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Design and synthesis of KNT-127, a δ-opioid receptor agonist effective by systemic administration

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ABSTRACT

We have reported previously the novel δ-opioid agonist, SN-28, which was more potent in in vitro assays than the prototype δ-agonists, TAN-67 and SNC-80. However, when administered by subcutaneous injection, this compound showed no analgesic effect at dosages greater than 30 mg/kg in the acetic acid writhing test. We speculated that SN-28 was not effective in the test because the presence of the charged ammonium groups prevented its penetration through the blood–brain barrier. On the basis of our proposal, we designed the novel δ-agonist, KNT-127, which was effective with systemic administration.

Three types of opioid receptors (μ, δ, κ) are now well established not only by pharmacological studies, but also through molecular biological studies.[1] For the past three decades, considerable effort has been expended on obtaining an opioid κ-selective agonist to eliminate undesirable morphine-like side effects like addiction. In 1982, U-50,488H was discovered to be a highly selective κ-agonist by researchers at Upjohn (now merged with Pfizer).[2–4] Since then, numerous research groups modified its structure and succeeded in preparing more selective and potent κ-agonists. These compounds had a potent antinociceptive effect in animal models and also succeeded eliminating the morphine-like side effects. However, all these compounds have structures

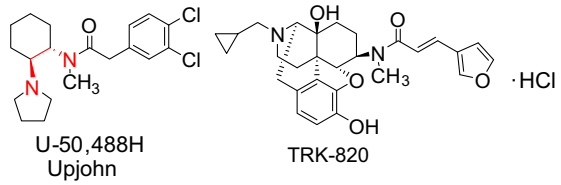

Figure 1. The structures of U-50,488H and TRK-820.

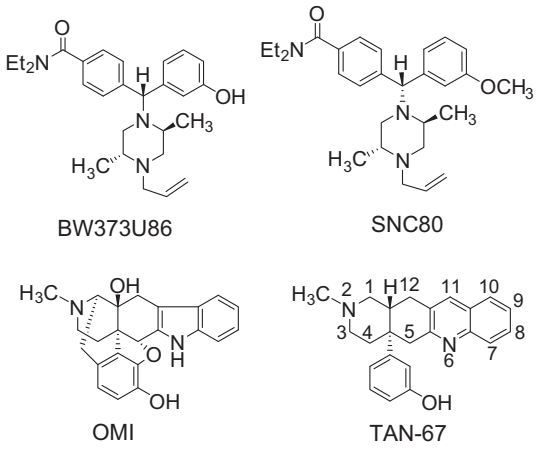

Figure 2. The structures of BW373U86, SNC-80, and TAN-67.

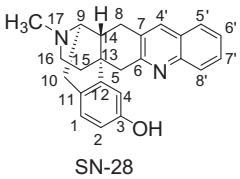

Figure 3. The structure of SN-28.

Abbreviations: BBB, blood–brain barrier; s.c., subcutaneous; i.t., intrathecal; CBM, cyclobutylmethyl.

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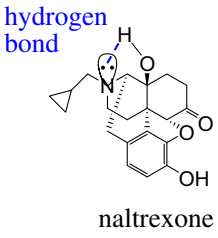
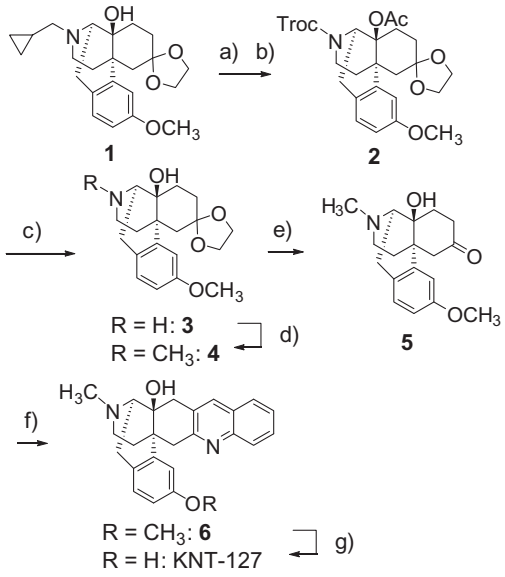


Figure 4. The hydrogen bond formed the lone electron pair on the 17-nitrogen and the 14-hydroxy group in naltrexone.



