 4.37 (1H, d, $J = 6.6$ Hz), 5.04 (1H, ddd, $J = 2.1$, 3.9, 10.3 Hz), 5.18 (1H, ddd, $J = 2.1$, 4.0, 17.2 Hz), 5.47 (1H, d, $J = 8.7$ Hz), 5.62 (1H, d, $J = 8.7$ Hz), 5.71–5.86 (1H, m), 6.45 (1H, d, $J = 8.1$ Hz), 6.54 (1H, d, $J = 8.1$ Hz), 7.83 (1H, br t, $J = 5.8$ Hz), 8.41 (1H, br s), 9.13 (1H, br s). MS (FAB) m/z 453 [M+H]⁺. Anal. Calcd for C₂₆H₃₀N₂O₄·0.3H₂O·1.1MeSO₃H: C, 59.65; H, 6.47; N, 5.13; S, 6.46. Found: C, 59.70; H, 6.47; N, 5.13; S, 6.32.

The following compounds were synthesized by method B:

9.8. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-(3-phenylpropyl)carboxamide-methane sulfonate (3f·MeSO₃H)

Quantitatively (the first step), 58% (the second step). IR (KBr) cm^{-1} : 3356, 2938, 1645, 1547, 1502, 1461, 1321, 1210, 1195, 1046, 784, 556. ¹H NMR (DMSO-*d*₆, 400 MHz) δ : 0.38–0.53 (2H, m), 0.59–0.77 (2H, m), 1.05–1.18 (1H, m), 1.55 (1H, dd, $J = 6.6$, 12.5 Hz), 1.62–1.75 (2H, m), 2.00–2.17 (2H, m), 2.33 (3H, s), 2.52–2.63 (3H, m), 2.78 (1H, dd, $J = 9.3$, 12.2 Hz), 2.90–3.12 (5H, m),

3.26–3.52 (3H, m), 4.28 (1H, s), 4.37 (1H, d, $J = 6.8$ Hz), 5.46 (1H, d, $J = 8.8$ Hz), 5.62 (1H, d, $J = 8.8$ Hz), 5.70 (1H, br s), 6.45 (1H, d, $J = 8.3$ Hz), 6.54 (1H, d, $J = 8.3$ Hz), 7.13–7.24 (3H, m), 7.324–7.32 (2H, m), 7.70 (1H, br t, $J = 5.4$ Hz), 8.37 (1H, br s), 9.09 (1H, br s). MS (FAB) m/z 513 [M+H]⁺. Anal. Calcd for C₃₂H₃₆N₂O₄·0.5H₂O·MeSO₃H: C, 64.16; H, 6.69; N, 4.53; S, 5.19. Found: C, 64.04; H, 6.84; N, 4.59; S, 5.28.

9.9. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-methyl-*N*-phenethyl)carboxamide-methane sulfonate (3g·MeSO₃H, TAN-821·MeSO₃H)

Quantitatively (the first step), 74% (the second step). IR (KBr) cm^{-1} : 3422, 2940, 1629, 1501, 1464, 1414, 1320, 1209, 1194, 1119, 1057, 934, 783. ¹H NMR (DMSO-*d*₆, 400 MHz) δ : 0.4–0.54 (2H, m), 0.6–0.68 (1H, m), 0.70–0.77 (1H, m), 1.06–1.15 (1H, m), 1.30 (0.6H, dd, $J = 6.4$, 12.2 Hz), 1.46 (0.4H, dd, $J = 6.4$, 12.2 Hz), 1.98 (0.6H, br d, $J = 11.7$ Hz), 2.04 (0.4H, br d, $J = 11.7$ Hz), 2.18–2.37 (1H, m), 2.32 (1.8H, s), 2.33 (1.2H, s), 2.67–2.78 (1H, m), 2.75 (1.2H, s), 2.82–3.08 (5H, m), 3.05 (1.8H, s), 3.12 (0.6H, t, $J = 7.8$ Hz), 3.26–3.40 (2.4H, m), 3.44–3.64 (3.6H, m), 3.81–3.89 (0.4H, m), 4.33 (0.6H, d, $J = 6.8$ Hz), 4.36 (0.4H, d, $J = 6.8$ Hz), 4.43 (0.4H, s), 4.46 (0.6H, s), 5.40 (0.6H, d, $J = 8.8$ Hz), 5.43 (0.4H, d, $J = 8.8$ Hz), 5.64 (0.4H, d, $J = 8.8$ Hz), 5.70 (0.6H, d, $J = 8.8$ Hz), 6.45 (1H, d, $J = 8.3$ Hz), 6.54 (0.6H, d, $J = 8.3$ Hz), 6.55 (0.4H, d, $J = 8.3$ Hz), 7.17–7.38 (5H, m), 8.36 (1H, br s), 9.07 (1H, br s). MS (FAB) m/z 513 [M+H]⁺. Anal. Calcd for C₃₂H₃₆N₂O₄·0.7H₂O·MeSO₃H: C, 63.79; H, 6.72; N, 4.51; S, 5.16. Found: C, 63.66; H, 6.79; N, 4.58; S, 5.29.

