

Opioids in Preclinical and Clinical Trials

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Abstract Since 1952, when Gates determined the stereo structure of morphine, numerous groups have focused on discovering a nonnarcotic opioid drug [1]. Although several natural, semisynthetic, and synthetic opioid ligands (alkaloids and peptides) have been developed in clinical studies, very few were nonnarcotic opioid drugs [2]. One of the most important studies in the opioid field appeared in 1976, when Martin and colleagues [3] established types of opioid receptors (these are now classified into μ , δ , and κ types). Later, Portoghese discovered a highly selective μ type opioid receptor antagonist, β -funaltrexamine [4]. This led to the finding that the μ type opioid receptor was correlated to drug dependence [5]. Consequently, δ , and particularly κ , opioid agonists were expected to lead to ideal opioid drugs. Moreover, opioid antagonists were evaluated for the treatment of symptoms related to undesirable opioid system activation. In this chapter, we provide a short survey of opioid ligands in development and describe the discovery of the two most promising drugs, TRK-851 [6] and TRK-820 (nalfurafine hydrochloride) [7].

Keywords Clinical development · Nonnarcotic · Opioid drug · TRK-820 · TRK-851

Contents

1	Opioid Ligands in Early Drug Development	31
1.1	Peripheral Antagonists	31
1.2	δ Opioid Agonists	32
1.3	δ Opioid Antagonists	33
1.4	κ Opioid Agonists	34

2	Antitussive δ Opioid Antagonist TRK-851	36
2.1	Antitussive Effects of Opioid Ligands	36
2.2	Mechanism of the Antitussive Effect of NTI	37
2.3	Improvement on the Antitussive Activity of NTI	38
2.4	Improvement of Metabolic Defect of TRK-850	42
2.5	Summary	45
3	Antipruritic κ Opioid Agonist TRK-820 (Nalfurafine Hydrochloride)	48
3.1	Design Rationale	48
3.2	Pharmacology and Medicinal Chemistry	50
3.3	Clinical Development of TRK-820 in Postoperative Surgery	56
3.4	Clinical Development of TRK-820 as an Antipruritic Agent	57
3.5	Concluding Remarks	60
	References	60

Abbreviations

AAW	Acetic acid writhing
ADME	Absorption, distribution, metabolism, and excretion
ADR	Adverse drug reaction
β -FNA	β -Funaltrexamine
BBB	Blood–brain barrier
BNTX	7-Benzylidenenaltrexone
cLog P	Calculated log P
CNS	Central nervous system
CTOP	H-D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH ₂
DAMGO	[D-Ala ² , N-Me-Phe ⁴ , Gly-ol ⁵]enkephalin
DPDPE	[D-Pen ² , D-Pen ⁵]enkephalin
DSLET	Tyr-D-Ser-Gly-Phe-Leu-Thr
GPI	Guinea pig ileum
HPLC	High-performance liquid chromatography
i.c.	Intracisternal
i.d.	Intradermal
i.p.	Intraperitoneal
i.t.	Intrathecal
MVD	Mouse vas deferens
nor-BNI	nor-Binaltorphimine
NTI	Naltrindole
PBS	Phosphate-buffered saline
p.o.	Peroral
SAR	Structure–activity relationship
s.c.	Subcutaneous
S.E.M.	Standard error of the mean
TF	Tail flick
VAS	Visual analog scale

1 Opioid Ligands in Early Drug Development

Representative opioid agonists and antagonists for μ , δ , and κ opioid receptors are listed in Table 1.

Recent drug development efforts in the opioid field have mainly focused on improving patient compliance by creating new formulations, including slow release, long acting tablets, and transdermal delivery [8–11]. Others have focused on preventing illegal use by combining an opioid agonist with an opioid antagonist, naloxone, in an oral formulation. Naloxone is immediately metabolized in its first pass through the liver clearance process. Therefore, when the combination drug is used orally, the opioid agonist has a normal analgesic effect. However, when the drug is ground to a powder for intravenous injection, the naloxone works to prevent the effect of the opioid agonist [12].

Recently, relatively small numbers of new opioid compounds have been developed. New chemicals for producing an opioid drug can be categorized into four classes:

- 1. Peripheral antagonists
- 2. δ Agonists
- 3. δ Antagonists
- 4. κ Agonists

We will provide short reviews of these drug candidates in the following subsections.

1.1 Peripheral Antagonists

Patients that take prescription opioid analgesics often experience intolerable side effects, including nausea, emesis, or constipation. Because opioid analgesics act primarily on the central nervous system (CNS), targeting an antagonist outside the CNS might prevent peripheral side effects without affecting the analgesic effects.

Table 1 Representative opioid ligands

Type	Selective		Nonselective		Endogenous
	Agonists	Antagonists	Agonists	Antagonists	
μ	DAMGO	CTOP	Etorphine	Naloxone	Endomorphin
	Morphine	β -FNA	Levorphanol	Naltrexone	Endorphin
	Fentanyl				
δ	DPDPE	NTI			Enkephalin
	DSLET	NTB			
	SNC-80	BNTX			
	TAN-67				
κ	U-50,488H	nor-BNI			Dynorphin
	U-62,066				
	TRK-820				

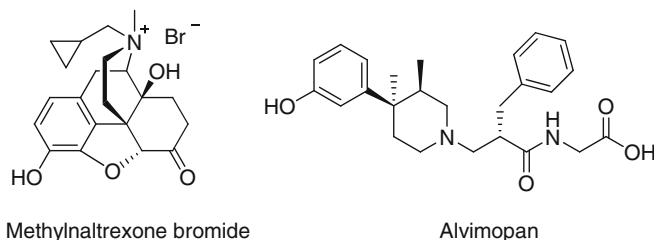


Fig. 1 Structures of methylnaltrexone bromide and alvimopan

This concept proved to be successful with methylnaltrexone bromide (Fig. 1) [13], which was launched in 2008 by Wyeth (originated at the University of Chicago) for the treatment of constipation caused by opioid analgesics. The addition of a quaternary cationic 17-nitrogen moiety prevented the compound from passing through the blood–brain barrier (BBB), but also reduced the antagonistic activity. Other examples of peripheral antagonists include Alvimopan (Fig. 1) [14] and S-297995¹ (in Phase I by Shionogi; chemical structure undetermined). Alvimopan was launched in 2008 by GSK (originated by Adolor) for recovery of normal gastrointestinal function after abdominal surgery and to accelerate upper and lower gastrointestinal recovery after partial large or small bowel resection surgery with primary anastomosis.

1.2 δ Opioid Agonists

Currently, there are no δ opioid pharmaceuticals on the market; moreover, the essential properties of this class of drugs remain unclear. It is unknown whether δ opioid agonists incur the side effects observed with other opioid drugs, like dependency and constipation.

SNC-80 (GSK, NIH) [15] is one of the leading δ opioid agonists in development. SNC-80 has a bisarylmethyl substituted piperazine substructure (Fig. 2). Some SNC-80 derivatives for treating pain or urinary incontinence are in clinical development, including DPI-3290 [16] (δ and μ opioid agonist; GSK, Enhance Biotech), PF-04856880 [17] (ADL-5859; Pfizer, Adolor), PF-04856881 [18] (ADL-5747; Pfizer, Adolor), and a dimethylpiperazine derivative [19] (University of Arizona, NIH) (Fig. 2).

¹ Shionogi reports Financial Results for the Third Quarter of Fiscal Year 2009, Shionogi Press Release 2010, February 1.

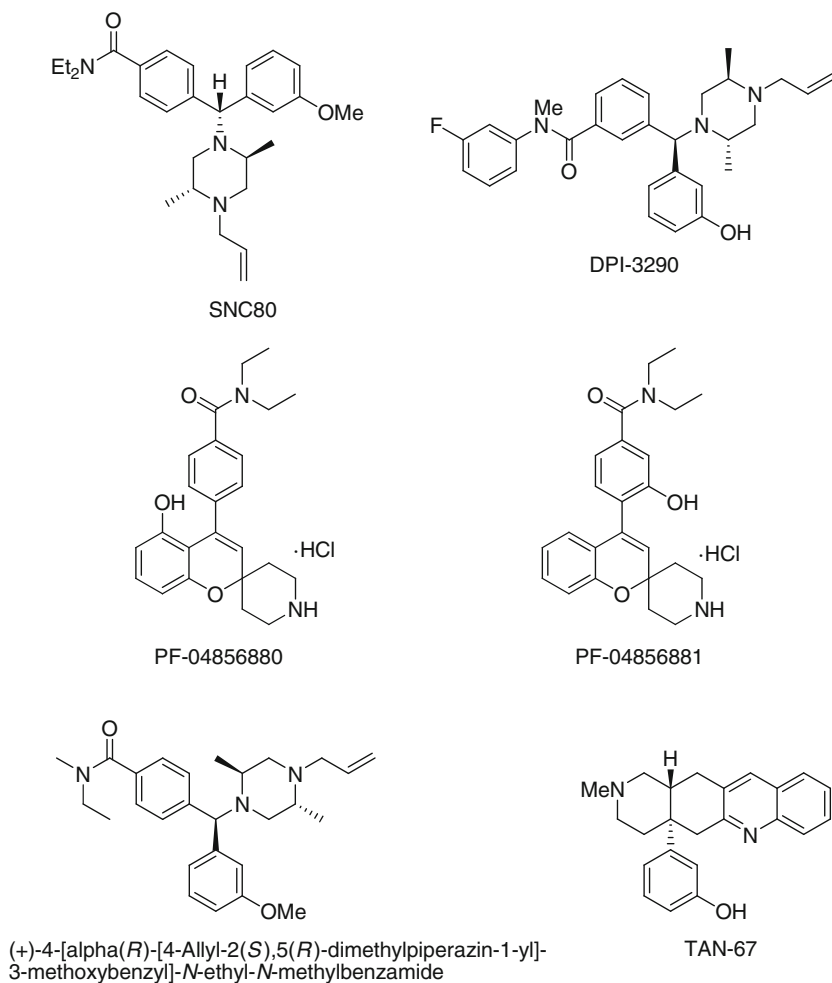


Fig. 2 Structures of δ opioid agonists in clinical development

Our group is currently developing TAN-67 (Toray), another lead δ opioid agonist with a characteristic 4a-aryl-decahydroisoquinoline structure. We previously discussed the design rationale and pharmacology of this compound [20, 21].

