DISCOVERY OF A STRUCTURALLY NOVEL OPIOID κ-AGONIST DERIVED FROM 4,5-EPOXYMORPHINAN

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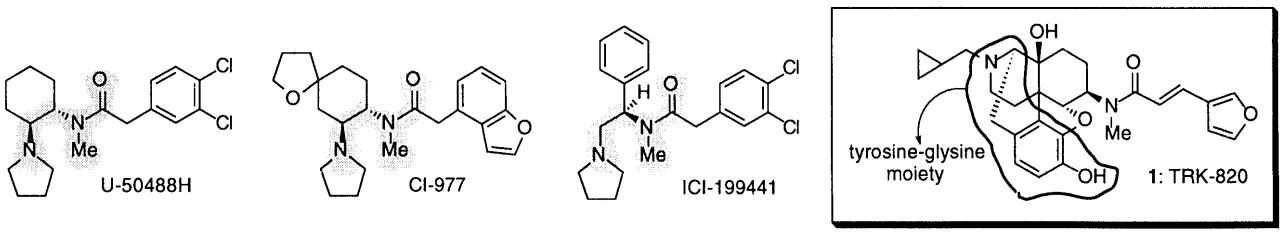
A new type of κ-agonist, 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[*N*-methyl-*trans*-3-(3-furyl)acrylamido]morphinan hydrochloride (**1**, TRK-820), was discoverd by a new working hypothesis. The "message-address concept" for opioid antagonists and the "accessory site" for general antagonists were applied to design TRK-820. A unique structural feature of TRK-820, which is different from other prototypical κ-opioid receptor agonists, is the existence of the 4,5-epoxymorphinan structure with a tyrosine-glysine moiety for endogenous opioid peptides such as dynorphins. TRK-820 exhibited high potency and high κ-selectivity in guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations. In the mouse acetic-acid-induced writhing model and mouse tail flick model of antinociception, TRK-820 was 85-140 times more potent than morphine and 85-350 times more potent than U-50488H. This structurally novel κ-agonist showed neither aversion nor preference in the Conditioned Place Preference test, in spite of the fact that prototypes of κ-agonists (U-50488H derivatives) demonstrated aversion.

KEY WORDS opioid; κ-agonist; message-address concept; accessory site; TRK-820

For the past two decades, considerable effort has been expended on obtaining an opioid κ-selective agonist to eliminate undesirable morphine-like side effects (*e.g.*, respiratory depression, constipation, physical dependence, etc.). In 1982, U-50488H was discovered to be a highly selective κ-agonist.[1] After that several research groups modified its structure and succeeded in preparing more selective and potent κ-agonists (see structures below).[2,3] All these compounds had a potent antinociceptive effect in animal models and also eliminated the morphine-like side effects. However, the compounds were not developed for clinical use since they probably cause different types of side effects, such as dysphoria and psychotomimetic effects.[4] All these compounds have a very similar structure to the [N-C-C-N(SP2)] pharmacophore sequence[5] (shaded parts of structures below) since they are analogues of U-50488H. They do not have the tyrosine-glysine part which is essential for opioid activity from the viewpoint of endogenous opioid chemistry. We had doubts concerning whether these were real κ-opioid agonists. Therefore we intended to design a new type of non-peptide κ-agonist with a novel chemical structure which has the tyrosine-glysine part in order to separate the side effects of the prototype κ-agonists. Here we wish to report the design of a κ-selective full agonist with a 4,5-epoxymorphinan structure.[6]



Portoghese et al. applied the "message-address concept" for synthesizing selective δ- and κ-antagonists (NTI and nor-BNI).[7,8] They pointed out that the 4,5-epoxymorphinan skeleton defined as the message subsite, which is commonly found in NTI and nor-BNI, is necessary for producing the opioid effect. The other structural part was defined as the address subsite and is involved in the receptor type selectivity. As shown in Fig. 1, the receptor type selectivity of these opioid antagonists can be regulated by alteration of the structural size of the address subsite. However, this concept is applicable for designing a selective opioid antagonist, and thus it is necessary to modify this concept to design a selective opioid agonist.