9.10. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-allyl-*N*-methyl)carboxamide-methane sulfonate (3h·MeSO₃H)

Quantitatively (the first step), 59% (the second step). IR (KBr) cm^{-1} : 3424, 3022, 2942, 1627, 1503, 1471, 1418, 1321, 1209, 1195, 1059, 785, 561. ¹H NMR (free base, CDCl₃, 300 MHz) δ : 0.08–0.24 (2H, m), 0.43–0.62 (2H, m), 0.74–1.00 (1H, m), 1.11–1.31 (1H, m), 1.84–2.08 (2H, m), 2.29–2.49 (4H, m), 2.64–2.70 (2H, m), 2.88 (1.2H, s), 2.96–3.36 (2H, m), 3.03 (1.8H, s), 3.51 (1H, d, $J = 6.3$ Hz), 3.90–4.05 (2H, m), 4.41 (0.4H, s), 4.45 (0.6H, s), 4.65 (1H, br s), 5.09–5.33 (2H, m), 5.37 (1H, d, $J = 8.5$ Hz), 5.44 (1H, br s), 5.64–5.88 (1H, m), 5.89–6.02 (1H, m), 6.46 (1H, d, $J = 8.0$ Hz), 6.61 (0.4H, d, $J = 8.2$ Hz), 6.62 (0.6H, d, $J = 8.0$ Hz). MS (free base, EI) m/z 448 [M]⁺. Anal. Calcd for C₂₇H₃₂N₂O₄·0.8H₂O·MeSO₃H: C, 60.15; H, 6.78; N, 5.01; S, 5.74. Found: C, 60.09; H, 6.65; N, 5.06; S, 5.90.

9.11. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-propargyl)carboxamide-methane sulfonate (3i·MeSO₃H)

Quantitatively (the first step), 56% (the second step). IR (KBr) cm^{-1} : 3442, 3394, 2944, 1657, 1541, 1504, 1471, 1321, 1209, 1193, 1049, 931, 783. ¹H NMR (DMSO-*d*₆,

300 MHz) δ : 0.36–0.54 (2H, m), 0.58–0.78 (2H, m), 1.03–1.19 (1H, m), 1.52 (1H, dd, $J = 6.3, 12.4$ Hz), 1.99–2.16 (2H, m), 2.33 (3H, s), 2.53–2.63 (1H, m), 2.72–2.84 (1H, m), 2.87–3.13 (3H, m), 3.10 (1H, t, $J = 2.5$ Hz), 3.25–3.55 (3H, m), 3.78 (1H, ddd, $J = 2.5, 5.0, 15.6$ Hz), 3.92 (1H, ddd, $J = 2.5, 5.6, 15.6$ Hz), 4.26 (1H, s), 4.37 (1H, d, $J = 6.9$ Hz), 5.48 (1H, d, $J = 8.5$ Hz), 5.62 (1H, d, $J = 8.5$ Hz), 6.46 (1H, d, $J = 8.2$ Hz), 6.54 (1H, d, $J = 8.2$ Hz), 8.10 (1H, t, $J = 5.3$ Hz), 8.38 (1H, br s), 9.11 (1H, br s). MS (free base, EI) m/z 432 $[M]^+$. Anal. Calcd for $C_{26}H_{28}N_2O_4 \cdot 0.7H_2O \cdot MeSO_3H$: C, 59.92; H, 6.22; N, 5.18; S, 5.92. Found: C, 59.76; H, 6.34; N, 5.11; S, 6.00.

