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# Synthesis of a novel 6,14-epoxymorphinan derivative and its pharmacology

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ABSTRACT

A novel 6,14-epoxymorphinan benzamide derivative (NS22) was synthesized, which showed opioid κ receptor agonistic activity in the [$^{35}$S]GTPγS binding assay. The antinociceptive effect of NS22 was evaluated in the tail-flick and the hot-plate test. This compound showed a potent antinociceptive activity in mice by sc administration, which was attenuated with nor-BNI (selective opioid κ receptor antagonist).


Three types of opioid receptors (μ, δ, κ) are now well-established not only by pharmacological studies but also by molecular biological characterizations.[1] In naltrexone, a μ antagonist (Fig. 1), the 4,5-epoxymorphinan structure (message site) is believed to influence the intrinsic activity of the ligand with the opioid receptor and the substituents (address site) may help the drug distinguish among the opioid receptor types.[2] Both the morphinan structure and the 4,5-epoxymorphinan structure may contribute to three points of association between the drug molecule and the receptor site, which include an ionic interaction, a π–π interaction, and formation of a hydrogen bond.[3,4]

To obtain potent analgesia without drug dependency, many highly selective and potent κ opioid receptor agonists were synthesized, and they are presently available for studies of this receptor. Many selective agonists were arylacetamide derivatives such as U50,488H and U69,593 in Figure 1,[5,6] but all of those derivatives have serious aversive side effects like psychotomimetic reactions and were thus excluded from clinical trials.

We previously reported a novel κ receptor selective agonist, TRK-820, which has a 4,5-epoxymorphinan skeleton, quite a different structure from that of the arylacetamide derivatives (Fig. 2).[7,8] Although TRK-820 had no aversive effects, the compound showed sedative effects and was not approved in clinical trials for postoperative pain. Eventually TRK-820 was applied as an antipruritics for patients undergoing dialysis and is now on application for ap-

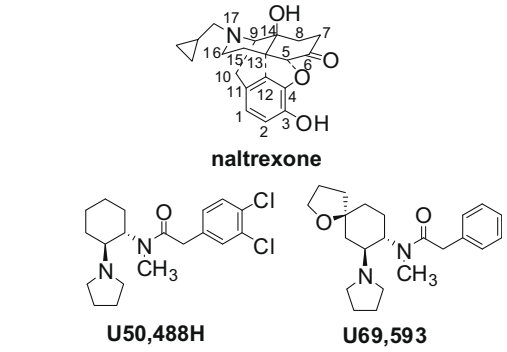


**Figure 1.** The structures of naltrexone and representative arylacetamide κ agonists.

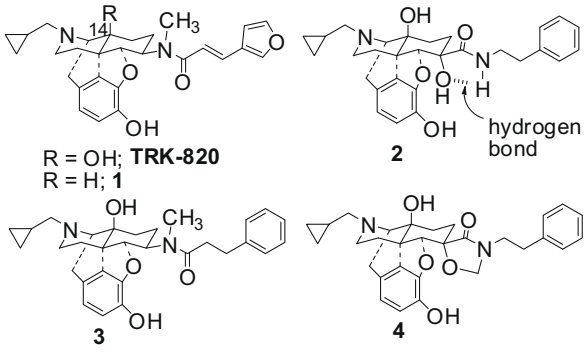


**Figure 2.** The structures of TRK-820 and compounds **1**–**4**.

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proval. Thus we were able to eliminate aversion effects from a κ agonist by using a different basic skeleton.

We then attempted to synthesize other potent κ agonists with a basic skeleton that differed from TRK-820 to obtain a strong analgesic without the side effects of addiction and sedation. In the course of the study, we found that TRK-820 derivative **1**, without the 14-hydroxy group, showed very low κ selectivity (Fig. 2).[8] Furthermore, the 6α-hydroxyl amide derivative **2** had neither analgesic activity nor κ selectivity [$K_i$ (nM) = 1.17 (μ), 2.89 (κ), 23.9 (δ)], in contrast to derivative **3**, which lacked the 6-hydroxy group, but retained κ selective analgesic activity ($ED_{50}$ = 0.04 mg/kg, sc, acetic acid writhing tests).[8] We postulated that the absence of analgesic activity for compound **2** resulted from formation of an intramolecular hydrogen bond with 6-hydroxy group (Fig. 2). The amide group in compound **2** would not participate in a hydrogen bond with the 14-hydroxy group as the hydrogen atom in the amido group can only interact with the 6-hydroxy group. To confirm the effect of 6-hydroxy group, oxazolidinone derivative **4** was synthesized. The $K_i$ value of compound **4** was 4.54 nM (μ), 8.66 nM (κ), and 4.82 nM (δ), respectively, and was higher than that of compound **2** (no analgesic effect).

