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Synthesis of novel twin drug consisting of 8-oxaendoethanotetrahydromorphides with a 1,4-dioxane spacer and its pharmacological activities: μ, κ, and putative ε opioid receptor antagonists

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ABSTRACT

A twin drug consisting of 8-oxaendoethanotetrahydromorphides with a 1,4-dioxane spacer, NS29, was synthesized from a naltrexone derivative. The structure of compound **8**, the precursor of NS29, was determined by X-ray crystallography. Monomeric NS28 showed μ opioid receptor antagonist activity, whereas dimeric NS29, consisting of two NS28 units, showed antagonist activities for μ, κ, and the putative ε opioid receptor agonists. Twin drug NS29 and its derivatives are expected to be unique pharmacological tools for investigation of opioid receptor types

Opioid receptors are generally classified into μ, δ, and κ types not only by pharmacological studies but also by molecular biological characterizations, and all receptor types are related to analgesic effect.[1] Based on the detailed investigation of pharmacological effects using selective ligands, each receptor type has been further divided into subtypes (μ_1, μ_2, δ_1, δ_2, κ_1, κ_2, and κ_3).[1] In addition to these three types, a putative ε opioid receptor was proposed as the receptor specifically binding the endogenous opioid peptide, β-endorphin, based on various pharmacological observations.[2] Although the three types of the opioid receptors (μ, δ, and κ) have been cloned, the opioid receptor subtypes and the putative ε opioid receptor have not yet. On the other hand, the dimerization of a variety of G-protein-coupled receptors (GPCRs) has been reported and the homo- or hetero-dimerization of GPCRs could modulate their pharmacological effects.[3] The opioid receptor is also known to form homo- and hetero-dimers.[3,4] A recent proposal has attributed the diversity of the opioid pharmacological effects to the dimerization of the corresponding receptor types, an idea which differs from the earlier concept that opioid receptor subtypes (perhaps including the putative ε receptor)[5] are responsible for the heterogeneous effects.

We have already synthesized ε-agonist TAN-821[6] and ε-antagonist TAN-1014 (Fig. 1).[7] Although these ligands showed selectivity for the putative ε opioid receptor in the mouse tail-flick and hot-plate assays, the selectivity was not sufficient in the binding assay. To obtain a more selective ligand for the putative ε opioid receptor, we designed and synthesized the 8-oxaendoethanotetrahydromorphide derivatives (Fig. 1) on the basis of the following working hypothesis: (1) the strong affinities of TAN-821 and TAN-1014 for the opioid receptors stemmed from their high lipophilicity,

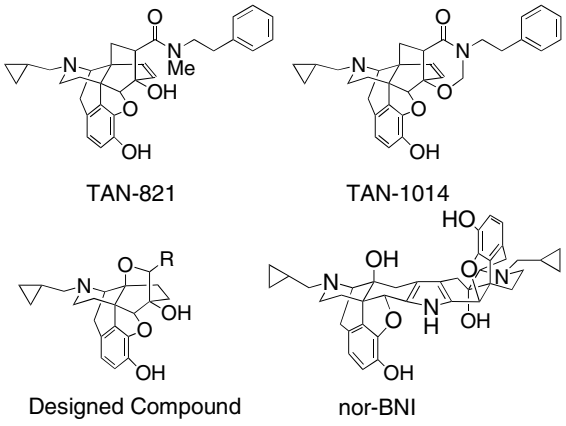

Figure 1. Structures of TAN-821, TAN-1014, designed compound, and nor-BNI.

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which may cause their low selectivity in vitro; (2) the introduction of a hydrophilic moiety into the basic bicyclo[2.2.2]octane skeleton of these ligands could decrease its lipophilicity.[8] In the course of our attempts to synthesize the designed compound, a novel twin drug with a 1,4-dioxane spacer was obtained unexpectedly. In general, a twin drug, dimeric ligand is one of the most useful tools for the investigation of pharmacological properties of some receptors and enzymes because a dimeric ligand can induce unique effects which a monomeric structure never shows.[9] An identical twin drug consisting of two identical pharmacophoric entities could increase the binding affinity and/or efficacy compared with that of the corresponding monomeric ligand, while a non-identical twin drug bearing two different pharmacophoric entities is expected to bind to the respective receptors for each monomeric ligand and could elicit the corresponding effects derived from the individual receptors.[8] Furthermore, a twin drug can sometimes show an unexpected effect, which may not be predicted from each monomeric unit. For example, nor-BNI (Fig. 1), consisting of two identical units of the μ opioid receptor antagonist naltrexone with a pyrrole spacer, showed selective κ opioid receptor antagonist activity.[10] Herein, we report synthesis of the novel twin drug NS29 and describe its pharmacological effects.