9.12. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dimethoxy-6,14-endoethenomorphinan-7 α -carbonitrile (7) and 17-(cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dimethoxy-6,14-endoethenomorphinan-7 β -carbonitrile (8)

A stirred solution of 17-(cyclopropylmethyl)northebaine (1) (1.01 g, 2.9 mmol) in acrylonitrile (15 mL) was refluxed for 18 h. After cooling to rt, the reaction mixture was concentrated in vacuo. The residue was chromatographed on silica gel to give 581 mg (50%) of **7** and 518 mg (44%) of **8** as an amorphous substance. Compound **7**: IR (KBr) cm^{-1} : 2908, 2243, 1632, 1503, 1445, 1283, 1208, 1165, 1102, 1060. 1H NMR ($CDCl_3$, 300 MHz) δ : 0.06–0.19 (2H, m), 0.45–0.60 (2H, m), 0.75–0.90 (1H, m), 1.55 (1H, dd, $J = 5.8, 13.2$ Hz), 1.80–1.98 (2H, m), 2.30–2.45 (3H, m), 2.45 (1H, dd, $J = 6.7, 18.8$ Hz), 2.67–2.77 (1H, m), 2.89 (1H, dd, $J = 5.6, 9.7$ Hz), 3.11 (1H, d, $J = 18.4$ Hz), 3.32 (1H, dd, $J = 9.7, 13.3$ Hz), 3.56 (1H, d, $J = 6.3$ Hz), 3.68 (3H, s), 3.82 (3H, s), 4.52 (1H, d, $J = 1.4$ Hz), 5.66 (1H, d, $J = 9.1$ Hz), 5.98 (1H, d, $J = 8.8$ Hz), 6.54 (1H, d, $J = 8.2$ Hz), 6.63 (1H, d, $J = 8.2$ Hz). HREI-MS $[M]^+$ m/z calcd for $C_{25}H_{28}N_2O_3$: 404.2100. Found: 404.2095. Compound **8**: IR (KBr) cm^{-1} : 2936, 2835, 2235, 1629, 1506, 1457, 1263, 1167, 1132, 1103, 1056. 1H NMR ($CDCl_3$, 300 MHz) δ : 0.09–0.18 (2H, m), 0.46–0.59 (2H, m), 0.77–0.92 (1H, m), 1.71 (1H, dd, $J = 11.8, 13.5$ Hz), 1.89 (1H, d, $J = 9.9$ Hz), 2.27–2.48 (5H, m), 2.75–2.85 (1H, m), 2.80 (1H, dd, $J = 3.6, 11.8$ Hz), 3.13 (1H, d, $J = 19.0$ Hz), 3.18 (1H, dd, $J = 3.8, 13.2$ Hz), 3.51 (1H, d, $J = 6.6$ Hz), 3.64 (3H, s), 3.83 (3H, s), 5.03 (1H, d, $J = 1.6$ Hz), 5.57 (1H, d, $J = 9.1$ Hz), 5.94 (1H, dd, $J = 1.5, 8.9$ Hz), 6.52 (1H, d, $J = 8.2$ Hz), 6.63 (1H, d, $J = 8.0$ Hz). HREI-MS $[M]^+$ m/z calcd for $C_{25}H_{28}N_2O_3$: 404.2100. Found: 404.2117.

9.13. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethenomorphinan-7 α -(*N*-*t*-butyl)carboxamide-maleate (3j·HOOCCH=CHCOOH)