1.3 δ Opioid Antagonists

Portoghese and Suzuki have studied the possibility of reducing the undesirable side effects of μ opioid agonists by combining them with a δ opioid antagonist. This concept is based on the cross-talk among all three types of opioid receptors.

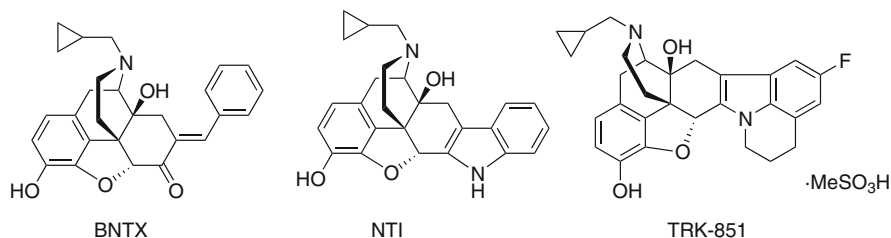


Fig. 3 Structures of δ opioid antagonists BNTX, NTI, and TRK-851

Portoghese and coworkers have shown that the dependency and tolerance induced by morphine could be treated with 7-benzylidenenaltrexone (BNTX), a δ_1 opioid antagonist (Fig. 3) [22]. Suzuki and colleagues also suggested that the psychological dependence induced by morphine could be reduced with naltrindole (NTI), another δ opioid antagonist (Fig. 3) [23, 24]. However, no drugs of this type are currently in clinical development. It has also been suggested that δ opioid antagonists might be potentially useful for immunosuppression [25], but no clinical development has been reported for this indication. The immunosuppressive effect of the δ antagonist NTI was suggested to arise from an alternative mechanism [26].

Kamei and colleagues discovered that an opioid network was involved in the cough reflex. A selective δ antagonist, TRK-851 (Toray, Fig. 3), is a clinical candidate in development as an antitussive agent [6]. This compound will be discussed in detail in Sect. 2.

1.4 κ Opioid Agonists

For the past three decades, considerable effort has been focused on developing an opioid κ selective agonist that could eliminate undesirable side effects of morphine-like drugs. In 1982, Upjohn (currently merged with Pfizer) discovered a highly selective κ agonist known as U-50,488H [27]. Subsequently, several research groups have modified this structure to create more selective and more potent κ agonists (Fig. 4) [29, 30].

In animal models, these κ agonists had potent antinociceptive effects and could eliminate the undesirable side effects of morphine-like drugs. They all had very similar structures that included the $[N-C-C-N(sp^2)]$ pharmacophore sequence (shaded parts in Fig. 4), because they were derived from U-50,488H. Moreover, they lacked the tyrosine moiety that is essential for opioid activity from the viewpoint of endogenous opioid chemistry (Fig. 5).

Currently, the only clinically successful κ agonist drug has been TRK-820 (nalfurafine hydrochloride) (Toray, Fig. 6). Almost all of the U-50,488H derivatives apparently had side effects that were different from that of morphine,

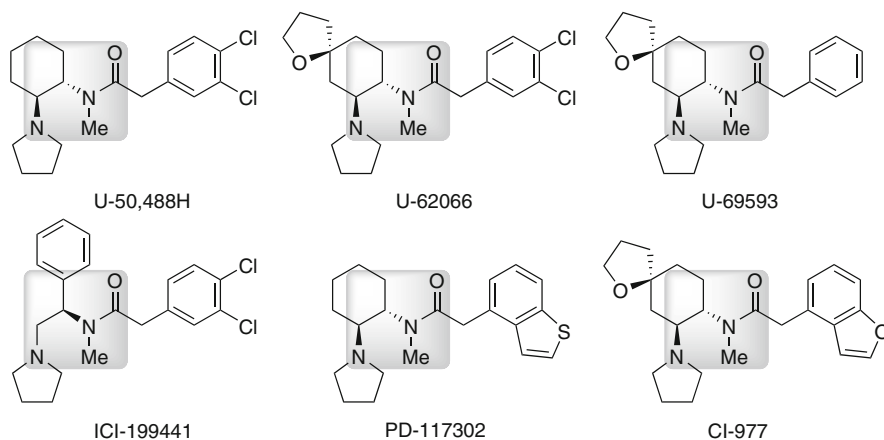


Fig. 4 Structures of κ agonist U-50,488H and its derivatives. Reprinted from [28], with permission from Elsevier. Copyright (2008)

Endomorphin-1(μ) **Tyr**-Pro-Trp-Phe-NH₂

Endomorphin-2(μ) **Tyr**-Pro-Phe-Phe-NH₂

[Met⁵]enkephalin(δ) **Tyr**-Gly-Gly-Phe-Met

[Leu⁵]enkephalin(δ) **Tyr**-Gly-Gly-Phe-Leu

Dynorphin A (κ) **Tyr**-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln

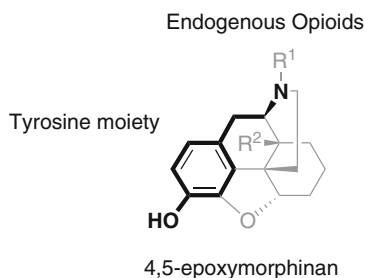


Fig. 5 Structures of 4,5-epoxymorphinan and typical opioid peptides. The tyrosine moiety is shown in *bold*. Reprinted from [28], with permission from Elsevier. Copyright (2008)

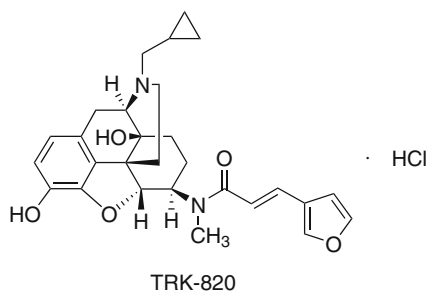


Fig. 6 Structure of κ agonist TRK-820

including dysphoria and psychotomimetic effects. We will describe the design rationale for TRK-820 in Sect. 3.

2 Antitussive δ Opioid Antagonist TRK-851

TRK-851 is a clinical candidate for an antitussive drug; it has a novel, complex morphinan ring system. The development of TRK-851 was motivated by the finding that NTI, a selective δ opioid receptor antagonist, showed antitussive effect. In this section we will describe the process of developing TRK-851, including the structure–activity relationship (SAR) studies on NTI derivatives and the difficulties encountered in overcoming a defect in the metabolism of a prototype clinical candidate, TRK-850.

2.1 Antitussive Effects of Opioid Ligands

It was recently recognized that opioid receptor agonists have antitussive effects [31]. Morphine (Fig. 7) is a potent μ opioid receptor agonist that exhibits marked antitussive effect, and it has been used for the treatment of severe coughs. Codeine (Fig. 7) is one of the most reliable centrally acting antitussive agents; its antitussive

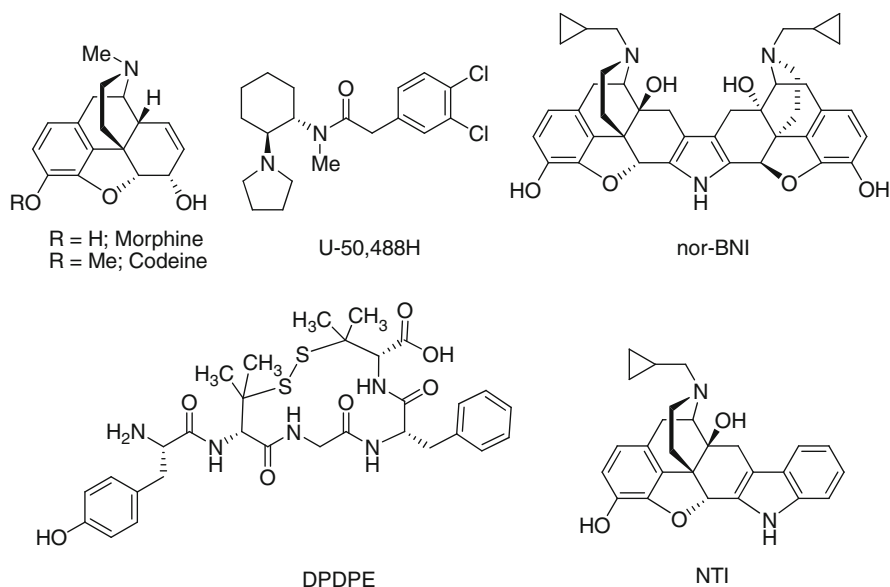


Fig. 7 Structures of opioid receptor ligands that have antitussive effects (agonists: morphine, codeine, U-50,488H; antagonists: NTI), δ opioid agonist DPDPE, and κ opioid antagonist nor-BNI

Table 2 Different opioid receptor ligands and their antitussive effects in rats

Opioid ligand (s)	Antitussive effect
μ Agonist	++
κ Agonist	+
δ Agonist	—
μ Agonist + δ agonist	—
κ Agonist + δ agonist	—
δ Antagonist	+

effect is thought to be derived from the activation of both a codeine specific receptor and the μ opioid receptor, because one of its active metabolites is morphine. Although these narcotic antitussive agents have sufficient therapeutic effects, they also exhibit μ opioid receptor-mediated side effects, *e.g.*, constipation, dependency, and respiratory depression. Therefore, the use of these drugs is limited [31, 32].

The effects of κ and δ opioid receptor activation on the cough reflex have been particularly investigated by Kamei and coworkers. They showed that U-50,488H (Fig. 7), a selective κ opioid receptor agonist, suppressed the cough reflex induced by capsaicin inhalation in rats. This antitussive effect was blocked by coadministration of the κ receptor antagonist, nor-BNI (Fig. 7). This suggested that the κ opioid receptor had mediated the antitussive effects (Table 2) [33].

On the other hand, the δ opioid receptor agonists exhibited activity in contrast to μ and κ agonists. One δ opioid receptor agonist, DPDPE ([D-Pen², D-Pen⁵]enkephalin) (Fig. 7), hardly induced antitussive effect, but it blocked the antitussive effect of morphine. The blockade could be antagonized by the coadministration of NTI (Fig. 7), a selective δ opioid receptor antagonist (Table 2) [34]. The antitussive activity of U-50,488H was also blocked by DPDPE, and that blockade was also antagonized by the coadministration of NTI [35]. These results suggested that activation of the δ opioid receptor suppressed the antitussive activities of μ and κ opioid agonists.

Furthermore, Kamei *et al.* reported that the administration of NTI alone also produced an antitussive effect (Table 2) [36].