In general, receptors can change their structure as they fit into the structure of ligands ("induced fit") when the agonist binds them. This change would lead to the next signal transduction so that the agonist shows its effect. Because it has an

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extra structural part that interferes with the structural change in the receptor, the antagonist does not show such an effect even if it binds a receptor. The structural site for participating in the interference of "induced fit" is called an "accessory site," which is usually a highly hydrophobic and sterically hindered site.[9] The structural difference between the antagonist and agonist lies mainly in whether it has an accessory site or not.

To design a new κ-opioid agonist from the κ-selective antagonist, nor-BNI, we applied the two concepts of message-address and accessory site. The accessory site of nor-BNI should be removed while maintaining the message-address concept. The message subsite of nor-BNI, a 4,5-epoxymorphinan skeleton with a cyclopropylmethyl substituent, could be indispensable for opioid activity because it corresponds to the tyrosine-glysine part of endogenous opioid peptides. Therefore it is expected that the accessory site of nor-BNI might be located in the address subsite of this antagonist. The key point for the design of the κ-selective agonist was to explore which part of the address subsite of nor-BNI would be selected as the accessory site.

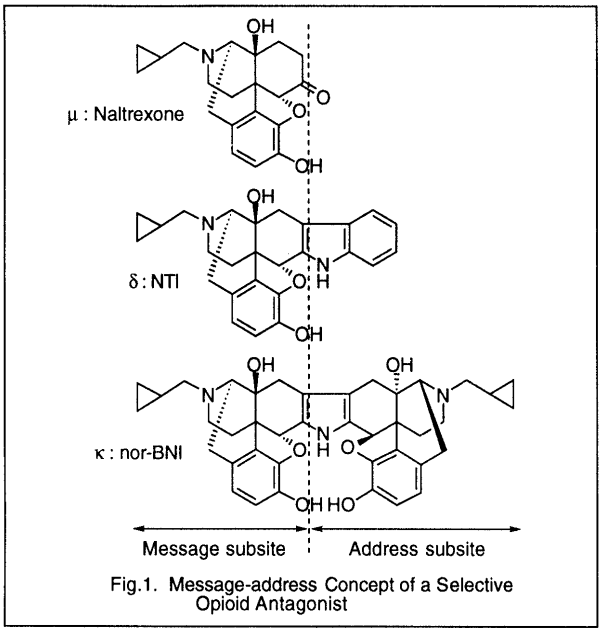


Fig.1. Message-address Concept of a Selective Opioid Antagonist

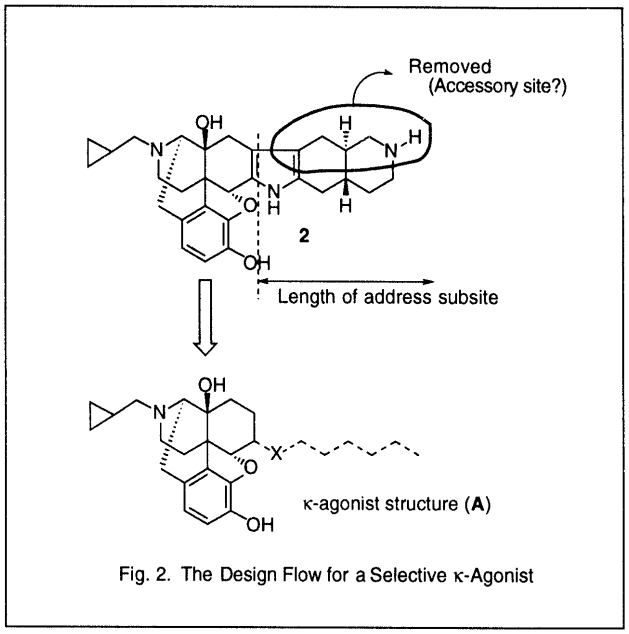


Fig. 2. The Design Flow for a Selective κ-Agonist

The meso form analogue of nor-BNI has κ-selectivity and antagonist activity similar to those of nor-BNI.[10] Furthermore, compound **2** in which the phenol ring in the address subsite of nor-BNI is removed also maintained κ-receptor antagonist selectivity as high as nor-BNI.[11] These facts indicate that the phenol ring and the hydroxy group of the ring junction in the address subsite of nor-BNI are not required for the κ-selectivity and antagonist activity. Upon comparing the κ-antagonist, compound **2**, with naltrexone and NTI, it was proposed that the essential factor of the address subsite for receptor selectivity would be the structural length of the address subsite from the message subsite (Fig. 2). From this point of view, it is necessary when designing a κ-selective agonist to remove the accessory site of compound **2** while maintaining the length of the address subsite for the κ-receptor. Therefore we make the address subsite slimmer and synthesized compound (**A**) to couple C6 (carbon atom of the 6-position) and X (hetero atom) with single bond (Fig. 2). This single bond gives structural flexibility to the ligand, so that the induced fit of the receptor for exhibiting the agonist activity could occur more easily.