A solution of **7** (581 mg, 1.4 mmol) in *t*-BuOH (3.2 mL), AcOH (4.3 mL), and an 85 vol% H_2SO_4 (2.0 mL) solution was stirred for 8 h at rt. After removing the solvent in vacuo, the reaction mixture was poured into a saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 . The organic layer was washed with brine and dried over $MgSO_4$. After removing the solvent in vacuo, the residue

was chromatographed on silica gel to give 460 mg (67%) of **2j**. To the stirred solution of **2j** (208 mg, 0.44 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise a 1.0 M solution of BBr_3 in dry CH_2Cl_2 (4.4 mL, 4.4 mmol) at 0 °C. After stirring at rt for 2 h, the reaction mixture was cooled to 0 °C. To the solution was added 6% aqueous ammonia (5 mL), then stirred vigorously at rt. The resulting mixture was separated and extracted with $CHCl_3$. The combined organic layers were dried over $MgSO_4$ and evaporated in vacuo. The residue was chromatographed on silica gel to give 121 mg (62%) of **3j**. To the stirred suspension of **3j** in MeOH was added dropwise a solution of maleic acid (31.2 mg, 0.27 mmol) in MeOH at 0 °C. Ethyl acetate was then added to the solution. The precipitated amorphous salt was filtered to give **3c**·maleate. IR (free base, KBr) cm^{-1} : 3388, 2924, 1644, 1538, 1455, 1364, 1322, 1223, 1025. 1H NMR (free base, $CDCl_3$, 400 MHz) δ : 0.08–0.20 (2H, m), 0.45–0.59 (2H, m), 0.77–0.91 (1H, m), 1.27–1.44 (2H, m), 1.33 (9H, s), 1.81–1.90 (1H, m), 1.92–2.05 (1H, m), 2.27–2.51 (5H, m), 2.67–2.79 (1H, m), 3.09 (1H, d, $J = 18.3$ Hz), 3.10–3.20 (1H, m), 3.54 (1H, d, $J = 5.9$ Hz), 4.37 (1H, s), 4.86 (1H, br s), 5.43 (1H, d, $J = 8.3$ Hz), 5.50–5.64 (1H, m), 5.81 (1H, d, $J = 8.3$ Hz), 6.46 (1H, d, $J = 8.1$ Hz), 6.61 (1H, d, $J = 8.1$ Hz). MS (free base, EI) m/z 450 $[M]^+$. Anal. Calcd for $C_{27}H_{34}N_2O_4 \cdot 2.1H_2O \cdot C_4H_4O_4$: C, 61.60; H, 7.04; N, 4.63. Found: C, 61.59; H, 6.88; N, 4.63.

9.14. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethanomorphinan-7 α -(*N*-phenethyl)carboxamide-methane sulfonate (10c·MeSO₃H)

A solution of **3c** (131 mg, 0.26 mmol) in MeOH (5 mL) with $MeSO_3H$ (20 μ L, 0.31 mmol) was stirred for 48 h with 10% Pd/C (50% wet, 20 mg) under H_2 atmosphere (1 atm) at rt. The catalyst was removed by filtration, and the filtrate was stirred for 20 h with 10% Pd/C (50% wet, 67 mg) under H_2 atmosphere (1 atm) at rt. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to half the volume. The resulting solution was poured into a saturated $NaHCO_3$ solution, and extracted with AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was chromatographed on silica gel to give 40 mg (30%) of **10c**. To a stirred solution of **10c** in MeOH was added dropwise $MeSO_3H$ at 0 °C until the solution became acidic. Ethyl acetate was then added to the solution. The precipitated amorphous salt was filtered to give **10c**· $MeSO_3H$: IR (KBr) cm^{-1} : 3404, 2942, 1653, 1547, 1504, 1463, 1323, 1209, 1048, 783, 703. 1H NMR ($DMSO-d_6$, 500 MHz) δ : 0.35–0.48 (2H, m), 0.55–0.73 (3H, m), 1.03–1.13 (2H, m), 1.33 (1H, dt, $J = 6.4, 12.5$ Hz), 1.73 (1H, t, $J = 12.5$ Hz), 1.86–1.93 (2H, m), 2.14 (1H, dt, $J = 4.4, 13.8$ Hz), 2.32 (3H, s), 2.40–2.63 (2H, m), 2.68–2.81 (3H, m), 2.82–2.97 (2H, m), 3.20–3.32 (3H, m), 3.34–3.45 (2H, m), 3.89 (1H, d, $J = 6.8$ Hz), 4.17 (1H, s), 5.02 (1H, br s), 6.54 (1H, d, $J = 8.3$ Hz), 6.68 (1H, d, $J = 7.8$ Hz), 7.17–7.33 (5H, m), 7.80 (1H, br t, $J = 5.1$ Hz), 8.11 (1H, br s), 9.28 (1H, br s). HRESI-MS $[M+H]^+$ m/z calcd for $C_{31}H_{36}N_2O_4$: 501.2753. Found: 501.2758.