2.2 Mechanism of the Antitussive Effect of NTI

Figure 8 shows the mechanism proposed for the antitussive effect of NTI [36]. When the respiratory tract is stimulated by irritants, *e.g.*, xenobiotics, the cough reflex occurs, and the stress of coughing triggers the secretion of endogenous opioid peptides, which may simultaneously stimulate three types of opioid receptors. In a disease state, the stimulation of the δ opioid receptor could block the μ and κ opioid receptor-mediated suppression of the cough reflex, which would result in continual coughing (Fig. 8a). When a selective δ opioid antagonist, *e.g.*, NTI, is administered,

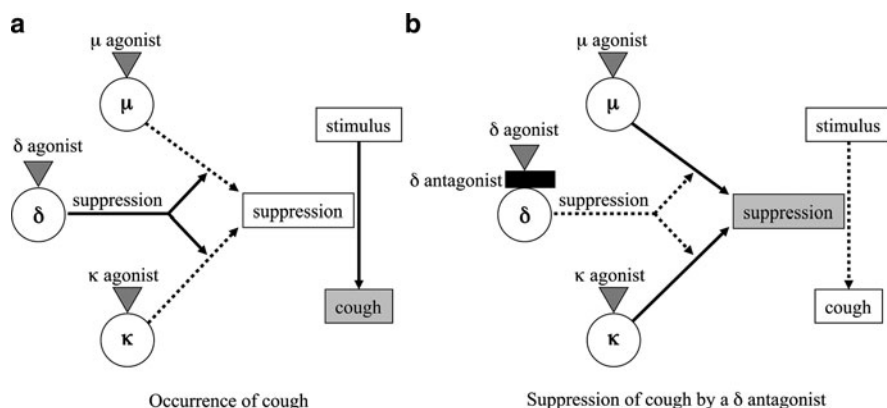


Fig. 8 Possible mechanism of the antitussive effect caused by δ opioid receptor antagonists. *Solid arrows indicate activated pathways; dotted lines indicate inactivated pathways*

it blocks the antagonism by δ agonist to cough suppression derived from the μ and κ receptors, and coughing is suppressed (Fig. 8b).

These observations strongly supported the feasibility of δ opioid receptor antagonists as antitussive drugs that did not have μ opioid receptor-mediated side effects.

2.3 Improvement on the Antitussive Activity of NTI

2.3.1 Rat Cough Model

In order to discuss the SARs of opioid ligands, we must first describe the rat cough model. In this model, rats are exposed to a capsaicin aerosol that induces coughing (Fig. 9) [36]. The coughs are detected as changes in air pressure inside the rat chamber, and the number of coughs can be counted as the number of peaks on the polygraph with careful distinction of coughs from wriggles. The ED_{50} values of NTI injected *via* intraperitoneal (i.p.) and peroral (p.o.) routes were 104 $\mu\text{g}/\text{kg}$ and 1,840 $\mu\text{g}/\text{kg}$, respectively, comparable to those of codeine.

This cough model was used to determine SARs of NTI derivatives. In addition to the antitussive efficacy of compounds, it is possible to assess not only compounds' intrinsic affinity for the receptor but their absorption, distribution, metabolism, and excretion (ADME) properties.

2.3.2 Design and Rationale of NTI

Improving a drug's permeability through the BBB can enhance its concentration in the CNS. Therefore, improving the BBB permeability of NTI might augment its antitussive activity.

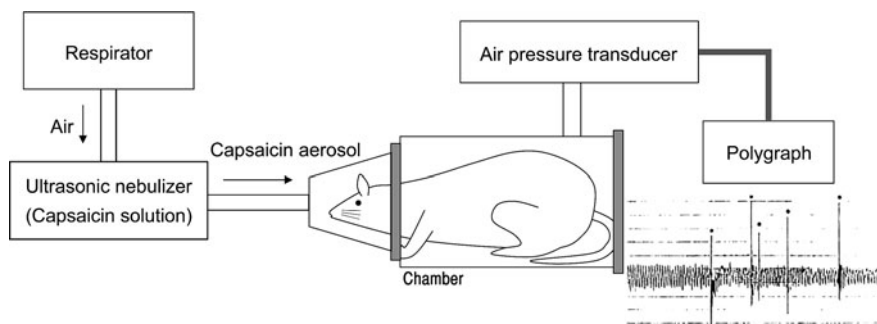


Fig. 9 Rat model of capsaicin-induced coughing

The BBB consists of microcapillary blood vessels that run throughout the brain in close proximity to brain cells [37, 38]. On the inner surface of the capillaries, the endothelial cells are held tightly together to each other to form a monolayer. Centrally acting drugs must permeate through these endothelial cells to penetrate into the brain.

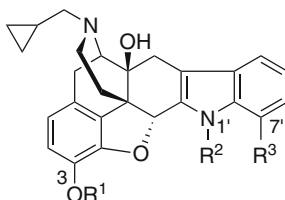
In general, the physicochemical properties of a compound can greatly affect its capacity for passive transcellular BBB permeation. General strategies for modifying drug structures to improve BBB permeability include the following [37, 38]:

1. Reduction of hydrogen bond donors
2. Augmentation of hydrophobicity
3. Reduction of molecular weight

In addition to passive transport, active transport can occur with proteins lodged in the BBB that facilitate uptake and efflux of compounds into and out of the CNS. Therefore, compounds can be modified to enhance their affinity for uptake transporters or reduce their affinity for efflux transporters (*e.g.*, *P*-glycoprotein) to improve BBB permeability [37]. However, these strategies required major structural transformations; thus, we focused on strategies related to changing the physicochemical properties of NTI, as mentioned below.

2.3.3 Introducing Alkyl Substituents into the Indole Ring of NTI

One strategy for improving the BBB permeability of NTI was to augment its hydrophobicity by introducing alkyl substituents into the indole ring. Several NTI derivatives with alkyl substituents were synthesized and tested for potentiation of the antitussive activity. Table 3 presents a comparison of the resulting NTI derivatives. Methylation of the 3-hydroxy group in NTI (compound 1) slightly increased the antitussive activity [39]. In contrast, *N*-methylation at the 1' position of NTI (compound 2) reduced the antitussive activity. However, simultaneous alkylations (compound 3) at both the 3-hydroxy group and the 1' position markedly improved the antitussive activity. Moreover, introduction of a third alkyl substituent at the 7'

Table 3 Antitussive effects of alkyl-substituted NTI derivatives in rats (administered i.p.)

Compound	R ¹	R ²	R ³	ED ₅₀ (μg/kg) ^a	cLog P
NTI	H	H	H	104 (20.3–553)	3.2
1	Me	H	H	63.9 (29.4–139)	3.8
2	H	Me	H	204 (41.8–999)	3.7
3	Me	<i>n</i> -Pr	H	14.8 (4.09–53.8)	5.3
4	Me	Me	Me	6.35 (2.05–19.6)	4.8
5	Me	Et	Me	1.79 (0.67–4.89)	5.3

^aED₅₀ value; the dose that reduces the number of coughs to 50% vs control, expressed as the mean (*n* = 8). Figures in parentheses indicate 95% confidence limits

position in compounds **4** and **5** led to further enhancement of potency by factors of 16 and 58, respectively, compared to the parent compound.

These results suggested that introducing alkyl groups into the indole substructure, especially at the 1' and 7' positions, increased the hydrophobicity of these compounds, which is supported by the calculated log P values (cLog P). This enhanced their BBB permeability and, as a result, the antitussive activities were enhanced. Reducing the number of hydrogen bond donors was another strategy for improving BBB permeability; however, although NTI derivatives **1** and **2** lacked a single hydrogen bond donor, they did not show improved antitussive activity compared to NTI.

2.3.4 NTI Derivatives with an Additional Fused Ring

Among the compounds in Table 3, compound **5** showed the highest antitussive activity; however, it showed a markedly reduced δ receptor antagonist activity in the mouse vas deferens (MVD) assay (Table 4). In the MVD assay, the *Ke* value indicated the potency of the test compound antagonist activity for each receptor type. Compound **5** showed a markedly high *Ke* value (low potency) for the δ receptor compared with that of unmodified NTI [39]. Moreover, compound **5** showed a reduced selectivity for the δ receptor over the μ and κ receptors. This suggested that the antitussive activity of compound **5** may be induced by its active metabolites (*e.g.*, 3-OH analog).

We suspected that the bulky substituents in the indole ring of compound **5** might have caused its reduced affinity for the δ receptor, which resulted in a reduction in δ receptor antagonist activity. Thus, to reduce the bulkiness of the indole ring on

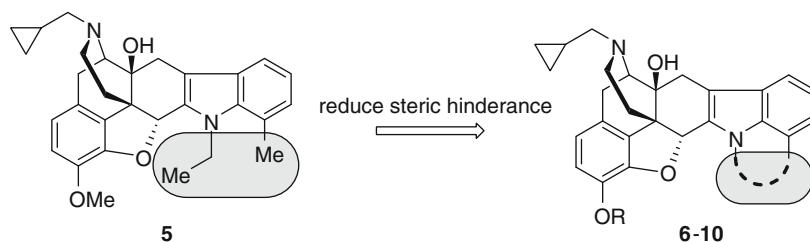
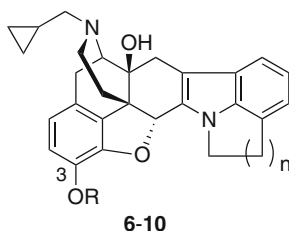


Fig. 10 Additional fused ring design of new NTI derivatives

Table 4 Antagonist effect of NTI and its derivatives (MVD assay)^a



Compound	R	n	<i>Ke</i> (nM) ^b		
			DPDPE (δ)	Morphine (μ)	U-50,488H (κ)
NTI	–	–	0.074	50	2.9
5	Me	–	204	263	213
6	H	1	0.61	17	8.6
7	H	2	0.80	13	28
8	Me	2	6.3	^c	68
9	H	3	3.1	18	53
10	Me	3	333	^d	17

^aDPDPE, morphine, and U-50,488H were used as agonists of δ, μ, and κ opioid receptors, respectively

^bThe *Ke* values were calculated according to the following equation. $Ke = [\text{test compound concentration}]/[\text{dose ratio}-1]$. The dose ratio was the ratio of agonist concentrations that elicited equal responses in the absence and presence of the test compound at increasing concentrations

^cNo antagonism at a test compound concentration of 100 nM

^dNo antagonism at a test compound concentration of 10 nM

compound **5**, we designed and synthesized a new type of NTI derivative, with an additional fused ring system (Fig. 10).