Scheme 1. Reagents and conditions: (a) Ac2O, reflux; (b) TrocCl, K_2CO_3, $CHCl_2CHCl_2$, reflux, 85% (two steps from 1); (c) 12 N KOH, DMSO, reflux; (d) HCHO, CH_3COONa, CH_3COOH, H_2, 10% Pd–C, rt, 72% (two steps from **2**); (e) 1 N HCl, 80 °C, 90%; (f) 2-aminobenzaldehyde; CH_3SO_3H, EtOH, reflux, 75%; (g) BBr_3, CH_2Cl_2, 0 °C → rt, 85%.

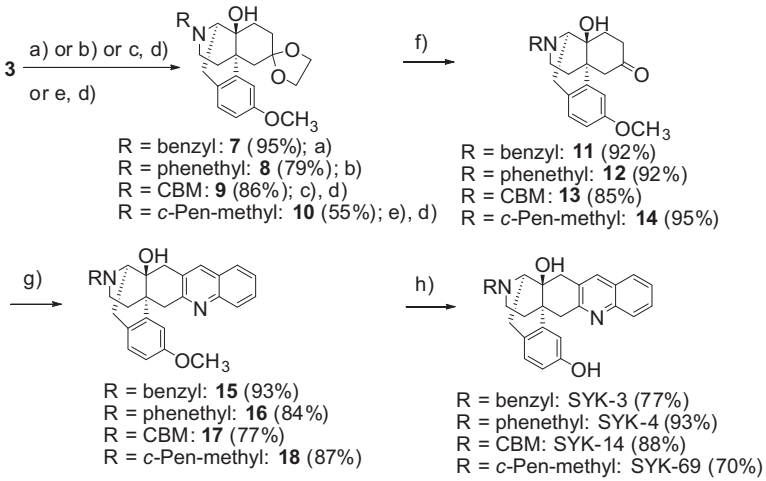
similar to that of U-50,488H, with the [N–C–C–N(sp^2)] pharmacophore sequence (Fig. 1), and showed severe aversion like psychotomimetic effect. We recently reported a novel κ-agonist, TRK-820 (Nalfurafine hydrochloride) which has a structure quite different from U-50,488H. Unlike U-50488H, TRK-820 bears a tyrosine–glycine dipeptide unit which is a structural motif commonly found in endogenous opioid peptides (Fig. 1).[5,6]

The new compound showed neither addiction nor aversion and was launched in Japan as an antipruritic agent for kidney dialysis patients last year. This is the first opioid drug that does not cause addiction. Now that we obtained a selective κ-agonist without morphine-like side effects and aversion, our next target was a highly selective and potent δ-agonist. Nonpetide δ-opioid agonists BW373U86,[7] SNC-80,[8,9] and OMI[10,11] have been described and we have also reported the highly selective δ-agonist, TAN-67(Fig. 2).[12,13]

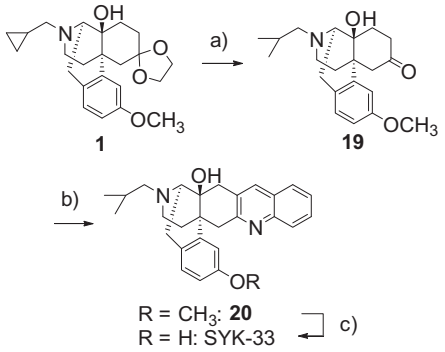
However, these prototypical compounds were not potent enough to examine the intrinsic pharmacological effects of the δ-receptor and even now we do not know if δ-receptor contributes to addiction, as occurs with morphine.

Quite recently, we have reported a novel δ-agonist, SN-28, which showed about 15 times and 334 times more potent agonist activities than TAN-67 and SNC-80, respectively and almost equivalent selectivity to TAN-67 in in vitro assays (Fig. 3).[14] However, at dosages over 30 mg/kg administered by subcutaneous (s.c.) injection, SN-28 showed no analgesic effect in the acetic acid writhing test.[6] To date, no δ-agonist exists that is therapeutically effective by systemic administration and this problem illustrates the difficulties of developing peptidic compounds as drugs. In an effort to understand why SN-28 (by the s.c. route) was not effective in the test, we proposed that SN-28 does not penetrate through the blood–brain barrier (BBB). To confirm this hypothesis, we examined the analgesic effect of SN-28, administered by intrathecal injection (i.t. injection), in the acetic acid writhing test. As we had expected, SN-28 showed a strong analgesic effect (ED_{50} = 0.095 nM) in this test, which supported the idea that the compound does not cross the BBB. On the basis of the above postulate, we designed a novel δ-agonist, KNT-127, which is effective in systemic administration. Here we report the design and synthesis of KNT-127.

