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Rational drug design and synthesis of a selective ε opioid receptor antagonist on the basis of the accessory site concept

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Abstract—To newly synthesize a selective ε opioid receptor antagonist, 17-(cyclopropylmethyl)-4,5α-epoxy-6β,21-epoxymethano-3-hydroxy-6,14-endoethenomorphinan-7α-(*N*-phenethyl)carboxamide was first designed from an ε opioid receptor agonist TAN-821 on the basis of the accessory site concept. The designed compound antagonized the agonistic effects induced by an ε opioid receptor agonist β-endorphin on the rat vas deference test. Moreover, the designed compound blocked the antinociception induced by β-endorphin given intracerebroventricularly.

β-Endorphin is an endogenous opioid peptide and generally classified as μ and δ opioid receptor agonist.[1] However, the detailed behavioral studies on β-endorphin provide evidence that β-endorphin also acts as the agonist for ε opioid receptor.[2–4] 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. Furthermore, selective agonists and antagonists for putative ε opioid receptor are not available so far described. It should be mentioned that the finding of the selective δ opioid receptor antagonist NTI[5] and the κ opioid receptor antagonist nor-BNI[6] can give us the valuable information on the pharmacological properties of δ and κ opioid receptors. Considering these backgrounds, we have recently synthesized the ε opioid receptor agonist, TAN-821, 17-(cyclopropylmethyl)-4,5α-epoxy-3,6β-dihydroxy-6,14-endoethenomorphinan-7α-(*N*-methyl-*N*-phenethyl)carboxamide.[7] Here, we report the rational drug design and synthesis of the selective ε opioid receptor antagonist on the basis of the accessory site concept.[8]

In general, receptors can change their structure as they fit into the structure of ligands ('induced fit') when the agonist binds to them. This change leads to the next signal transduction so that the agonist shows a pharmacological effect. When the antagonist has an extra structural part that interferes with the structural change of the receptor, it does not show such effect even if it binds to a receptor. The structural site for participating in the interference of 'induced fit' is called an 'accessory site', which is usually a hydrophobic and sterically hindered site.[8] The structural difference between an antagonist and agonist lies mainly in whether it has an accessory site or not. We have already reported the selective κ opioid receptor agonist TRK-820[9] and the δ opioid receptor agonist (−)-TAN-67[10–12] from the corresponding antagonist on the basis of the accessory site concept. In this study, we tried to design a selective ε opioid receptor antagonist from the ε opioid receptor agonist TAN-821[7] on the basis of this concept. Compound **1**,17-(cyclopropylmethyl)-4,5α-epoxy-6β,21-epoxymethano-3-hydroxy-6,14-endoethenomorphinan-7α-(*N*-phenethyl)carboxamide, was designed in which the tethering structure between the 6 and 21 positions

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was incorporated into TAN-821 as a possible accessory site (Fig. 1).

The syntheses of 1,3-oxazinan-4-ones from 2-hydroxycycloalkane-1-carboxamides have been reported by Fülöp and Bernáth.[13] According to their report, although the *N*-methylcarboxamides gave the cyclized products, the *N*-phenyl, benzyl, or phenethylcarboxamides were recovered unchanged. In contrast to their report, heating compound **2** with *para*-formaldehyde in dioxane gave the target compound **1** in 61% yield (Scheme 1).[14] At present, it is not clear why our result was different from their report. However, it might be easy to carry out the acid-catalyzed cyclization of compound **2** because the rigid bicyclo[2.2.2]octene structure led the 6 and 21 positions to be in close contact with each other.

The ε opioid activity and the antinociceptive effect of compound **1** was evaluated on the rat vas deferens (RVD) test and the mouse tail-flick test, respectively. The putative ε opioid receptor is present in the RVD.[15,16] Compound **1** exhibited no agonistic effects on the RVD test, however, it antagonized the agonistic effects induced by an ε opioid receptor agonist β-endorphin (IC_{50} ratio = 15, $pA_2 = 9.1$).[17] On the tail-flick test, although compound **1** at doses from 1 to 20 μg given intracerebroventricularly (i.c.v.) alone did not produce any appreciable tail-flick inhibition, the tail-flick inhibition induced by i.c.v.-administration of β-endorphin was blocked by compound **1** preinjected i.c.v. in a dose-dependent manner (0.01–1 μg) (Fig. 2). Furthermore, the i.c.v.-pretreatment with compound **1** failed to attenuate the antinociception induced by the i.c.v.-injection of either the μ opioid receptor agonist DAMGO, the δ opioid receptor agonist DPDPE, or the κ opioid receptor agonist U-50,488H (Table 1). These results suggest that compound **1** may act as the selective ε opioid receptor antagonist. It has been reported that β-endorphin [1–27] could act as an ε opioid receptor