Based on the above discussion, we hypothesized that the 14-hydroxy group forms a hydrogen bond with the 6-amide side chain and this orientation of amide side chain assists the adaptation of the ligand to the κ receptor (Fig. 3). The TRK-820 derivative **1**, lacking the 14-hydroxy group, cannot form this hydrogen bond. The hydrogen bond in TRK-820 may fix the conformation of the C-ring of the compound into the boat form. The above working hypothesis led us to design an oxabicyclo[2.2.1]heptane skeleton **5** having the boat form in the C-ring (Fig. 3).[9]

We have already reported the synthetic method of the key compound **5** with the novel skeleton by the treatment of morphinan derivative **6** with a stable sulfur ylide (Scheme 1).[9] The amide group of the resulting amide derivative prepared from compound **10** may have similar orientation to that of TRK-820 in Figure 3, as the oxabicyclo[2.2.1]heptane skeleton fixes the C-ring to more rigid boat form than that of TRK-820 (Fig. 3).

Herein, we report the synthesis of a 6,14-epoxymorphinan derivative and its pharmacology.

Compound **5** was synthesized from compound **6** by a previously reported method.[9] The hydroxymethyl group of compound **5** was treated with $CH_3SO_2Cl$ in pyridine, and the resulting mesylate **7**

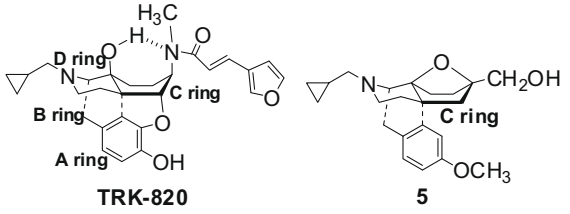
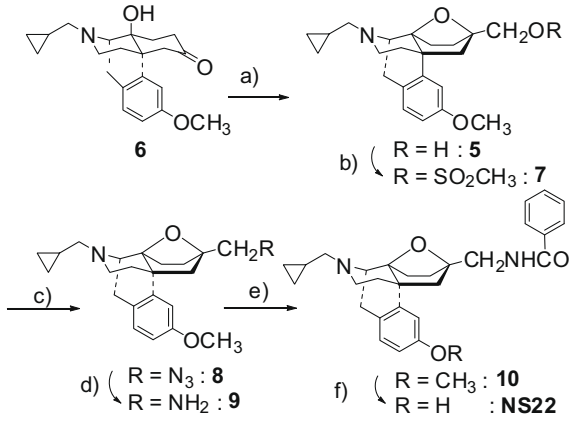



**Figure 3.** The structures of TRK-820 and compound **5**. TRK-820 could form postulated hydrogen bond between 14-OH and 6-N to fix boat conformation.




**Scheme 1.** Reagents and conditions: (a) $CH_3S^+O(CH_3)_2I^-$, NaH, THF, rt, 60%; (b) $CH_3SO_2Cl$, Py, 0 °C; (c) $NaN_3$, DMSO, 110 °C, 89% (2 steps); (d) Pd–C, CSA, MeOH, $H_2$, rt; (e) $(PhCO)_2O$, Py, rt, 60% (2 steps); (f) $C_3H_7SH$, $(CH_3)_3COK$, DMF, 150 °C, 61%.