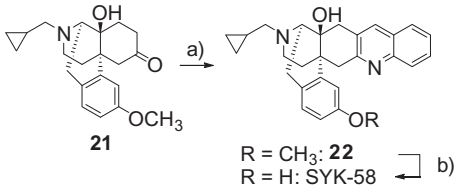
TAN-67 and SN-28 have two basic nitrogens: a quinoline moiety and a 17-nitrogen. Both would contribute to high polarity of the compounds. The quinoline group is considered to be an essential part of the address for δ-receptor agonist activity.[12,13] Therefore, when we designed and synthesized TAN-67 by use of the message-address concept[15] and the accessory site hypothesis,[16] we chose the quinoline group as the address moiety. On the other



Scheme 2. Reagents and conditions: (a) BnBr, K_2CO_3, DMF, rt, 95%; (b) $PhCH_2CHO$, $NaBH(OAc)_3$, CH_2Cl_2, rt, 79%; (c) *c*-BuCOCl, NEt_3, CH_2Cl_2, rt; (d) $LiAlH_4$, THF, 0 °C → rt, **9**: 86% (two steps from **3**), **10**: 55% (three steps from **2**); (e) *c*-PenCOCl, NEt_3, CH_2Cl_2, rt; (f) 1 N HCl, 80 °C; (g) 2-aminobenzaldehyde, CH_3SO_3H, EtOH, reflux; (h) BBr_3, CH_2Cl_2, 0 °C → rt.



Scheme 3. Reagents and conditions: (a) 48%HBr, MeOH, PtO_2, H_2, rt, 56%; (b) 2-aminobenzaldehyde, CH_3SO_3H, EtOH, reflux, 70%; (c) BBr_3, CH_2Cl_2, 0 °C → rt, 72%.



Scheme 4. Reagents and conditions: (a) 2-aminobenzaldehyde, CH_3SO_3H, EtOH, reflux, 71%; (b) BBr_3, CH_2Cl_2, 0 °C → rt, 61%.

hand, the basic 17-nitrogen could form ammonium ions under physiological conditions, and the resulting ionized compound may be less likely to penetrate through the BBB. We postulated that the lone electron pair on the 17-nitrogen in SN-28 requires protection from forming of an ammonium ion. We reported already that the 14-hydroxy group in naltrexone could form a hydrogen bond with the lone electron pair on the 17-nitrogen to produce a less polar compound (Fig. 4).[17] Based on the above discussion, we designed and synthesized 14-hydroxy-SN-28, that is, KNT-127. The morphinan **1** was synthesized from naltrexone by the reported method.[18–20] The 17-cyclopropylmethyl group of compound **1** was converted to a 17-methyl group by a previously reported method.[21,22] The treatment of compound **5** with 2-aminobenzaldehyde in the presence of CH_3SO_3H provided compound **6**.[12] The resulting compounds **6** was demethylated with BBr_3 to afford KNT-127 (Scheme 1).[23,24]

Thus obtained KNT-127 showed higher selectivity for the δ-receptor [K_i (nM) = 21.3 (μ), 153 (κ), 0.16 (δ) and K_i ratio: 133.5 (μ/δ), 960.5 (κ/δ)] than did SN-28 [K_i (nM) = 10.8(μ), 49.4 (κ), 0.14 (δ) and K_i ratio: 77.1 (μδ), 352.9 (κδ)].

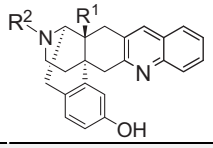
Next, using i.t. injection, we compared the analgesic activity of the newly obtained compound with that of SN-28 in the acetic acid writhing method in mice. This compound showed almost the same potent agonistic activity (ED_{50} = 0.149 nM) as did SN-28 (ED_{50} = 0.095 nM) in this test. Finally, we examined the analgesic effect of KNT-127 given by s.c. injection in the acetic acid writhing test in mice. As we expected, a very strong dose dependent analgesia was observed (ED_{50} = 1.2 mg/kg), which was about 30 times more potent than that of TAN-67 (ED_{50} = 31.4 mg/kg).[12]

Thus, we confirmed our above postulation that SN-28 could not readily cross the BBB and our design to improve the defect of SN-28 led to the potent analgesic agent (KNT-127), with effective systemic administration. Having obtained a lead compound for the δ-agonist, we next attempted to vary the substituent at 17-nitrogen in an effort to address the structure–activity relationship. 14-Hydroxymorphinan derivatives with various 17-substituents were synthesized in a similar manner to the synthesis of KNT-127 (Schemes 2–4). 17-Isobutyl derivative **19** was obtained by the reductive cleavage of the 17-cyclopropylmethyl substituent in compound **1** (Scheme 3).[25,26] The results of binding assays of the thus obtained compounds SYK-3, SYK-4, SYK-14, SYK-33, SYK-58, and SYK-69 along with those of reference compounds SN-28, KNT-127, and TAN-67 appear in Table 1.