9.15. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dihydroxy-6,14-endoethanomorphinan-7 α -(*N*-methyl-*N*-phenethyl)carboxamide-methane sulfonate (10g·MeSO₃H)

Yield (53%). IR (KBr) cm⁻¹: 3454, 3428, 2940, 1625, 1498, 1462, 1421, 1204, 1048. ¹H NMR (DMSO-*d*₆, 300 MHz) δ : 0.33–0.51 (2H, m), 0.51–0.75 (3H, m), 0.98–1.15 (2H, m), 1.29–1.48 (1H, m), 1.72–1.94 (3H, m), 2.25–2.40 (1H, m), 2.32 (3H, s), 2.52–3.00 (4.5H, m), 2.79 (1.5H, s), 3.06 (1.5H, s), 3.09–3.30 (3H, m), 3.30–3.76 (4H, m), 3.84–4.04 (1.5H, m), 4.38 (1H, s), 6.54 (0.5H, d, *J* = 8.2 Hz), 6.55 (0.5H, d, *J* = 8.2 Hz), 6.68 (0.5H, d, *J* = 8.0 Hz), 6.69 (0.5H, d, *J* = 8.0 Hz), 7.17–7.35 (5H, m), 8.14 (1H, br s), 9.32 (1H, br s). MS (FAB) *m/z* 515 [M+H]⁺. Anal. Calcd for C₃₂H₃₈N₂O₄·0.7H₂O·1.1MeSO₃H: C, 62.81; H, 6.97; N, 4.43; S, 5.57. Found: C, 62.90; H, 6.70; N, 4.43; S, 5.65.

9.16. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dimethoxy-6,14-endoethenomorphinan-7 α -carbohydrazide (11)

To a stirred solution of **4** (870 mg, 1.9 mmol) in 2-ethoxyethanol (10 mL) was added NH₂NH₂·H₂O (10 mL) and the mixture was refluxed for 30 h. After cooling to rt, the reaction solution was poured into distilled water and extracted with AcOEt. The combined organic layer was dried over MgSO₄ and evaporated in vacuo. The residue was chromatographed on silica gel to give 763 mg (91%) of **11** as an amorphous substance: IR (KBr) cm⁻¹: 3456, 3322, 2932, 1655, 1633, 1503, 1443, 1285, 1260, 1208, 1166, 1103, 1055. ¹H NMR (CDCl₃, 300 MHz) δ : 0.06–0.18 (2H, m), 0.43–0.57 (2H, m), 0.75–0.90 (1H, m), 1.58 (1H, dd, *J* = 6.3, 13.2 Hz), 1.84 (1H, dd, *J* = 2.5, 13.2 Hz), 1.99 (1H, dt, *J* = 5.5, 12.5 Hz), 2.28–2.48 (4H, m), 2.59 (1H, dd, *J* = 6.3, 9.3 Hz), 2.71 (1H, dd, *J* = 4.8, 11.7 Hz), 3.11 (1H, d, *J* = 19.0 Hz), 3.13 (1H, dd, *J* = 9.9, 12.9 Hz), 3.57 (1H, d, *J* = 6.6 Hz), 3.64 (3H, s), 3.82 (3H, s), 3.87 (2H, d, *J* = 4.1 Hz), 4.54 (1H, d, *J* = 1.4 Hz), 5.60 (1H, d, *J* = 8.8 Hz), 5.90 (1H, d, *J* = 8.8 Hz), 6.52 (1H, d, *J* = 8.0 Hz), 6.62 (1H, d, *J* = 8.2 Hz), 7.28 (1H, br s). HREI-MS [M]⁺ *m/z* calcd for C₂₅H₃₁N₃O₄: 437.2315. Found: 437.2335.