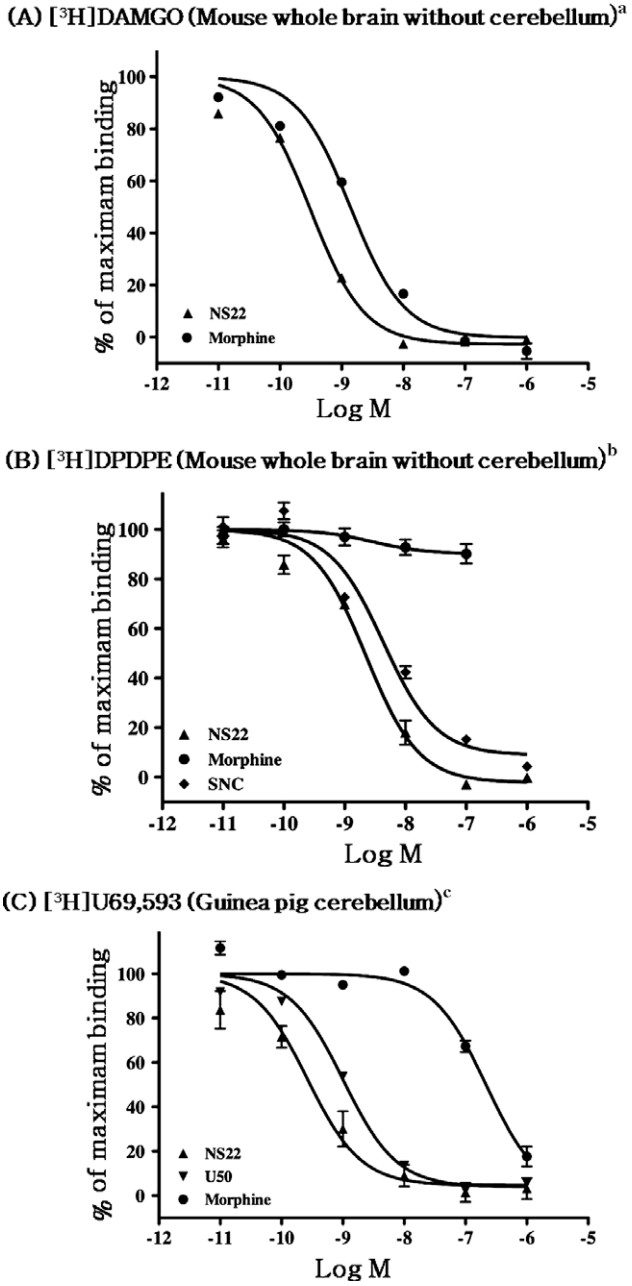



**Figure 4.** Displacement by NS22 of [$^3$H]DAMGO (A), [$^3$H]DPDPE (B) and [$^3$H]U69,593 (C) binding to membranes of the mouse whole brain or the guinea pig cerebellum. Values are expressed as % of specific binding of specific radioligand binding against the log concentration of each compound and values are mean ± SEM of 2–4 observations. [a]$IC_{50}$ (nM); NS22 0.288, Morphine 1.271, [b]$IC_{50}$ (nM); NS22 2.073, Morphine >1000, SNC80 ((+)-4-[(α-*R*)-α-[2*S*,5*R*]-4-allyl[2,5-dimethyl-1-piperazinyl]-3-methoxybenzyl]-*N*,*N*-diethylbenzamide) 6.411, [c]$IC_{50}$ (nM); NS22 0.303, U50,488 1.136, Morphine 213.1.

**Table 1**
Effect of NS22 on G-protein activation in the mouse whole brain without cerebellum membranes.[a]

| Treatment | % Stimulation |
|---|---|
| NS22 | 29.8 ± 2.0 |
| NS22 + nor-BNI | 10.7 ± 8.1** |
| NS22 + β-FNA | 19.2 ± 8.9 |

[a] Membranes were incubated with [$^{35}$S]GTPγS and GDP with NS22 ($10^{-5}$ M) in the presence or absence of β-FNA (β-funaltrexamine) (μ opioid receptor antagonist, $10^{-6}$ M) or nor-BNI ($10^{-6}$ M). The data are shown as the percentage of basal [$^{35}$S]GTPγS binding measured in the presence of GDP and absence of NS22; $n = 6$.
** $p < 0.01$ versus NS 22 alone.

was converted to azide **8** with sodium azide in DMSO. The reduction of compound **8** with Pd–C under $H_2$ afforded amine **9**. The resulting compound **9** was amidated with benzoic anhydride in pyridine followed by demethylation of amide **10** with 1-$C_3H_7SK$ in DMF (Scheme 1).[10,11] Finally NS22 was converted to NS22·hydrochloride to evaluate the pharmacological activity.[11]