An acetal exchange reaction of dithiane **1** derived from naltrexone methyl ether **10** with $HC(OMe)_3$ and $CuCl_2$/CuO afforded orthoester **2** predominantly (Scheme 1).[8] On the other hand, the acetal exchange reaction of **1** with $HC(OMe)_3$ and $CuCl_2$ in the presence of camphorsulfonic acid (CSA) provided a mixture of acyclic acetal **3** and cyclic acetal **4** instead of the orthoester **2** (Scheme 1). Hydrolysis of the mixture of acetals **3** and **4** gave an inseparable mixture of α-hydroxyl aldehyde **5**, its hemiacetal dimer **7**, and cyclic hemiacetal **6**. As the α-hydroxy aldehyde is known to be prone to transform into five and six membered dimers,[11] we attempted to lead the mixture of compounds **5**, **6**, and **7** toward the acetal dimer **8**. After extensive investigations, we found that treatment of the mixture of compounds **5**, **6**, and **7** with CSA under azeotropic distillation provided the objective dimer **8**. Compound **8** was chemically stable and subsequently converted to dimer **9** (NS29) by demethylation. The structure of compound **8**, confirmed by X-ray crystallography (Fig. 2), consisted of two 8-oxa-endoethanotetrahydromorphide skeletons with a 1,4-dioxane spacer.[12] For comparison with the pharmacological profile of dimer **9**, compound **13** (NS28), which corresponded to the monomeric component of dimer **9**, was also synthesized from naltrexone methyl ether **10** in two steps according to a reported method (Scheme 2).[13]

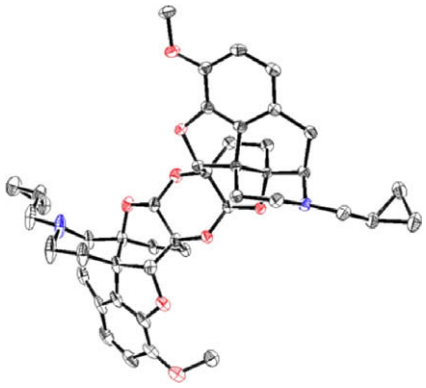
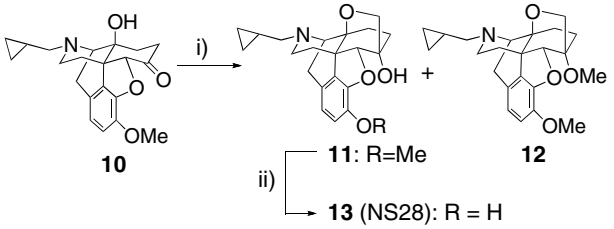
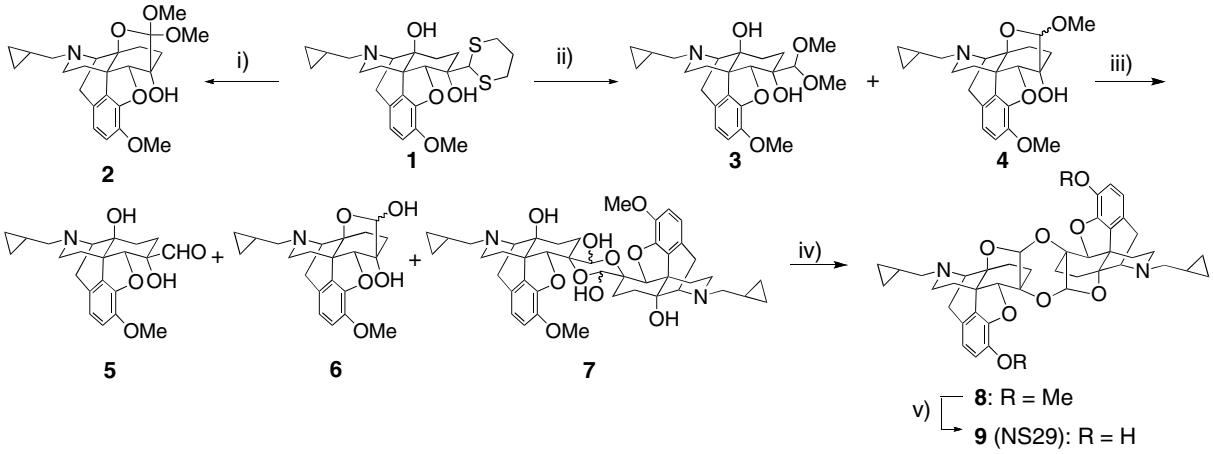


Figure 2. X-ray crystallography of compound **8**. Crystal water in the crystal structure was omitted for clarity.