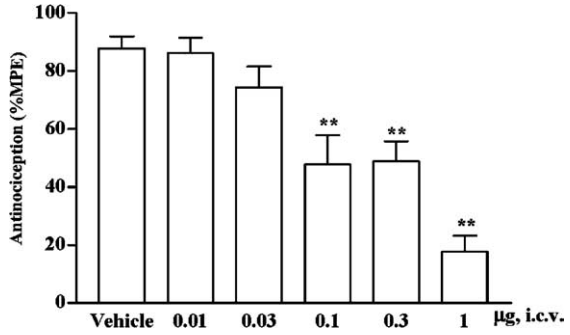


Figure 2. Effects of compound **1** on the antinociception induced by β-endorphin in the mouse tail-flick test. Groups of mice were i.c.v.-injected various doses (0.01–1 μg, i.c.v.) of compound **1** or vehicle 10 min prior to β-endorphin (2 μg) i.c.v.-injection. The tail-flick inhibition were then measured 30 min thereafter. The statistical significance of difference between the groups was assessed with one-way ANOVA followed by Dunnett's post-test. $^{**}p < 0.001$.

antagonist, whereas the high dose of β-endorphin [1–27] itself produces antinociception,[18,19] indicating that β-endorphin [1–27] could be the partial agonist for putative ε opioid receptor. In contrast to β-endorphin [1–27], compound **1** had no agonistic activity because compound **1** showed no antinociceptive effect. It is also of interest to note that the i.c.v.-treatment with bupurenorphine suppresses the antinociception induced by β-endorphin administered i.c.v., whereas the i.c.v.-injection of bupurenorphine itself at high doses produces the antinociception, which can be attenuated by the i.c.v.-pretreatment with the μ opioid receptor antagonist β-FNA.[20] These findings indicate that bupurenorphine is not only an ε opioid receptor antagonist but also a partial agonist for μ opioid receptor. On the contrary, compound **1** alone exhibited no antinociceptive effect, and selectively attenuated the antinociception induced by β-endorphin. Taken together, compound **1** is the first synthesized selective

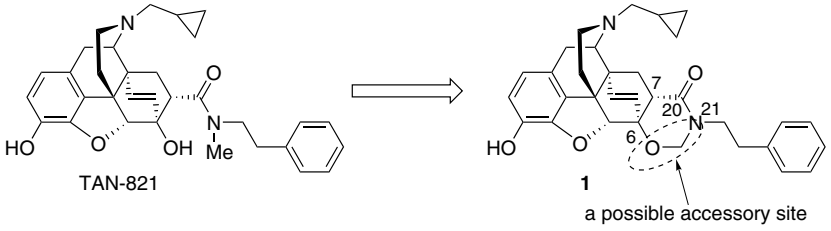
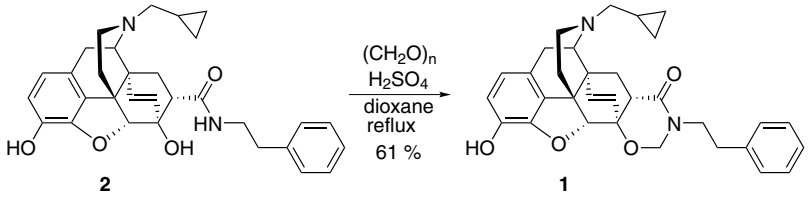


Figure 1. An incorporation of a possible accessory site into an ε opioid receptor agonist TAN-821. The accessory site interferes with the structural change of a receptor ('induced fit'), when an antagonist binds to the receptor.



Scheme 1.