To evaluate the binding affinities of NS22 for opioid receptors, we demonstrated the competitive displacement of bound [$^3$H]DAMGO ([D-Ala$^2$, D-Leu$^5$]-enkephaline) (the μ opioid receptor ligand) (Fig. 4A), [$^3$H]DPDPE (cyclic[D-Pen$^2$, D-Pen$^5$]-enkephalin) (the δ opioid receptor ligand) (Fig. 4B), or [$^3$H]U69,593 (the κ opioid receptor ligand) (Fig. 4C), using membranes of the mouse whole brain or the guinea pig cerebellum (μ and δ: whole brain; κ: cerebellum). NS22 showed binding affinity for all three opioid receptors (Fig. 4).

We then investigated the ability of NS22 to activate G-proteins in the mouse whole brain or the guinea pig cerebellum membranes. NS22 showed the GTPγS binding activity which was attenuated with a selective κ opioid receptor antagonist nor-BNI (norbinaltrphimine) (Table 1). We next evaluated the antinociceptive effects induced by sc administration of NS22 using the hot-plate and tail-flick tests. A sc administration of NS22 produced an antinociceptive effect and this analgesia was antagonized by the higher level of nor-BNI (κ antagonist) (Fig. 5), but was not antagonized by β-FNA (μ antagonist) or NTI (naltrindole) (δ antagonist) (Fig. 6).[12]

NS22 showed a potent agonistic activity for κ opioid receptor in the GTPγS binding test and analgesic activity in the mouse hot-plate and tail-flick tests. An analgesic effect induced by NS22 was antagonized by nor-BNI, but not by β-FNA or NTI. These results indicated that NS22 showed its antinociceptive effect only through the κ opioid receptor, even though NS22 showed binding affinity for all three types of opioid receptors. These results suggest that NS22 may possess κ opioid receptor agonistic activity, but may also have antagonistic activity toward μ and δ receptors.

The pronounced analgesic activity of NS22 via the κ receptor supports our hypothesis that the orientation of the amide side chain in NS22, forced by the oxabicyclo[2.2.1]heptane skeleton, promotes the ligands adaptation to the κ receptor. However, NS22 still exhibits affinity for μ and δ receptor. We are now examining the SARs to improve κ selectivity, and are currently synthesizing many other 6,14-epoxymorphinan derivatives and 17-cyclopropylmethyl analogs with various 6-amide side chains. We have also converted the 17-cyclopropylmethyl group to other substituents and, among them, the 17-benzyl derivatives showed high κ selectivity. These syntheses and the pharmacological data will be reported in full detail in the near future.

With a more complete investigation of the SARs of the 6,14-epoxymorphinan compounds, we expect to clarify the most favorable spatial location of the 6-amide side chain for optimum κ selectivity. We will further examine the contribution of the boat form of the C-ring to κ selectivity. We anticipate that various derivatives of this skeleton will yield new analgesics with limited side effects of addiction, aversion and sedation.

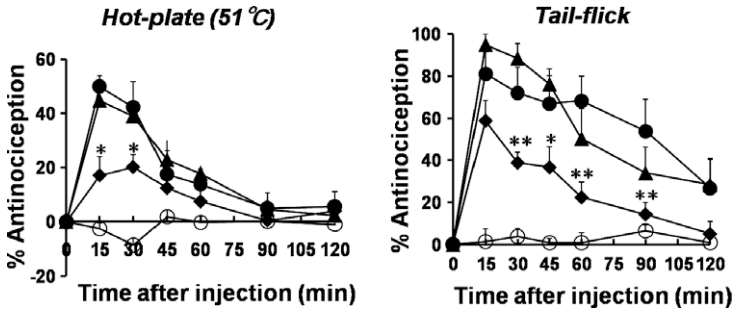



**Figure 5.** Effect of pre-treatment with nor-BNI (5 or 20 mg/kg, sc) on the NS22 (10 mg/kg, sc)-induced antinociception. Groups of mice were pre-treated with nor-BNI at 24 h, respectively, before NS22 injection. Each value represents the mean with SEM, $n = 6$–8. ** $p < 0.01$ or * $p < 0.05$ versus saline–NS22 group. ○ SAL (saline) (sc)–SAL, ● SAL (sc)–NS22 (10 mg/kg, sc), ▲ nor-BNI (5 mg/kg, sc)–NS22 (10 mg/kg, sc), ♦ nor-BNI (20 mg/kg, sc)–NS22 (10 mg/kg, sc).