Scheme 2. (i) NaH, $Me_3S^+I^-$, THF, DMSO, rt, 62% for **11**, 26% for **12**; (ii) *t*-BuOK, *n*-PrSH, DMF, 150 °C, 96%.

The effects of NS29 and NS28 on G-protein activation introduced by selective opioid agonists were evaluated by [^{35}S]GTPγS binding to mouse whole brain without cerebellum membranes (μ, δ, and putative ε receptors) or to the guinea pig cerebellum (κ receptor). NS29 blocked the stimulation of [^{35}S]GTPγS binding induced by μ-agonist morphine (10^{-5} M), κ-agonist U50,488H (10^{-5} M), and putative ε-agonist β-endorphin[14] (10^{-6} M) (Fig. 3(A), (C), and (D), respectively) in a concentration-dependent manner. However, the stimulation of [^{35}S]GTPγS binding induced by δ-agonist SNC-80 (10^{-5} M) was not affected by NS29 (Fig. 3(B)). These results indicated that NS29 may be an antagonist against the μ-, κ-, and putative ε-receptors. Antagonist activity of NS29 for β-endorphin was strong, but weaker for morphine and U50,488H. On the other hand, NS28 inhibited the stimulation of



Scheme 1. Reagents and conditions: (i) $CH(OMe)_3$, $CuCl_2$/CuO, MeOH, 50 °C, 78%; (ii) $CH(OMe)_3$, $CuCl_2$/CSA, MeOH, 50 °C, 9 h, 65% for **3**, 18% for **4**; (iii) 1 M HCl, reflux; (iv) CSA, toluene, reflux, 23%; (v) *t*-BuOK, *n*-PrSH, DMF, 150 °C, 82%.

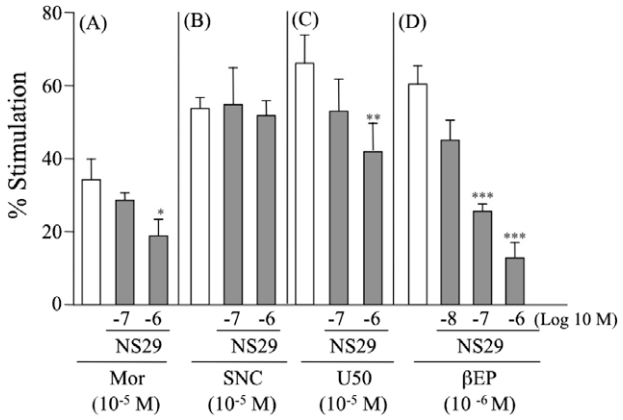


Figure 3. Effects of NS29 on G-protein activation induced by μ (A), δ (B), κ (C), and putative ε (D) opioid receptor agonist.

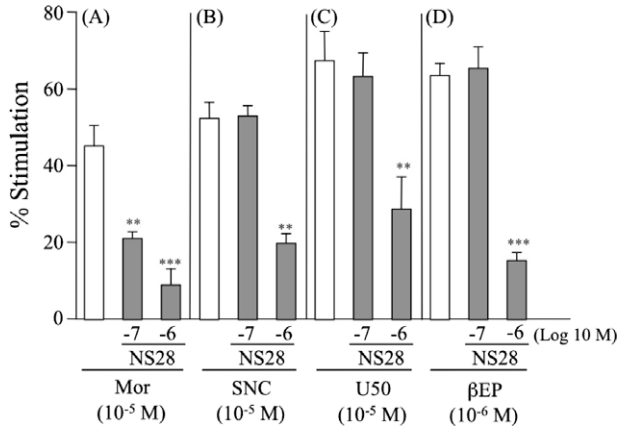


Figure 4. Effects of NS28 on G-protein activation induced by μ (A), δ (B), κ (C), and putative ε (D) opioid receptor agonist.

[^{35}S]GTPγS binding induced by μ-agonist morphine (10^{-5} M) concentration–dependently (Fig. 4(A)). However, the stimulation of [^{35}S]GTPγS binding induced by δ-agonist SNC-80 (10^{-5} M), κ-agonist U50,488H (10^{-5} M), and putative ε-agonist β-endorphin (10^{-6} M) was blocked by only the highest concentration of NS28 (10^{-6} M), but the effect was not concentration dependent (Fig. 4(B), (C), and (D)). These results suggested that NS28 would be a μ-antagonist.