As expected, the fused ring compounds **6–9** exhibited higher δ receptor antagonist activity than compound **5** (Table 4) [39]. In the 3-OMe derivatives, compound **8** (a propylene bridge) showed higher δ antagonist potency than compound **10** (a butylene bridge). In the 3-OH derivatives, the δ antagonist potency order was **6** (an ethylene bridge) \approx **7** (a propylene bridge) $>$ **9** (a butylene bridge). These results strongly suggested that the δ receptor binding affinity was reduced with the

Table 5 Antitussive effects of NTI derivatives that had an additional fused ring structure (administered i.p. in rats)

Compound	ED ₅₀ (μg/kg) ^a	cLog P
NTI	104 (20.3–553)	3.2
	1,830 (890–3,820) ^b	
6	9.14 (4.23–19.8)	3.8
7	8.58 (2.33–31.7)	4.2
8 (TRK-850)	3.43 (0.93–12.6)	4.8
	6.40 (1.54–26.5) ^b	
9	12.3 (3.92–38.3)	4.8
10	9.68 (1.98–47.4)	5.3

^aED₅₀ value; the dose that reduces the number of coughs to 50% vs control, expressed as the mean (*n* = 8). *Figures in parentheses indicate 95% confidence limits*

^bp.o. administration

bulkiness of the indole ring, and a compact fused ring facilitated a higher δ receptor binding affinity.

Table 5 shows the antitussive activities of the fused ring NTI derivatives [39]. All the fused ring compounds **6–10** showed dramatically improved antitussive activity compared to NTI. In particular, the 3-OMe analog **8**, whose methanesulfonic acid salt was selected as a prototype clinical candidate, TRK-850, showed very high antitussive activity; its ED₅₀ value was 30-fold lower than that of NTI. Moreover, when administered p.o., TRK-850 also exhibited a highly potent antitussive effect; its ED₅₀ value was 6.40 μg/kg, or 280 times lower than that of NTI given p.o. The increased hydrophobicity and lower bulkiness of TRK-850 led to potent antitussive effects.

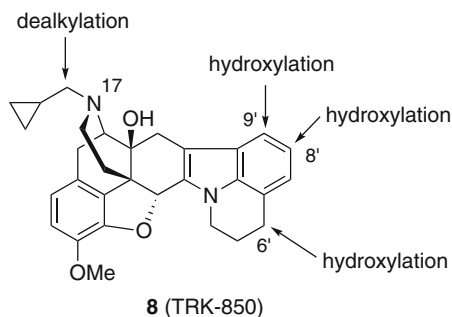
In summary, the introduction of alkyl chains into NTI resulted in increased antitussive activities of these compounds, but it simultaneously reduced the δ receptor antagonist activity. The introduction of an extra fused ring created a compact-sized hydrophobic moiety attached to the indole ring in NTI. This led to a compound, TRK-850, with both high δ receptor antagonism and potent antitussive effects.

2.4 Improvement of Metabolic Defect of TRK-850

2.4.1 Strategies

As mentioned above, TRK-850 had excellent pharmacological properties (potent δ antagonist activity and antitussive effect by p.o. administration). However, a serious defect in TRK-850 was observed during the ADME study. After p.o. administration of tritiated TRK-850 in a rat, the plasma sample showed only a small parent peak with HPLC [³H]-detection. This indicated that TRK-850 was

Fig. 11 Structure of compound **8** (TRK-850) and its main metabolic pathways. Reprinted from [6] with permission from Elsevier. Copyright (2008)



metabolized at an extremely high rate, which later proved to be caused by cytochrome P450 [6]. Therefore, we concluded that TRK-850 was inadequate as a long-lasting drug. The metabolism of TRK-850 was investigated in detail by isolating the main metabolites in the plasma samples of rats that received oral TRK-850. We identified four primary metabolites: 17-decyclopropylmethyl, 9'-hydroxy, 8'-hydroxy, and 6'-hydroxy derivatives (Fig. 11) [6].

On the basis of the structures of the four metabolites, we designed strategies for improving the oxidative metabolic profile of TRK-850.

In general, oxidative metabolism may be prevented with the following strategies [38]:

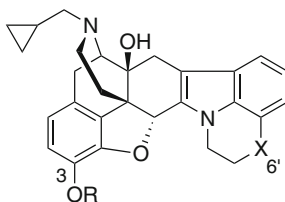
1. Incorporating blocking groups at the labile site.
2. Modifying the labile site with bulky substituents, which prohibit the metabolic enzyme from approaching its substrate through steric hindrance.
3. Reducing the lipophilicity of the compound.
4. Introducing electron-withdrawing groups around the labile site to reduce electron density at the labile site and, consequently, prohibit an oxidative metabolic reaction.

2.4.2 Structure–Metabolic Stability Relationships of TRK-850 Derivatives

Several TRK-850 derivatives were synthesized based on the general strategies mentioned above, and their metabolic stabilities were evaluated in the presence of human liver microsomes. Metabolic rates were determined by incubating the compounds with human liver microsomal enzymes and then quantifying the remaining parent peak by HPLC analysis. The data were expressed as the elimination rate constant (K_{EL}) and the relative metabolic rate, or the K_{EL} of the compound compared with the K_{EL} of TRK-850 (K_{EL} ratio).

Replacement of one of the metabolic sites, the 6'-methylene group of TRK-850, with an oxygen atom or dimethylmethylene group improved the metabolic stability (Table 6) [6].

The derivatization that most effectively lowered the metabolic rate was the demethylation of the 3-methoxy group (Table 6). The conversion of the 3-methoxy

Table 6 Metabolic rates of TRK-850 derivatives in the presence of human liver microsomes

Compound	X	R	Metabolic rate ^a		cLogP
			K_{EL} (min ⁻¹)	$(K_{EL} \text{ ratio})^{-1}$	
8 (TRK-850)	CH ₂	Me	0.341	1	4.8
11	CMe ₂	Me	0.0618	5.51	5.8
12	O	Me	0.0627	5.43	4.2
7	CH ₂	H	0.0249	13.7	4.2
13	CMe ₂	H	0.0758	4.5	5.2
14	O	H	0.0334	10.2	3.6

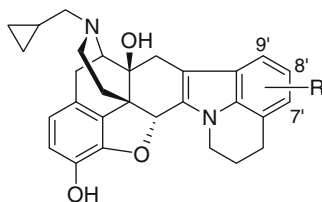
^a K_{EL} , an elimination rate constant calculated from the time course of compound elimination. K_{EL} ratio = (K_{EL} of a given compound)/(K_{EL} of TRK-850)

group of TRK-850 to a hydroxyl group (compound **7**) dramatically improved metabolic stability. This stabilizing effect was also observed in compound **14**. These results might be due to the reduced lipophilicity of these compounds, as suggested by the cLog P values.

Next, we evaluated the metabolic rates of compound **7** derivatives, which had a substituent in the indole benzene ring (**15–28**; Table 7) [6]. Introduction of an electron-withdrawing substituent into the indole moiety tended to suppress the metabolism of a compound (**16**, **18**, **19**, **21–23**, **27**). On the other hand, an electron-donating substituent tended to enhance the metabolism of a compound (**20**, **26**, **28**). These observations suggested that the electron density around the indole moiety was correlated to the cytochrome P450 oxidative metabolic rate. In addition, the position of substitution was important. For example, blocking at the 8'-position suppressed the metabolism more effectively than blocking at the 7'- or 9'-positions.

2.4.3 Antitussive Activities of TRK-850 Derivatives: Journey to TRK-851

The antitussive effects of selected TRK-850 derivatives were evaluated (Table 8) [6]. Incorporation of a substituent into the indole moiety resulted in lower potency than that of compound **7**; nevertheless, some derivatives still exhibited high antitussive activity (**19–21**, **24**, **26**). Incorporation of bulky substituents at the 8'-position also tended to reduce potency (**22**, **23**). Incorporation of fluorine, the smallest substituent with the greatest electron-withdrawing capacity, resulted in the

Table 7 Metabolic rates of compound **7** derivatives in the presence of human liver microsomes

Compound	R	Metabolic rate ^a	
		K_{EL} (min ⁻¹)	$(K_{EL} \text{ ratio})^{-1}$
7	H	0.0249	13.7
15	7'-Cl	0.0290	11.7
16	7'-Br	0.0218	15.6
17	7'-OMe	0.0262	13.0
18	8'-Cl	0.0177	19.2
19	8'-Br	0.0151	22.6
20	8'-OMe	0.0331	10.3
21	8'-F	0.0174	19.6
22	8'-OCF ₃	0.00909	37.5
23	8'-CF ₃	0.0162	21.0
24	9'-Cl	0.0270	12.6
25	9'-Br	0.0371	9.20
26	9'-OMe	0.0285	12.0
27	9'-CF ₃	0.0167	20.4
28	9'-Me	0.0339	10.1

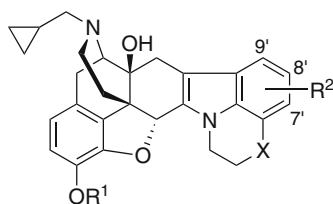
^a K_{EL} , an elimination rate constant calculated from the time course of compound elimination. K_{EL} ratio = (K_{EL} of a given compound)/(K_{EL} of TRK-850)

minimum loss of potency (**21**). Among these derivatives, compound **21** showed the most potent antitussive activity and had a metabolic rate 20 times lower than that of the parent compound, TRK-850. The methanesulfonic acid salt of compound **21** was called TRK-851. Figure 12 shows that p.o. administration of TRK-851 reduced the number of coughs in a dose-dependent manner with an ED₅₀ value of 35.5 µg/kg. Thus, the potency of TRK-851 was over 50-fold higher than that of the starting compound, NTI [6].

The opioid receptor selectivity of TRK-851 was tested in the MVD assay (Table 9). TRK-851 strongly antagonized the agonist activity of DPDPE (δ), and it showed over 200 times greater selectivity for the δ opioid receptor than for µ or κ receptors. These results strongly suggested that the antitussive effect of TRK-851 was derived from its marked antagonism of δ opioid receptors [6].

2.5 Summary

NTI derivatives that possessed hydrophobic substituents were designed and synthesized based on the hypothesis that increased hydrophobicity might improve their

Table 8 Antitussive effects of TRK-850 derivatives in rats (administered s.c.)