9.17. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dimethoxy-6,14-endoethenomorphinan-7 α -(*N*-benzyloxycarbonyl)amine (12)

To a mixture of **11** (752 mg, 1.7 mmol) in 6 *M* HCl (10 mL) and Et₂O (15 mL) was added dropwise an aqueous solution of NaNO₂ (202 mg, 2.9 mmol) at 0 °C and stirred vigorously for 30 min at the same temperature. After being neutralized with NaHCO₃, the reaction mixture was extracted with Et₂O and dried over MgSO₄. Benzyl alcohol (5 mL) was added to the solution, and then Et₂O was removed in vacuo. The resulting solution was refluxed for 1 h, then cooled to rt. The reaction mixture was poured into distilled water and extracted with AcOEt. The combined organic layer was dried over MgSO₄ and evaporated in vacuo. The residue was chromatographed on silica gel to give 696 mg (72%) of **12** as a viscous oil: IR (neat) cm⁻¹: 3434, 3316, 2940,

2838, 1715, 1506, 1454, 1259, 1230, 1104, 1058, 1006, 911, 733. ¹H NMR (CDCl₃, 300 MHz) δ : 0.08–0.18 (2H, m), 0.46–0.56 (2H, m), 0.76–0.90 (1H, m), 1.06 (1H, dd, *J* = 4.4, 13.7 Hz), 1.83 (1H, dd, *J* = 2.2, 12.9 Hz), 2.05 (1H, dt, *J* = 5.6, 12.6 Hz), 2.27–2.46 (4H, m), 2.73 (1H, dd, *J* = 5.1, 12.2 Hz), 3.08 (1H, d, *J* = 18.4 Hz), 3.42–3.56 (2H, m), 3.51 (3H, s), 3.81 (3H, s), 4.05 (1H, br s), 4.71 (1H, s), 4.81 (1H, br d, *J* = 7.7 Hz), 5.10 (2H, s), 5.56 (1H, d, *J* = 8.8 Hz), 5.64 (1H, d, *J* = 8.8 Hz), 6.51 (1H, d, *J* = 8.0 Hz), 6.61 (1H, d, *J* = 8.0 Hz), 7.27–7.42 (5H, m). HREI-MS [M]⁺ *m/z* calcd for C₃₂H₃₆N₂O₅: 528.2624. Found: 528.2602.

9.18. 17-(Cyclopropylmethyl)-4,5 α -epoxy-3,6 β -dimethoxy-6,14-endoethenomorphinan-7 α -amine (13)

To a stirred solution of **12** (300 mg, 0.53 mmol) in AcOH (15 mL) and concentrated HCl (7.5 mL) was heated at 80 °C for 1 h. After cooling to rt, the reaction mixture was concentrated in vacuo. The residue was poured into 15% aqueous ammonia and extracted with AcOEt. The combined organic layer was dried over MgSO₄ and evaporated in vacuo. The residue was chromatographed on silica gel to give 171 mg (82%) of **13** as a viscous oil: IR (neat) cm⁻¹: 3418, 2948, 1633, 1505, 1455, 1289, 1266, 1167, 1105, 947. ¹H NMR (CDCl₃, 300 MHz) δ : 0.88–0.19 (2H, m), 0.44–0.58 (2H, m), 0.76 (1H, dd, *J* = 4.7, 13.5 Hz), 0.77–0.92 (1H, m), 1.79 (1H, dd, *J* = 2.5, 13.2 Hz), 2.00 (1H, dt, *J* = 5.7, 12.6 Hz), 2.29–2.46 (4H, m), 2.70 (1H, dd, *J* = 5.1, 11.9 Hz), 3.03 (1H, ddd, *J* = 1.4, 4.7, 8.8 Hz), 3.08 (1H, d, *J* = 19.0 Hz), 3.31 (1H, dd, *J* = 8.8, 13.5 Hz), 3.49 (1H, d, *J* = 6.6 Hz), 3.64 (3H, s), 3.83 (3H, s), 4.53 (1H, d, *J* = 1.1 Hz), 5.57 (1H, d, *J* = 8.8 Hz), 5.74 (1H, d, *J* = 8.8 Hz), 6.51 (1H, d, *J* = 8.2 Hz), 6.62 (1H, d, *J* = 8.0 Hz). HREI-MS [M]⁺ *m/z* calcd for C₂₄H₃₀N₂O₃: 394.2256. Found: 394.2252.