The monomeric NS28 showed μ-antagonist activity, while dimeric NS29 exhibited strong antagonism for putative ε opioid receptors and weak antagonism for μ and κ opioid receptor in the [^{35}S]GTPγS binding assay. The results indicated that the dimerization of NS28 into the twin drug NS29 afforded an additional pharmacological effect, a strong putative ε-antagonist activity. In the opioid field, the 'message–address concept' is a well-known means to characterize ligands that are specific for different receptor types,[15] and several selective ligands have been designed and synthesized on the basis of this concept.[16] According to the concept, selective opioid ligands can be divided into two parts, the message-site and the address-site. The former site could be the necessary structure to produce the opioid intrinsic effects while the latter site could participate in the receptor type selectivity. Figure 5 illustrates the individual message sites and the address sites in putative ε-agonist TAN-821 and in NS29. Previously, we developed a working hypothesis for the selective binding of TAN-821 with the putative ε receptor: the address site for the putative ε receptor would be located above the C ring of 4,5-epoxymorphinan skeleton.[6] The 7α-carboxamide side chain in TAN-821 would

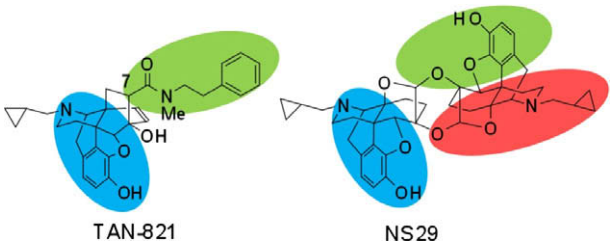


Figure 5. Message, address, and possible accessory site of TAN-821 and NS29 are indicated by blue, green, and red circles, respectively.

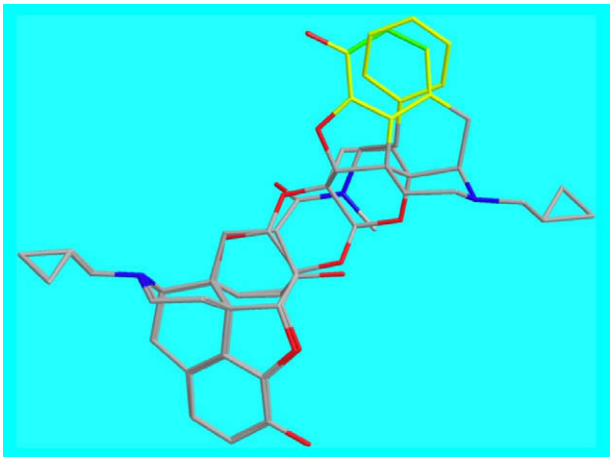


Figure 6. Superimposition of the 3D-aligment of TAN-821 onto that of NS29. Notable benzene rings, and nitrogen and oxygen atoms are indicated by yellow, blue, and red color, respectively.

act as the address site (Fig. 5). Particularly, the phenyl group in the address site was deduced to play an important role in binding to the putative ε receptor.

Each benzene ring of TAN-821 and NS29 in the address site (indicated by yellow color in Fig. 6) appears to occupy a similar location when the 3D-alignment of TAN-821 was superimposed onto that of NS29 (Fig. 6). The benzene ring shown at the upper right side in NS29 may act as the address site for the putative ε receptor. Moreover, NS29 has a bulkier lipophilic structure than TAN-821. Antagonists generally possess bulky, lipophilic moieties, the so-called accessory site,[17] which could interfere with the conformational change in the receptor which is required to induce agonistic activity. The bulky lipophilic moiety of NS29 indicated by a red circle in Figure 5 could function as an accessory site.

A twin drug such as NS29 may clarify whether various pharmacological effects of opioids derive from opioid receptor subtypes or whether they can be to the result of the dimerization of the corresponding receptor types, especially δ–κ and μ–δ heterodimers.

In conclusion, identical twin drug NS29, consisting of two NS28 molecules linked by a 1,4-dioxane spacer, was synthesized from naltrexone methyl ether, whose structure was determined by X-ray crystallography. NS29 attenuated the [^{35}S]GTPγS binding induced by μ-, κ-, or the putative ε-agonist, while NS28 blocked only the μ-agonist induced [^{35}S]GTPγS binding. Dimer NS29 and its derivatives are expected to be unique pharmacological tools for the investigation of opioid receptors.

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