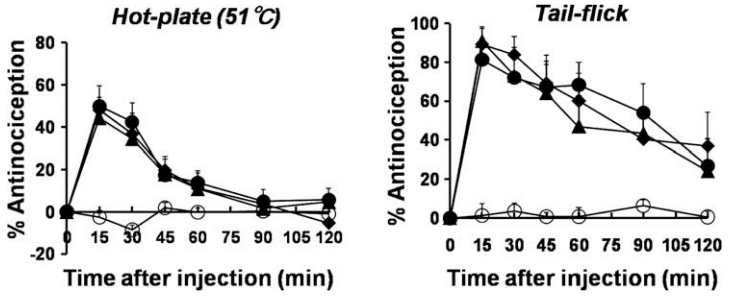



**Figure 6.** Effect of pre-treatment with β-FNA (20 mg/kg, sc) or NTI (3 mg/kg, sc) on the NS22 (10 mg/kg, sc)-induced antinociception. Groups of mice were pre-treated with β-FNA or NTI at 24 h or 30 min before NS 22 injection. Each value represents the mean with SEM of 6–8 trials. ○ SAL (sc)–SAL, ● SAL (sc)–NS22 (10 mg/kg, sc), ▲ β-FNA (20 mg/kg, sc)–NS22 (10 mg/kg, sc), ♦ NTI (3 mg/kg, sc)–NS22 (10 mg/kg, sc).

In conclusion, the novel 6,14-epoxymorphinan derivative, NS22 was designed and synthesized to show affinity for all three opioid receptors and induce its antinociceptive effect only through κ opioid receptor.

## Acknowledgments

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11. *N-[(17-Cyclopropylmethyl-6β,14-epoxy-3-hydroxymorphinan-6α-yl)methyl]benzamide (NS22):* IR (film): 1645 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$: 0.04–0.18 (2H, m), 0.42–0.60 (2H, m), 0.93 (1H, m), 1.28 (1H, dt, *J* = 12.5, 2.0 Hz), 1.37 (1H, m), 1.51–1.90 (5H, m), 2.09 (1H, dt, *J* = 2.0, 12.5 Hz), 2.23 (1H, dt, *J* = 4.5, 12.5 Hz), 2.34 (1H, dd, *J* = 7.0, 12.58 Hz), 2.52–2.62 (3H, m), 3.10 (1H, d, *J* = 18.0 Hz), 3.70 (1H, d, *J* = 5.5 Hz), 3.85–4.02 (2H, m), 6.61 (1H, d, *J* = 2.5 Hz), 6.66 (1H, dd, *J* = 2.5, 8.0 Hz), 6.89 (1H, t, *J* = 6.0 Hz), 6.93 (1H, d, *J* = 8.0 Hz), 7.40–7.56 (3H, m), 7.81–7.88 (2H, m), one proton (OH) was not observed. MS (FAB) *m*/*z* = 445 $[M+H]^+$. HRMS (FAB) Calcd for $C_{28}H_{33}N_2O_3$ $[M+H]^+$: 445.2491. Found 445.2504.*N-[(17-Cyclopropylmethyl-6β,14-epoxy-3-hydroxymorphinan-6α-yl)methyl]benzamide hydrochloride (NS22·HCl):* Mp 181–185 °C (dec). Anal. Calcd for $C_{28}H_{32}N_2O_3 \cdot HCl \cdot 0.8H_2O$: C, 67.88; H, 7.04; N, 5.65. Found: C, 67.85; H, 7.16; N, 5.46.
12. *Assessment of antinociception:* 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 antinociception according to the following formula: % antinociception = (test latency − pre-drug latency)/(cut-off time − pre-drug latency) × 100. Antinociceptive response represents as the mean ± SEM of % antinociception.