9.19. 17-(Cyclopropylmethyl)-4,5 α -epoxy-7 α -(3-phenylpropionyl)aminol-6,14-endoethenomorphinan-3,6 β -diol-methane sulfonate (15·MeSO₃H)

To a solution of **13** (393 mg, 1.0 mmol) in CH₂Cl₂ (10 mL) with Et₃N (0.85 mL, 6.1 mmol) was added dropwise a solution of 3-phenylpropionyl chloride (0.74 mL, 5.0 mmol) in CH₂Cl₂ (5 mL) at 0 °C and then stirred for 1 h at the same temperature. The reaction mixture was poured into a saturated NaHCO₃ solution and extracted with AcOEt. The combined organic layer was dried over MgSO₄ and evaporated in vacuo. The residue was chromatographed on silica gel to give 440 mg (84%) of **14**. To the stirred solution of **14** (440 mg, 0.83 mmol) in dry CH₂Cl₂ (15 mL) was added dropwise a 1.0 *M* solution of BBr₃ in dry CH₂Cl₂ (5 mL, 5.0 mmol) at 0 °C. After stirring at rt for 1 h, the reaction mixture was cooled to 0 °C. To the reaction mixture was added 6% aqueous ammonia (100 mL), then stirred vigorously at rt. The resulting mixture was separated and extracted with CHCl₃. The combined organic layers were dried over MgSO₄ and evaporated in vacuo. The residue was chromatographed on silica gel to give 61 mg (15%) of **15**. To the stirred suspension of **15** in MeOH

was added dropwise MeSO_3H at 0°C until the solution became acidic. Ethyl acetate was then added to the solution. The precipitated amorphous salt was filtered to give **16**· MeSO_3H : IR (KBr) cm^{-1} : 3426, 1639, 1546, 1502, 1461, 1322, 1210, 1195, 1048, 931, 785, 703. ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ : 0.37–0.53 (2H, m), 0.59–0.77 (2H, m), 0.92 (1H, dd, $J = 4.4, 13.2$ Hz), 1.05–1.17 (1H, m), 1.96–2.05 (1H, m), 2.18 (1H, dt, $J = 4.4, 13.9$ Hz), 2.32 (3.3H, s), 2.42 (1H, dt, $J = 3.1, 7.8$ Hz), 2.79 (2H, t, $J = 7.8$ Hz), 2.87–3.05 (3H, m), 3.19 (1H, dd, $J = 8.8, 13.2$ Hz), 3.28–3.40 (2H, m), 3.42–3.52 (1H, m), 3.91–4.00 (1H, m), 4.26 (1H, d, $J = 6.8$ Hz), 4.41 (1H, s), 5.58 (1H, d, $J = 8.3$ Hz), 5.65 (1H, d, $J = 8.3$ Hz), 6.45 (1H, d, $J = 7.8$ Hz), 6.55 (1H, d, $J = 8.3$ Hz), 7.13–7.31 (5H, m), 7.52 (1H, d, $J = 7.3$ Hz), 8.38 (1H, br s), 9.11 (1H, br s). MS (FAB) m/z 499 $[\text{M}+\text{H}]^+$. Anal. Calcd for $\text{C}_{31}\text{H}_{34}\text{N}_2\text{O}_4 \cdot 0.5\text{H}_2\text{O} \cdot 1.1\text{MeSO}_3\text{H}$: C, 62.86; H, 6.47; N, 4.57; S, 5.75. Found: C, 62.64; H, 6.51; N, 4.56; S, 5.99.

All pharmacological experiments were approved by and conformed to the guidelines for Care and Use of Laboratory Animals in Toray Pharmaceutical Research Laboratories or the guidelines of the Medical College of Wisconsin Animal Care Committee.