Compound	X	R ¹	R ²	ED ₅₀ (μg/kg) ^a
7	CH ₂	H	H	0.20 (0.06–0.6)
8 (TRK-850)	CH ₂	Me	H	8.58 (2.33–31.7) ^b
15	CH ₂	H	7'-Cl	3.43 (0.93–12.6) ^b
16	CH ₂	H	7'-Br	13.8 (0.62–310)
17	CH ₂	H	7'-OMe	27.4 (8.81–85.1)
18	CH ₂	H	8'-Cl	54.2 (18.6–158)
19	CH ₂	H	8'-Br	78.4 (11.5–538) ^b
20	CH ₂	H	8'-OMe	5.05 (2.58–9.86)
21 (TRK-851)	CH ₂	H	8'-F	2.0 (0.4–9.4)
				15.7 (5.31–46.4) ^b
22	CH ₂	H	8'-OCF ₃	>1,000
23	CH ₂	H	8'-CF ₃	>1,000
24	CH ₂	H	9'-Cl	3.5 (1.4–8.7)
25	CH ₂	H	9'-Br	94.6 (5.11–389)
26	CH ₂	H	9'-OMe	8.67 (1.02–73.6)

^aED₅₀ value; the dose that reduces the number of coughs to 50% vs control, expressed as the mean (*n* = 8). Figures in parentheses indicate 95% confidence limits

^bAdministered i.p.

permeability through the BBB. This was expected to increase their antitussive activities compared with that of unmodified NTI. Although the introduction of alkyl groups into the NTI skeleton increased the antitussive activities of these derivatives, it simultaneously decreased the δ opioid receptor antagonist activity. Thus, the antitussive activities of these compounds may be affected more potently by brain exposure of the compounds rather than by their δ antagonist activity.

The introduction of an extra fused ring, a compact-sized moiety, into the indole ring of NTI conferred both suitable hydrophobicity and low steric hindrance. Consequently, compound **8** (TRK-850) was identified as a prototype clinical candidate that exhibited both potent antitussive activity and selective δ receptor antagonist activity.

However, TRK-850 was found to be susceptible to oxidative metabolism by cytochrome P450. The results from structure optimization studies suggested that a drug candidate must have sufficiently low hydrophobicity to prevent a high metabolic rate, but also have sufficiently high hydrophobicity to retain potent antitussive activity. Therefore, additional compact indole substituents that were expected to

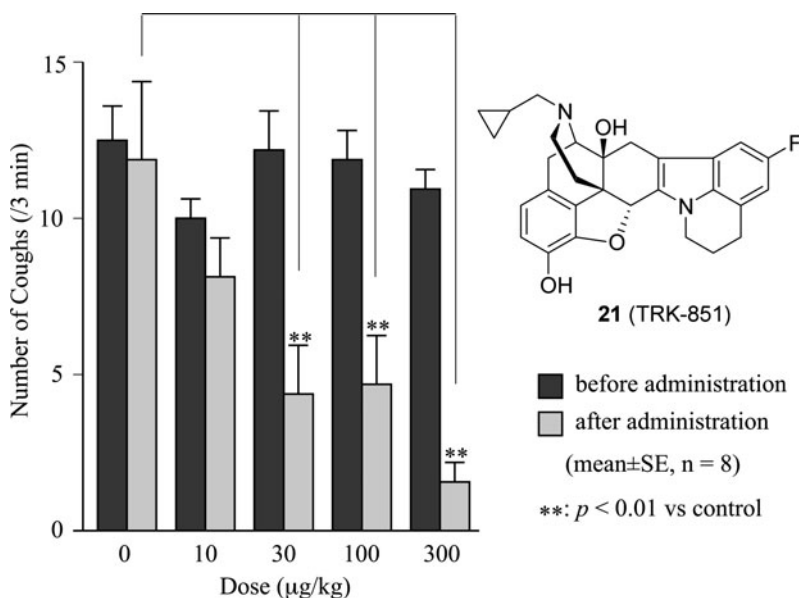


Fig. 12 Antitussive effect of compound **21** (TRK-851) on capsaicin-induced coughing in rats (p.o. administration). The number of coughs over 3 min were counted in the same rats before and 60 min after p.o. administration. The ED_{50} value (the dose that reduced the number of coughs to 50% vs control) for the inhibition of coughing was calculated to be 35.5 µg/kg (95% confidence limits: 16.8–72.9 µg/kg). Reprinted from [6] with permission from Elsevier. Copyright (2008)

Table 9 Compound **21** (TRK-851) antagonism of opioid receptors (MVD assay)^a

Compound	Ke (nM) ^b		
	DPDPE (δ)	Morphine (μ)	U-50,488H (κ)
21 (TRK-851)	1.46	333	^c

^aDPDPE, morphine, and U-50,488H were used as agonists of δ , μ , and κ opioid receptors, respectively

^bThe Ke values were calculated according to the following equation: $Ke = [\text{test compound concentration}]/[\text{dose ratio}-1]$. The dose ratio was the ratio of agonist concentrations that elicited equal responses in the absence and presence of the test compound at increasing concentrations

^cNo antagonism at test compound concentration of 10 nM

metabolize slowly were examined for their antitussive activity. Modifications of TRK-850, including demethylation of the 3-methoxy group and introduction of 8'-fluorine, provided the appropriate hydrophobicity and potent δ antagonist activity. Finally, TRK-851, a potent, selective δ receptor antagonist that metabolized slowly, was identified as a highly potent oral antitussive agent, and was chosen for further clinical evaluation.

3 Antipruritic κ Opioid Agonist TRK-820 (Nalfurafine Hydrochloride)

Nalfurafine hydrochloride is a first-in-class, nonaddictive opioid drug that is used as an antipruritic for severe itching associated with hemodialysis. This drug was launched in Japan in 2009. It has a unique morphinan structure that is rarely observed in typical and traditional opioid κ agonists. The design rationale for this characteristic compound was described in a previous study [7, 28], and would be of interest to researchers in the opioid field. We will summarize the rationale in Sect. 4.

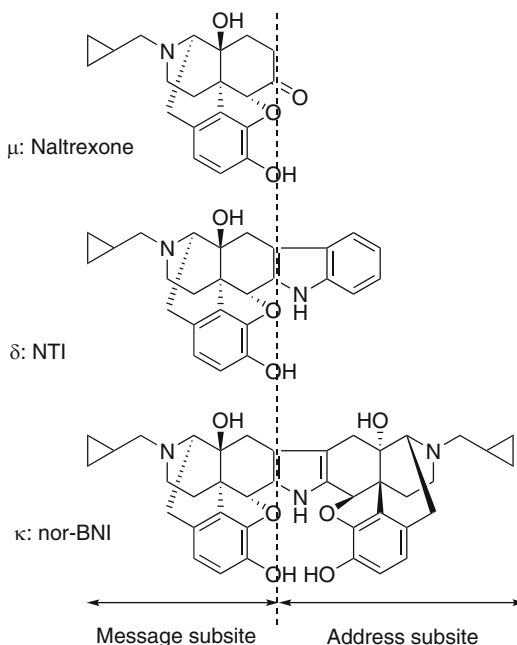
3.1 Design Rationale

Since the Upjohn Group first described the synthesis of U-50,488H (Fig. 4) [27], numerous U-50,488H derivatives have been synthesized and examined for pharmacological effects. However, none of these drugs was appropriate for practical indications. We questioned whether U-50,488H derivatives were actually κ opioid agonists. Therefore, we designed and synthesized a new type of nonpeptide κ agonist with a novel chemical structure. This compound retained the tyrosine moiety in order to reduce the side effects of the prototype κ agonists, including dysphoria and psychotomimetic effects [7, 40].

Portoghesi and colleagues at Minnesota University proposed the “Message-Address Concept” for designing selective δ and κ antagonists (Fig. 13: NTI and nor-BNI) [41, 42]. They pointed out that the 4,5-epoxymorphinan skeleton, defined as the “message” subsite, which is commonly found in NTI and nor-BNI, was necessary for the intrinsic activity of an opioid. The other part of the structure was defined as the “address” subsite, and it is involved in the receptor type selectivity. As shown in Fig. 13, the receptor type selectivity of these opioid antagonists can be regulated by altering the size of the address structure. Although this concept was suitable for designing a selective opioid antagonist, a further strategy was necessary to design an opioid agonist.

The strategy for designing an opioid agonist included a working hypothesis of an “accessory site”. This hypothesis holds that the receptor can change its shape to accommodate the structure of an agonist (“induced fit”) and facilitate agonist binding to the receptor. This change would lead to activating the signal transduction pathway that expressed the effects of the agonist. On the other hand, the antagonist might be considered to be a compound with an extra structural part that interferes with the accommodating changes in the receptor. As a result, the antagonist shows no agonistic effects. The structural moiety that could interfere with the induced fit is called an “accessory site” [43], and it comprises a highly hydrophobic moiety and a sterically hindered structure.

Fig. 13 Message-Address
Concept proposed for opioid
antagonists. Reprinted from
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To design a new agonist, we first attempted to remove the accessory site of nor-BNI and maintain the message and address sites. The message site of nor-BNI, a 4,5-epoxymorphinan skeleton with a cyclopropylmethyl substituent, was considered to be indispensable for opioid activity because it corresponded to the tyrosine of endogenous opioid peptides. Therefore, we postulated that the accessory site of nor-BNI was located in the address subsite of this antagonist.

The meso isomer of nor-BNI, **27** (Fig. 14), showed κ selectivity and antagonist activity similar to those of nor-BNI [44]. Compound **28**, which had a nor-BNI address site, but lacked the phenol ring, also maintained high κ receptor selectivity, similar to that of nor-BNI [45]. These facts indicated that neither the phenol ring nor the hydroxyl group of the ring junction in the address site of nor-BNI were required for κ selectivity or antagonist activity. Based on a comparison between the κ antagonist **28**, naltrexone, and NTI, we proposed that the feature of the address subsite that was essential for receptor selectivity was the length of the address site (Fig. 14). From this point of view, the design of a κ selective agonist required the removal of the accessory site from compound **28**, but the length of the address site must be maintained for binding the κ receptor. On the basis of that concept, we designed and synthesized compounds (**A**) that had a C6 (carbon atom in the 6-position) and an X (a hetero atom) with a single bond (Fig. 14). The single bond was expected to give structural flexibility to the ligand, which would facilitate the ability of the receptor to assume an induced fit for binding the agonist. Based on these design principles, a number of compounds were synthesized (**29–33**) with the proper side chain structure, size, and length (address subsite) for a κ agonist activity.