Based on these ideas for design, a number of compounds were synthesized to grasp the proper structural size and length on the side chain (address subsite) for a κ-agonist. We found that the side-chain structure of the 6-position had a major influence to receptor selectivity and opioid activity (the SARs are stated later). As a result, we finally found compound **1**, 17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6β-[*N*-methyl-*trans*-3-(3-furyl)acrylamido]morphinan hydrochloride (TRK-820),[12] which was synthesized from 6β-N-Methyl-naltorexamine[13] (**3**) and 3-(3-furyl)acryloyl chloride,[14] to be a potent and selective κ-agonist.

The agonist activity of TRK-820 was tested on the electrically stimulated guinea pig ileal longitudinal muscle (GPI) and mouse vas deferens (MVD). Receptor selectivity was determined using each receptor-selective antagonist: naloxone for the μ site, NTI for the δ site, and nor-BNI for the κ site. Morphine and U-50488H were also tested as reference compounds.

The agonistic potency of TRK-820 was 4000-fold that of morphine in both preparations (Table 1). In comparison to U-50488H, TRK-820 was 200 times and 70 times more potent on GPI and MVD, respectively. The Ke ratios of TRK-820, both μ/κ and δ/κ, were more than 100 and that of U-50488H were more than 1000 on MVD. It seems that U-50488H has superior κ-selectivity compared to TRK-820 as long as the κ_1-selective antagonist, nor-BNI, is used. However, this suggests the posibility that TRK-820 would have an affinity to a κ-receptor subtype other than U-50488H. TRK-820 was also evaluated for its antinociceptive effect in the acetic acid writhing method (Ac-WR) and tail flick method in mice. TRK-820 showed highly antinociceptive activity in both tests (Table 2). The ED_{50} values of TRK-820 were 0.0033 mg/kg and 0.062 mg/kg, respectively. These activities were 85-140 times more potent than morphine and 85-350 times more potent than U-50488H. This analgesic effect was antagonized only by the κ-receptor antagonist, nor-BNI, but not by NTI or by low-dose naloxone.[15]

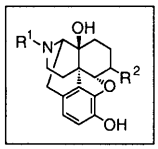
Some SARs of related compounds of TRK-820 are summarized in Table 2. The antinociceptive effect of TRK-820 was greatly reduced by converting the substituent groups of amide nitrogen from methyl to hydrogen (**3**). Compound **4**, which is a 17-position substituent group, converted from a cyclopropylmethyl gruop to a methyl group, reduced not only the analgesic effect but also the κ-selectivity (Ke ratio: μ/κ=1.3, δ/κ=5.3). A thioester derivative of TRK-820 (**5**) did not show any analgesic effect in the Ac-WR, although KT-90[16] (a thioester derivative of morphine) had an effect on this assay. Both **6** and **7** were κ-selective compounds, but their analgesic effect was reduced with the lengthening of the address subsite.

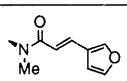
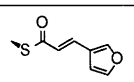
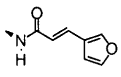
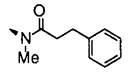
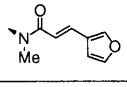
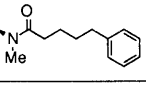
Table 1. Agonist Potency and Selectivity in Guinea Pig Ileum (GPI) and Mouse Vas Deferens (MVD), and Agonist Activity in the Acetic Acid Writhing Test (Ac-WR) and Tail Flick Test (TF) in Mice