KNT-127 and SYK-4 showed the highest κ/δ selectivity, whereas the highest μ/δ ratio was obtained in SYK-33 and SYK-69. Although SYK-58 had the strongest affinity for the δ-receptor, its selectivities for the δ-receptor over the μ- and κ-receptors were low. Among these newly synthesized 14-hydroxymorphinan derivatives, KNT-127 had sufficient affinity and the most balanced and highest selectivity for the δ-receptor. Moreover, its affinity and selectivity for the δ-receptor was superior to those of the prototypical δ-agonist, TAN-67. These results suggested that the 17-substituent would be a very important structural determinant for the study of 14-hydroxymorphinan derivatives with the quinoline moiety that serve as δ-selective opioid ligands.

In conclusion, we designed and synthesized 14-hydroxymorphinan derivatives to improve the penetration of SN-28 through the BBB by use of the hydrogen bond formed between the 14-hydroxy group and the lone electron pair on the 17-nitrogen. One of these compounds, KNT-127, showed the most balanced and highest

Table 1
The binding affinity and selectivity of SN-28, KNT-127, TAN-67, SYK-3, 4, 14, 33, 58, and 69 for the opioid μ-, δ-, and κ-receptors[a]



Compounds	R1	R^2	Affinity (K_i, nM)			Selectivity	
			μ	δ	κ	μ/δ	κ/δ
SN-28	H	Methyl	10.8	0.14	49.4	77.1	352.9
KNT-127	OH	Methyl	21.3	0.16	153	133.5	960.5
TAN-67[b]			284.14	1.44	732.45	197.5	508.7
SYK-3	OH	Benzyl	924.3	6.38	122.8	144.8	19.2
SYK-4	OH	Phenethyl	53.2	0.57	558	93.7	983.4
SYK-14	OH	Cyclobutylmethyl	10.6	0.21	5.72	51.3	27.7
SYK-33	OH	Isobutyl	153.9	0.51	48.8	301.7	95.7
SYK-58	OH	Cyclopropylmethyl	3.55	0.14	1.95	25.4	13.9
SYK-69	OH	Cyclopentylmethyl	277.9	0.78	33.6	357.7	43.2

[a] The binding affinity of each compound was evaluated by displacement of [^{3}H]DAMGO (μ), [^{3}H]DPDPE (δ), and [^{3}H]U-69,593 (κ) binding to membranes of mouse whole brain (μ and δ) or the guinea pig cerebellum (κ).
[b] Ref. 14.

selectivity for the δ-receptor and a very potent analgesic effect in the acetic acid writhing test even when it was given by s.c. administration. Its systemically active antinociceptive effect was about 30 times more potent than that of a prototypical δ-agonist, TAN-67. The pharmacological character of the δ-receptor has not yet been clarified due to unavailability of a sufficiently active, especially systemically active, δ-agonist. In contrast, the μ-receptor is known to contribute to the analgesic effects and to severe side effects like addiction, while the κ-agonist has antinociceptive and antipruritic effects without addiction. As KNT-127 induced a marked effect at low dose through systemic administration, it is expected to be a useful tool to clarify the real pharmacological effects via the δ-receptor including analgesia and addiction.

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24. *KNT-127*: mp 250–254 °C (decomp.); IR (KBr): 3430 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ: 1.34–1.39 (1H, m), 2.25–2.32 (2H, m), 2.45 (3H, s), 2.47–2.51 (1H, m), 2.90–2.98 (2H, m), 3.01 (1H, d, J = 6.0 Hz), 3.14 (1H, d, J = 17.5 Hz), 3.25–3.31 (1H, m), 3.46 (1H, d, J = 17.0 Hz), 3.63 (1H, d, J = 17.0 Hz), 6.50 (1H, dd, J = 2.5, 8.0 Hz), 6.74 (1H, d, J = 2.5 Hz), 6.95 (1H, d, J = 8.0 Hz), 7.44 (1H, ddd, J = 1.0, 7.0, 7.5 Hz), 7.63 (1H, ddd, J = 1.5, 7.0, 7.5 Hz), 7.69–7.73 (1H, m), 7.83–7.85 (1H, m), 7.89–7.93 (1H, m). MS (ESI) m/z = 373 $[M+H]^+$. HRMS (FAB) calcd for $C_{24}H_{25}N_2O_2$ $[M+H]^+$: 373.1916; found 373.1906.
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