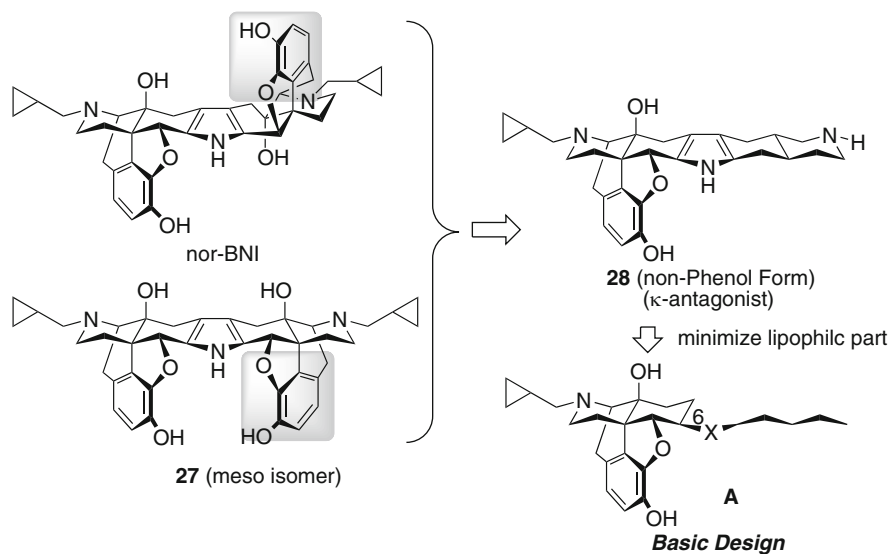


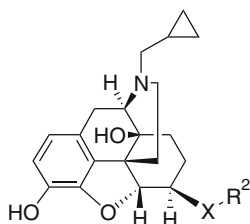
Fig. 14 Basic design of a new chemical class opioid κ agonist. Reprinted from [28] with permission from Elsevier. Copyright (2008)

3.2 Pharmacology and Medicinal Chemistry

Table 10 shows the results of screening for the most appropriate X spacer for κ agonist activity. The side chain was fixed at a 6-carbon length, which is similar to the size of the address site of nor-BNI. Opioid receptor selectivity and agonistic activity were evaluated with the MVD assay [46]. The agonist potencies were expressed as IC_{50} values. The Ke value indicated the receptor selectivity of test agonists. After detecting the opioid receptor agonist activity of test compounds *in vitro*, the acetic acid writhing (AAW) test [47] was conducted in mice to estimate the *in vivo* potency. The analgesic effects of test compounds could be effectively antagonized by pretreatment with the opioid κ antagonist, nor-BNI.

Almost all the compounds showed similar agonistic activity in the MVD assay. Although KT-90 and KT-95 (Fig. 15) [48–50] which had thioester structures, reportedly showed opioid κ agonist activity, compound 31, also with a thioester, showed almost no agonistic activity *in vitro* or *in vivo*. This finding revealed the importance of the chemical structure of the “address” site of the analog. Amide compounds, particularly the *N*-methyl amido compound 32b, showed the most potent agonistic activity in the AAW test and the highest κ selectivity in the MVD assay.

Table 11 shows that both κ agonistic activity and selectivity were affected by the introduction of an unsaturated bond and a lipophilic moiety into the *N*-methyl amide side chain. Often, the binding affinity or agonistic activity could be increased by introducing an unsaturated bond into the side chain. This might fix a favorable

Table 10 SAR studies on the variation of the spacer X

Compound	X-R ²	MVD ^a				AAW, ED ₅₀ (mg/kg) ^j		
		IC ₅₀ (nM)	Ke (nM) ^b			s.c.	p.o.	p.o./s.c.
			μ ^c	δ ^e	κ ^h			
29	O-C ₆ H ₁₃	16	4.5	1.5	0.12	1.8	ND ^k	ND ^k
30	S-C ₆ H ₁₃	38% inhibition (1 μM)				16	ND ^k	ND ^k
31	S(CO)-C ₅ H ₁₁	Not effective				Not effective		
32a	NH(CO)-C ₅ H ₁₁	2.0	14	NC ^f	0.3	0.29	ND ^k	ND ^k
33	NH-C ₆ H ₁₃	25	4.4	2.3	0.26	25% inhibition (10 mg/kg)		
32b	NMe(CO)-C ₅ H ₁₁	15	UD ^d	90 ^g	0.046 ⁱ	0.77	0.63	8.2

^aData were expressed as the mean ($n = 4-9$)

^bThe Ke values were calculated from the following equation: $Ke = [\text{antagonist}]/(\text{IC}_{50} \text{ ratio}-1)$; [antagonist]: concentration of the antagonist; IC₅₀ ratio: the IC₅₀ without the antagonist over the IC₅₀ with the antagonist. Each preparation was incubated with a selective antagonist for 20 min before the addition of a test compound

^cNaloxone (30 nM) was used as a μ selective antagonist

^dUndetectable (IC₅₀ did not change with up to 100 nM of naltrexone)

^eNTI (10 nM) was used as a δ selective antagonist

^fNot calculated (the IC₅₀ ratio was too small to calculate the Ke value)

^gNTI (100 nM) was used

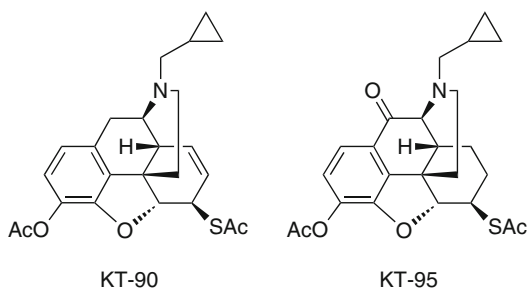
^hnor-BNI (10 nM) was used as a κ selective antagonist

ⁱnor-BNI (20 nM) was used

^jData were expressed as the mean ($n = 10$)

^kNot determined

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**Fig. 15** Structures of KT-90 and KT-95

conformation (Fig. 16) and may contribute to π - π interactions with the receptor. We also attempted to optimize the length of the side chain. In the course of these SAR studies, we found an attractive lead compound, **32f** (Fig. 16; Table 11),

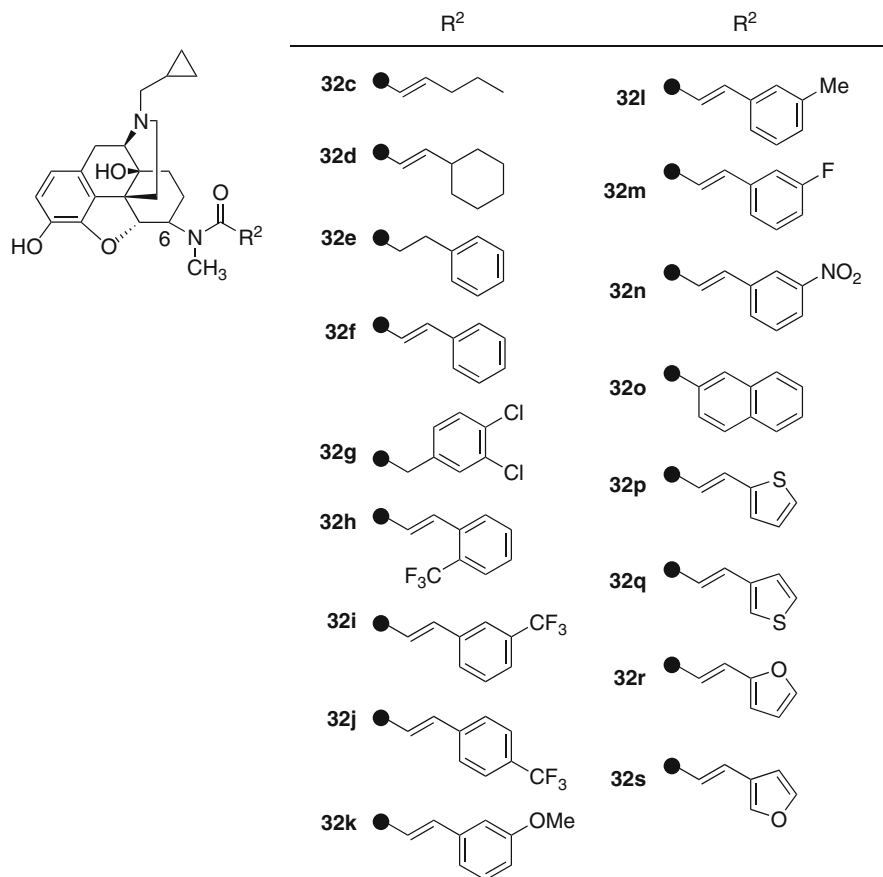


Fig. 16 Potential κ agonist structures: 6-amide derivatives of compound **32b**. Reprinted from [28] with permission from Elsevier. Copyright (2008)

a 6 β -cinnamamido derivative. Compound **32f** showed high selectivity for the κ receptor in the MVD assay and significant *in vivo* κ agonist potency in the AAW test, even with p.o. administration. Furthermore, the agonist effect of **32f** showed adequate duration in the *in vivo* test. However, **32f** had significant side effects.