Compound	GPI				MVD						Ac-WR (sc)	TF (sc)
	IC50 (nM) [a]	Ke (nM)[b]		μ/κ	IC50 (nM)[a]	Ke (nM)[b]			μ/κ	δ/κ	ED_{50} (mg/kg)[a]	ED_{50} (mg/kg)[a]
		κ	μ			κ	μ	δ				
TRK-820	0.0048 (0.0034-0.0066)	0.052[c]	14.5[f]	279	0.036 (0.029-0.043)	0.20[c]	20.7[f]	26.9[h]	104	135	0.0033 (0.0025-0.0043)	0.062 (0.032-0.119)
Morphine	49.3 (37.6-64.6)	20.5[d]	5.06[g]		145.1 (111.2-189.3)	53.5[d]	3.29[g]	7.35[h]	0.06	0.14	0.58 (0.48-0.71)	5.26 (3.79-7.32)
U-50488H	1.12 (0.70-1.80)	0.031[c]	12.1[f]	390	2.35 (1.91-2.90)	0.031[e]	31.5[f]	41.2[h]	1016	1329	1.16 (0.90-1.51)	5.18 (2.35-11.43)

[a]Values are arithmetic means of five to seven experiments with 95% confidence limits in parentheses. [b]Ke values were calculated from the following equation: Ke = [antagonist]/(IC_{50} ratio - 1). Each preparation was incubated with a selective antagonist for 20 min before the addition of a test compound. [c]5 nM nor-BNI. [d]100 nM nor-BNI. [e]1 nM nor-BNI. [f]100 nM naloxone. [g]20 nM naloxone. [h]100 nM NTI.

Table 2. SARs of TRK-820-related compounds



Compound	R^1	R^2	Ac-WR (sc) ED_{50} (μg/kg)	Compound	R^1	R^2	Ac-WR (sc) ED_{50} (μg/kg)
1 (TRK-820)	cyclopropylmethyl		3.3	5	cyclopropylmethyl		>10,000
3	cyclopropylmethyl		950	6	cyclopropylmethyl		40
4	Methyl		1030	7	cyclopropylmethyl		380

To apply for development of an opioid agonist, we modified the message-address concept of an opioid antagonist combined with the concepts of the accessory site and induced fit. Finally, we determined for the first time that TRK-820 is a highly potent and κ-selective agonist with the 4,5-epoxymorphinan structure as the tyrosine-glysine moiety. As shown above, TRK-820 has a very different chemical structure from the prototype of a κ-agonist, its pharmaceutical profile is different, ex. selectivity to κ-receptor subtype, behavior,[17] *etc.* The details of these aspects are under investigation and will be reported later.

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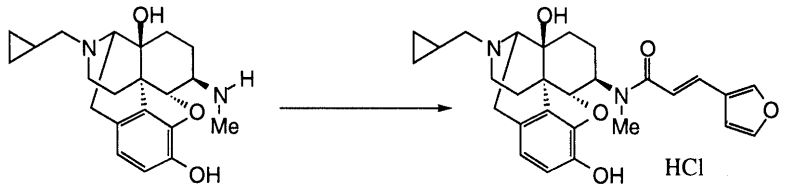
12) A solution of 3-(3-furyl)acryloyl chloride in anhydrous THF was added to a two-phase solution of compound **8** in THF and distilled water containing sodium carbonate, and stirred for 1 hour. The sodium hydroxide solution was then added to the reaction solution to hydrolyze the phenol ester. The obtained free base of TRK-820 by extraction and concentration of the solvent was purified by recrystallization from ethyl acetate/methanol. The purified compound was converted to hydrochloride salt using HCl to give TRK-820 in 75% yield.



8 **1**: TRK-820

13) Mohamed M. S., Larson D. L., Takemori A. E., Portoghese P. S., *J. Med. Chem.*, **29**, 1551-1553 (1986).
14) 3-(3-Furyl)acrylic acid is commercially available from Aldrich. It was converted to acid chloride using oxalyl chloride. The acid chloride could be purified by distillation (bp. 80 °C/1 mmHg).
15) Nor-BNI (20 mg/kg) was administered 1 day before the injection of TRK-820. Nor-BNI antagonized the antinociceptive effect of TRK-820 (dose ratio: 20.3), while TRK-820 was only slightly antagonized by NTI (2 mg/kg) and naloxone (0.3 mg/kg).
16) Tsksysnsgi I., Goromaru N., Koike K., Konno F., Mori T., Yoshida M., Kanematsu K., *Gen. Pharmacol.*, **21**, 541-546 (1990).
17) TRK-820 shows neither an aversive nor dependence effect in the Conditioned Place Preference test, in spite of the fact that an aversive effect is observed with prototype κ-agonists.

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