Table 1. Effects of compound **1** on the antinociception induced by DAMGO, DPDPE, or U-50,488H in the mouse tail-flick test

Agonists, i.c.v.	Pretreatment, i.c.v.	Antinociception (%MPE)
DAMGO (20 ng)	Vehicle	71 ± 10%
	Compound **1** (1 μg)	59 ± 11%
DPDPE (10 μg)	Vehicle	68 ± 13%
	Compound **1** (1 μg)	68 ± 12%
U-50,488H (50 μg)	Vehicle	48 ± 10%
	Compound **1** (1 μg)	35 ± 6%

Groups of mice were i.c.v.-injected compound **1** (1 μg) or vehicle 10 min prior to DAMGO (20 ng), DPDPE (10 μg), or U-50,488H (50 μg) i.c.v. injection. The tail-flick inhibition were then measured 30 min thereafter.

ε opioid receptor antagonist. It is worthwhile in future study to further investigate the pharmacological effects induced by compound **1**.

In conclusion, a selective ε opioid receptor antagonist was first designed from an ε opioid receptor agonist TAN-821 on the basis of the accessory site concept. The designed compound **1**, 17-(cyclopropylmethyl)-4,5α-epoxy-6β,21-epoxymethano-3-hydroxy-6,14-endoethenomorphinan-7α-(*N*-phenethyl)carboxamide, which possessed an epoxymethano moiety between the 6 and 21 positions as a possible accessory site, was synthesized by acid-catalyzed cyclization using *para*-formaldehyde. The i.c.v.-injection of compound **1** alone produced no antinociception, whereas the i.c.v.-pretreatment with compound **1** can attenuate the antinociception induced by β-endorphin given i.c.v. The present data suggest that compound **1** may be a useful tool for the investigation on the pharmacological properties of the putative ε opioid receptor.

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14. A stirred solution of compound **2** (50 mg, 0.10 mmol) and *para*-formaldehyde (10 mg) in dioxane (3 mL) was refluxed for 11 h in the presence of one drop of concentrated sulfuric acid. After cooling to rt, the reaction solution was poured into saturated sodium bicarbonate solution and extracted with ethyl acetate. The combined organic layers were washed with brine and dried over magnesium sulfate, then concentrated in vacuo. The residue was purified using a preparative TLC to give 31 mg (61%) of **1**. To the stirred suspension of **1** in MeOH was added dropwise $MeSO_3H$ until the solution became acidic at 0 °C. Ethyl acetate was then added to the solution. The precipitated salt was filtered to give **1**·CH_3SO_3H: IR (KBr) cm^{-1}: 3230, 2921, 1623, 1499, 1455, 1317, 1148, 1024, 926, 887. ^1H NMR (free base, $CDCl_3$, 300 MHz) δ: 0.03–0.10 (2H, m), 0.40–0.50 (2H, m), 0.70–0.90 (1H, m), 1.75 (1H, dd, $J = 6.3$, 13.4 Hz), 1.80 (1H, m), 1.95 (dt, $J = 5.3$, 12.7 Hz), 2.20–2.50 (5H, m), 2.65 (dd, $J = 4.2$, 12.0 Hz), 2.70–2.90 (2H, m), 3.00 (1H, dd, $J = 3.7$, 9.5 Hz), 3.03 (1H, d, $J = 18.3$ Hz), 3.40–3.70 (3H, m), 4.43 (1H, s), 4.52 (1H, d, $J = 9.3$ Hz), 4.76 (1H, d, $J = 9.3$ Hz), 5.48 (2H, s), 6.41 (1H, d, $J = 8.3$ Hz), 6.54 (1H, d, $J = 8.3$ Hz), 7.05–7.30 (5H, m). MS (EI) m/z 510 $[M+H]^+$. Anal. Calcd for $C_{32}H_{34}N_2O_4 \cdot 0.4H_2O \cdot 1.1CH_3SO_3H$: C, 63.66; H, 6.55; N, 4.40; S, 5.28. Found: C, 63.66; H, 6.55; N, 4.57; S, 5.46.
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17. The antagonist activity is expressed as IC_{50} ratio and pA_2. The IC_{50} ratio is calculated from equation:

$$IC_{50}\ \text{ratio} = (IC_{50}\ \text{value of the agonist in the presence of the antagonist})/(IC_{50}\ \text{value of the agonist in the absence of the antagonist}).$$

The pA_2 is calculated from equation:

$$pA_2 = -\log\{[\text{antagonist}]/(IC_{50}\ \text{ratio} - 1)\}$$

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