Therefore, a lead optimization study was performed by focusing on the stereochemistry, length, and aromatic ring structure of the side chain. A number of **32f** derivatives were synthesized by condensing α - and β -*N*-methylnaltrexamine with various carboxylic acids (Fig. 16; Table 12). Compounds α - and β -**32g** were considered the simple combination of a morphinan structure with U-50,488H, because U-50,488H had the same side chain (3,4-dichlorophenylacetamido). However, their analgesic activities, especially when administered p.o., were neither potent nor long lasting. Both the compounds that were simple derivations of the

Table 11 SAR study on the variation of the side chain R² (structures shown in Fig. 16)

Compound	MVD ^a				AAW, ED ₅₀ (mg/kg) ⁱ			
	IC ₅₀ (nM)	Ke (nM) ^b			s.c.	p.o.	p.o./s.c.	Duration ^j
		μ ^c	δ ^e	κ ^g				
32b	15	UD ^d	89.6 ^f	0.046 ^h	0.77	0.63	8.2	ND ^k
32c	0.65	1,797	UD ^d	0.072	0.024	0.21	8.8	S
32d	0.46	5,713	143	1.08	0.004	0.071	17.0	L
32e	0.019	UD ^d	188	0.008	0.040	0.29	7.3	S
32f	0.17	922	263	0.861	0.002	0.011	5.5	L

^aData were expressed as the mean ($n = 4-9$)^bThe Ke values were calculated from the following equation: $Ke = [\text{antagonist}]/(\text{IC}_{50} \text{ ratio}-1)$; [antagonist]: concentration of the antagonist; IC₅₀ ratio: IC₅₀ without the antagonist over the IC₅₀ with the antagonist. Each preparation was incubated with a selective antagonist for 20 min before the addition of a test compound^cNaloxone (30 nM) was used as a μ selective antagonist^dUndetectable^eNTI (10 nM) was used as a δ selective antagonist^fNTI (100 nM) was used^gnor-BNI (10 nM) was used as a κ selective antagonist^hnor-BNI (20 nM) was usedⁱData were expressed as the mean ($n = 10$)^jDose that inhibited 90% of the AAW behavior at 30 min after test compound administration (s.c.). AAW test was performed at time points of 60, 120, 180, and 240 min postadministration. S (Short): effect decreased within 120 min; L (Long): effect continued for 180 min or more^kNot determined

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U-50,488H chemical structure proved to be unsuccessful. In the course of the lead optimization process, we found the two following tendencies: (1) compounds with a 6β-amido structure showed a low s.c./p.o. potency ratio and a long p.o. duration in the AAW test and (2) the aromatic ring structure in the C6-side chain was one of the most important components for optimizing the pharmacological profile, particularly for attenuating the psychotomimetic effect.

Finally, we found that the compound with a furanacrylamido side chain, β-**32s**, had the best properties. Thus, the pharmacological profile of compound β-**32s**, or TRK-820, was examined in detail. The agonist potency of β-**32s** was 6,000-fold and 1,800-fold stronger than that of morphine in the guinea pig ileum (GPI) and MVD preparations, respectively (Table 13) [28]. Compared to U-50,488H, TRK-820 was approximately 140 times and 30 times more potent in the GPI and MVD assays, respectively.

We also evaluated TRK-820 for its antinociceptive effects in the AAW and tail flick (TF) tests in mice. TRK-820 showed high antinociceptive activity in both tests (Table 13) [28], with ED₅₀ values of 0.0033 mg/kg (AAW) and 0.062 mg/kg (TF). These activities were 85–175 times more potent than that of morphine and 80–350 times more potent than that of U-50,488H. This analgesic effect was antagonized by nor-BNI (κ antagonist) but not by NTI (δ antagonist) or by low-dose naloxone (μ antagonist). Furthermore, TRK-820 was notable for its low psychotomimetic effects. In contrast, morphine showed a significant preferential (addictive) effect

Table 12 SAR study on the variation of the side chain configuration and R^2 (structures shown in Fig. 16)

Compound	6- α or 6- β	MVD ^a				AAW, ED ₅₀ (mg/kg) ^h			
		IC ₅₀ (nM)	Ke (nM) ^c			s.c.	p.o.	p.o./s.c.	Duration ⁱ
			μ ^d	δ ^f	κ ^g				
32f	β	0.166	922	263	0.861	0.002	0.011	5.5	L
	α	0.12	16.5	0.43	4.90	0.0058	0.23	39.3	L
32g	β	17.0	17,000	1,113	1.37	0.46	17.99	39.1	ND ^b
	α	0.40	53.0	17.1	0.55	0.017	0.83	48.0	S
32h	β	ND ^b	–	–	–	0.18	ND ^b	–	ND ^b
32i	β	0.47	UD ^e	291	3.20	0.0046	0.034	7.4	ND ^b
32j	β	0.07	524	78.0	0.20	0.023	0.026	1.1	L
32k	β	0.75	60.9	96.4	1.60	0.0011	0.0048	4.4	L
32l	β	0.32	1,778	64.5	1.95	0.0049	0.064	9.7	L
32m	β	1.60	UD ^e	276	2.37	0.0019	0.0070	3.7	L
32n	β	0.23	185	89.0	1.40	0.017	0.220	12.9	ND ^b
32o	β	ND ^b	ND ^b	ND ^b	ND ^b	3.6	ND ^b	–	ND ^b
32p	β	0.10	UD ^e	89.6	0.28	0.0027	0.026	9.6	L
32q	β	0.10	UD ^e	UD ^e	1.71	0.0042	0.040	9.5	L
32r	β	0.55	UD ^e	UD ^e	0.20	0.0016	0.015	9.4	L
32s	α	0.049	31.9	245	0.91	0.0081	0.13	16.0	ND ^b
	β	0.42	14,000	41.6	0.16	0.0033	0.032	9.7	L

^aData were expressed as the mean ($n = 4-9$)^bNot determined^cThe Ke values were calculated from the following equation: $Ke = [\text{antagonist}]/(\text{IC}_{50} \text{ ratio}-1)$. [antagonist]: concentration of the antagonist; IC₅₀ ratio:IC₅₀ without the antagonist over IC₅₀ with the antagonist. Each preparation was incubated with a selective antagonist for 20 min before the addition of a test compound^dNaloxone (30 nM) was used as a μ selective antagonist^eUndetectable^fNTI (10 nM) was used as a δ selective antagonist^gnor-BNI (10 nM) was used as a κ selective antagonist^hData were expressed as the mean ($n = 10$)ⁱDose that could inhibit 90% of the AAW behavior at 30 min after test compound administration (s.c.). AAW test was performed at time points of 60, 120, 180, and 240 min postadministration. S (Short): effect decreased within 120 min; L (Long): effect continued for 180 min or more
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and U-50,488H appeared to induce an aversive (dysphoric) behavior in test animals. It was concluded that TRK-820 had a neutral effect (neither preferential nor aversive effect) on behavior and, thus, it was expected to cause less psychotomimetic side effects [25].

Next, we attempted to clarify which part of the TRK-820 structure was associated with its excellent pharmacological profile. The AAW test (s.c.) in mice was selected to evaluate structure–activity and structure–ADME relationships. Table 14 shows a summary of the results of a SAR study on compound TRK-820 derivatives (34–42).

Although the main factor that determined activity on the AAW test remains to be clarified, these data implied the importance of the followings: (1) the tyrosine components (17-nitrogen and the 3-hydroxy group) were indispensable for the

Table 13 Detailed comparison of compound TRK-820 (β -32s) with morphine and U-5,0488H

Compound	GPI			MVD			AAW (s.c.)		TF (s.c.)
	IC ₅₀ ^a (nM)	K _e (nM) ^b		IC ₅₀ ^a (nM)	K _e (nM) ^b		μ/κ	δ/κ	
		κ	μ		κ	μ			
β-32s	0.0081	0.070 ^c	5.5 ^f	0.080	0.049 ^c	48 ^f	980	–	0.0033
Morphine	49.3	20.5 ^d	5.06 ^g	145.1	53.5 ^d	3.29 ^g	0.06	0.14	0.58
U-50,488H	1.12	0.031 ^c	12.1 ^f	2.35	0.031 ^e	31.5 ^f	1016	1329	1.16
									5.18

^aData were expressed as the mean ($n = 5-7$)

^bThe K_e values were calculated from the following equation: $K_e = [\text{antagonist}]/([\text{IC}_{50} \text{ ratio}-1])$. [antagonist]: concentration of the antagonist; IC_{50} ratio: IC_{50} without the antagonist over IC_{50} with the antagonist. Each preparation was incubated with a selective antagonist for 20 min before the addition of a test compound

^cnor-BNI (10 nM) was used as a κ selective antagonist

^dnor-BNI (100 nM) was used as a κ selective antagonist

^enor-BNI (1 nM) was used as a κ selective antagonist

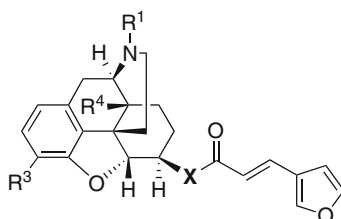
^fNaloxone (100 nM) was used as a μ selective antagonist

^gNaloxone (20 nM) was used as a μ selective antagonist

^hNTI (100 nM) was used as a δ selective antagonist

ⁱNot calculated

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Table 14 SAR study on compound TRK-820 derivatives

Compound	R ¹	R ³	R ⁴	X	MVD ^a				AAW, ED ₅₀ (mg/kg) ^b
					IC ₅₀ (nM)	K _e (nM) ^c			
						μ ^d	δ ^f	κ ^g	
TRK-820	CPM	OH	OH	NMe	0.42	14,000	41.6	0.16	0.0033
34	Me	OH	OH	NMe	55	8.1	33	6.3	1.03
35	H	OH	OH	NMe	ND ^b	—	—	—	7.07
36	Phenethyl	OH	OH	NMe	ND ^b	—	—	—	1.03
37	CPM	H	OH	NMe	27.2	12.0	8.40	0.07	0.013
38	CPM	NH ₂	OH	NMe	ND ^b	—	—	—	0.20
39	CPM	NHAc	OH	NMe	ND ^b	—	—	—	>10
40	CPM	OH	H	NMe	0.035	8.04	1.31	0.07	0.02
41	CPM	OH	OH	NH	0.87	UD ^c	8,100	0.44	0.95
42	CPM	OH	OH	S	ND ^b	—	—	—	>10

^aData were expressed as the mean ($n = 10$)^bNot determined^cThe K_e values were calculated with the following equation: $K_e = [\text{antagonist}]/(\text{IC}_{50} \text{ ratio} - 1)$. [antagonist]: concentration of the antagonist; IC₅₀ ratio: IC₅₀ without the antagonist over IC₅₀ with the antagonist. Each preparation was incubated with a selective antagonist for 20 min before the addition of a test compound^dNaloxone (30 nM) was used as a μ selective antagonist^eUndetectable^fNTI (10 nM) was used as a δ selective antagonist^gnor-BNI (10 nM) was used as a κ selective antagonist^hData were expressed as the mean ($n = 4-9$)

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in vivo activity of TRK-820 and (2) the 17-cyclopropylmethyl, 14-hydroxy, and *N*-methyl substituents were also important for its agonist activity.