9.20. Inhibition of smooth muscle contraction

Male SD rats were sacrificed by bleeding under anesthesia or after clubbing the head, and the vas deferens were excised. Male ICR mice were sacrificed by cervical vertebra dislocation, and the vasa deferentia were excised. For the assay using RVD or MVD, a Magnus tube was filled with Ringer's solution (NaCl 154 mM, KCl 5.66 mM, CaCl_2 2.54 mM, glucose 2.77 mM, NaHCO_3 5.95 mM, tyrosine 0.0018 mM) or Krebs Henseleit solution without Mg (NaCl 120.7 mM, KCl 5.9 mM, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 1.2 mM, glucose 11.5 mM, NaHCO_3 15.5 mM, CaCl_2 2.5 mM), respectively, kept at 36 – 37°C , and gassed with 5% CO_2 and 95% O_2 . Electrical stimulation was given through ring-shaped platinum electrodes located above and below the RVD or the MVD preparations at 70–100 mA, 0.2 Hz for 5 ms or 150–250 mA, 0.1 Hz for 1 ms, respectively. The contraction of the preparations was recorded on a polygraph using an isometric transducer. Compounds were added into the Magnus tube cumulatively. The agonistic activity was shown in terms of IC_{50} value or the degree of inhibition of contraction with the concentration of compounds. For the assay using MVD, effects of compounds in the presence of naloxone (30 nM), NTI (10 nM), or nor-BNI (10 nM), were also described. IC_{50} ratio was calculated from the equation: $\text{IC}_{50} \text{ ratio} = (\text{IC}_{50} \text{ value of the agonist in the presence of the antagonist}) / (\text{IC}_{50} \text{ value of the agonist in the absence of the antagonist})$.

9.21. [^{35}S]GTP γS binding assay

Male ICR mice were sacrificed by decapitation, and the pons/medulla was rapidly excised at 4°C , and the tissue

was homogenized with 15 volume (w/v) of ice-cold 0.32 M sucrose using a Potter–Elvehjem tissue grinder with a Teflon pestle. The homogenate was centrifuged at 4°C for 10 min at 1000g. The pellet was discarded and supernatant was centrifuged at 4°C for 20 min at 20,000g. The pellet was resuspended in 15 volumes of an ice-cold 50 mM Tris–HCl buffer (pH 7.4) and centrifuged at 4°C for 20 min at 20,000g. The resultant pellet was resuspended in an ice-cold assay buffer [Tris–HCl (pH 7.4), 50 mM, MgCl_2 , 5 mM, EGTA, 1 mM, NaCl, 100 mM] and then stored at -70°C until used. The homogenized membrane fractions (3–8 μg of protein/assay) were incubated at 25°C for 2 h in assay buffer with various concentrations of the agonist, 30 μM guanosine-5'-diphosphate and 50 pM [^{35}S]GTP γS in a total volume of 1 mL. The reaction was terminated by filtering through Whatman GF/B glass filters, which had been previously soaked in a soaking buffer [Tris–HCl (pH 7.4), 50 mM, MgCl_2 , 5 mM] at 4°C for 2 h, using a Brandel cell harvester. The filters were then washed three times with 5 mL of an ice-cold Tris–HCl buffer (pH 7.4) and transferred to scintillation counting vials. Then 0.5 mL of the tissue solubilizer Soluene-350 and 4 mL of the scintillation cocktail Hionic Fluor were added to the vials. After a 12 h equilibration period, the radioactivity in the samples was determined with a liquid scintillation analyzer. Nonspecific binding was measured in the presence of 10 μM unlabeled GTP γS .

9.22. Assessment of antinociception

Antinociception was determined by the tail-flick and hot-plate tests. For measurement of the latency of the tail-flick response, male ICR mice were gently held by hand with their tail positioned in an apparatus for radiant heat stimulation on the dorsal surface of the tail. The intensity of heat stimulus was adjusted so that the animal flicked its tail after 3–5 s. For the hot-plate test, male ICR mice were individually placed on the hot-plate (55°C) and the reaction time starting from the placement of the mouse on a $30 \times 30 \times 30$ cm hot-plate to the time of licking the paw was measured. Control latencies for the paw-licking response were 7–10 s. The inhibition of the tail-flick and paw-licking responses was expressed as percent maximum possible effect, %MPE, which was calculated as: $[(T^1 - T^0) / (T^2 - T^0)] \times 100$, where T^0 and T^1 were the tail-flick or hot-plate latencies before and after the treatments and T^2 was the cutoff time. Cutoff times were set at 10 and 30 s for the tail-flick and hot-plate tests, respectively.

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