3.3 Clinical Development of TRK-820 in Postoperative Surgery

TRK-820 was first developed as an analgesic for postoperative pain. However, in a Phase II clinical trial, it was concluded that, although the analgesic effect could be sufficiently potent, the safety margin was found to be insufficient. During that study, a physician suggested that patients who received a μ opioid receptor

agonist, like morphine often complained of an intolerable itch; in contrast, patients who were treated with TRK-820 rarely complained of itching. In addition to this pharmacological observation, μ opioid agonists and κ opioid agonists often showed contrary effects; for example, a preference for an aversive stimulus or hyperactivity in response to sedation. These findings led us to estimate the antipruritic effect of TRK-820. After confirming a significant antipruritic effect of TRK-820 in various animal itching models established by Kuraishi *et al.* [51–58], we initiated another clinical development study with the intent to develop an antipruritic agent.

3.4 Clinical Development of TRK-820 as an Antipruritic Agent

3.4.1 Antipruritic Activity of TRK-820 in an Animal Model

Epidural or intrathecal (i.t.) administration of morphine produced an itching sensation in humans [59, 60]. It has been reported that intradermal (i.d.) injections of histamine or substance P and intracisternal (i.c.) injections of morphine induced scratching in mice [61–63]. Scratching behavior in mice is considered to represent the itching sensation observed in humans. The administration of TRK-820 (p.o.) inhibited the scratching behavior in mice induced by histamine (i.d.), with an ED_{50} of 7.3 $\mu\text{g/kg}$ (Fig. 17a) [51, 54, 57]. Moreover, TRK-820 (p.o.) inhibited substance P (i.d.) induced scratching behavior in mice (ED_{50} = 19.6 $\mu\text{g/kg}$, p.o.), which was resistant to treatment with antihistamine drugs (chlorpheniramine and ketotifen) (Fig. 17b) [51, 54, 57]. The antipruritic effect of TRK-820 was antagonized by nor-BNI (Fig. 18) [51, 54]. Based on these studies, TRK-820 was expected to be a more effective antipruritic than antihistamines. Furthermore, TRK-820 inhibited morphine (i.c.) induced scratching behavior in mice, but ketotifen did not [52, 54, 57]. This indicated that TRK-820 might also have an antipruritic effect on antihistamine-resistant pruritus of CNS origin. In addition, scratching behavior in monkeys induced by morphine (i.v. or i.t. injections) could be attenuated by i.v. or intramuscular injections of TRK-820 [53, 58]. TRK-820 was also reported to exhibit antipruritic effects on scratching induced by compound 48/40 [56] or chloroquine [55], and itching observed in disease models for autoimmune disease [64] or cholestasis induced by chronic ethynylestradiol injections [65].

3.4.2 Clinical Effectiveness on Uremic Pruritus

Patients on hemodialysis often complain of an intractable pruritus (uremic pruritus). Indeed, the consequent sleep disorder can impair the quality of life. Clinical and preclinical data suggested that the μ opioid system induced itching, but not the κ system. The antipruritic efficacy of nalfurafine hydrochloride (general name of TRK-820) was demonstrated for patients on hemodialysis in two multicenter, randomized, double-blind, placebo-controlled, clinical studies in Europe [66].

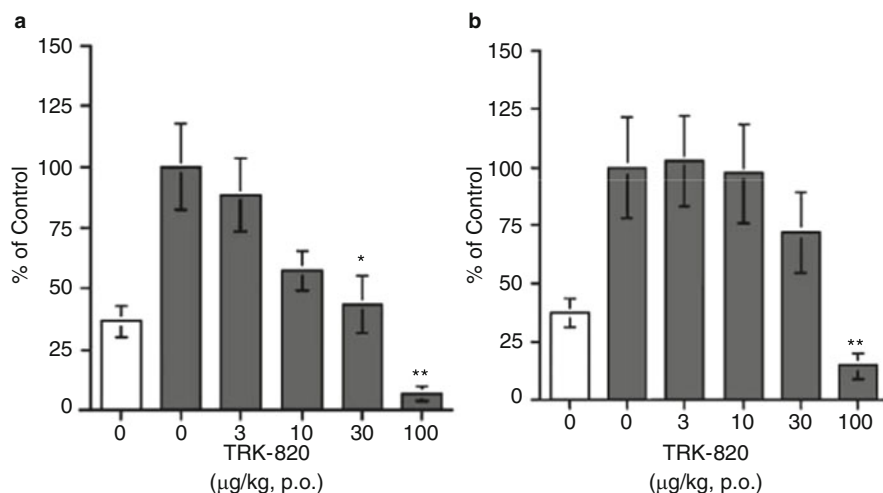


Fig. 17 Effects of TRK-820 on the scratching behavior in mice induced by histamine (a) or substance P (b). Mice (ICR strain) were given a p.o. administration of vehicle (*open bars*) or TRK-820 (*filled bars*). Then, 30 min later, phosphate-buffered saline, pH 7.4 (PBS), histamine (a), or substance P (b) was i.d.-injected. Immediately after the injection of PBS, histamine, or substance P, the number of scratching events was recorded and counted over a 30-min period. The numbers of scratching events observed with histamine or substance P injections in the absence of TRK-820 were considered 100% on the vertical axes. Each value represents the mean \pm standard error of the mean (S.E.M.) ($n = 8$). ** $P < 0.01$ compared between the PBS and histamine- or substance P-injected groups (Dunnett's test). Reprinted from [51] with permission from Elsevier. Copyright (2002)

Recently, Kumagai and colleagues also published results that clearly showed that nalfurafine hydrochloride was an effective treatment for itching in humans [67, 68]. The effects of the drug on severe itching in patients on hemodialysis was evaluated in a Phase III, randomized, double-blind, placebo-controlled study in Japan. In that study, the efficacy and safety of nalfurafine hydrochloride were prospectively investigated in 337 patients on hemodialysis with itching that was resistant to currently available treatments, *e.g.*, antihistamines. They randomly (1:1:1) administered 5 μ g (high dose) or 2.5 μ g (low dose) of the drug or a placebo orally for 14 days in a double-blind study design. The primary endpoint was the mean decrease in the visual analog scale (VAS)² from baseline. Decreases in the VAS were significantly larger in both the high dose ($n = 114$) and the low dose ($n = 112$) groups compared to the placebo group ($n = 111$, $P = 0.0002$ and $P = 0.0001$, respectively; one-sided test at 2.5% significance level; Fig. 19). The incidence of adverse drug reactions (ADRs) was 35.1%, 25.0%, and 16.2% in the

² The VAS test consisted of a 100-mm horizontal line without scale markings. The patients were asked to mark the intensity of itching on the scale, where the right end of the line (100 mm) indicated the strongest possible itching and the left end (0 mm) indicated no itching. The VAS has been widely accepted as a quantitative index of subjective sensations like pain and itching.

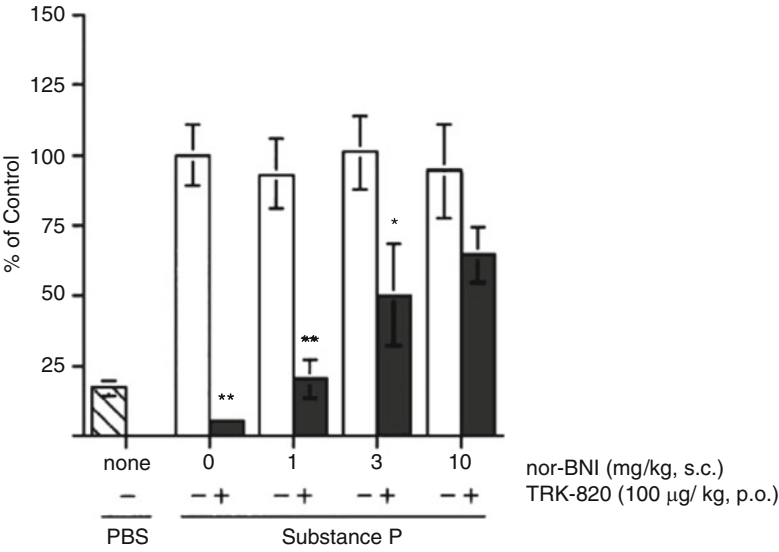


Fig. 18 Effect of nor-BNI on the antipruritic effect of TRK-820. Mice (ICR strain) were given an s.c. injection of nor-BNI or PBS (*hatched bar*). The next day, mice were given a p.o. administration of 100 µg/kg of TRK-820 (*filled bars*) or vehicle (*open bars*), and 30 min later, either PBS (*hatched bar*) or substance P was i.d. injected. Immediately after the latter, the number of scratching events was recorded and counted over a 30-min period. The number of scratching events recorded with substance P in the absence of nor-BNI and TRK-820 was considered to be 100% on the vertical axis. Each value represents the mean \pm S.E.M. ($n = 8$). * $P < 0.05$, ** $P < 0.01$ when compared with the corresponding vehicle-injected group (unpaired t -test). Reprinted from [51] with permission from Elsevier. Copyright (2002)

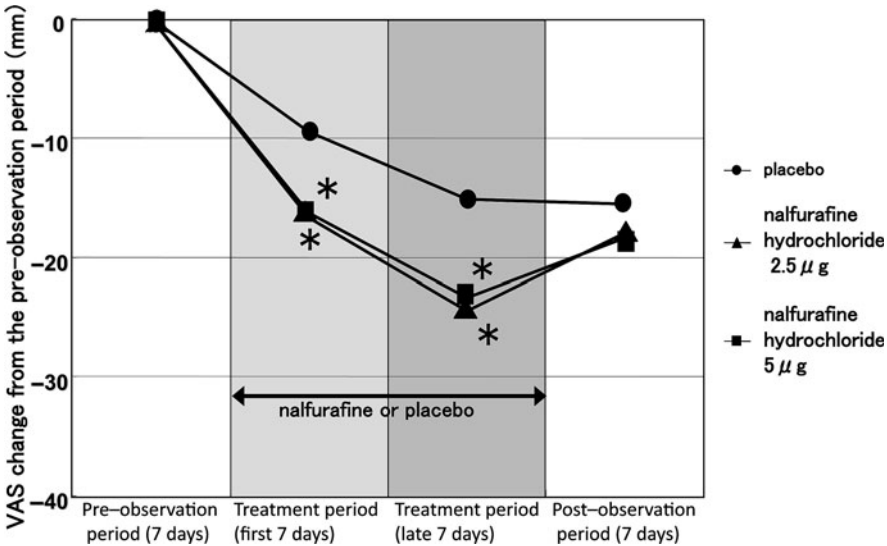


Fig. 19 Changes in VAS values from the preobservation period. All symbols show the mean values of VAS changes. * $P < 0.025$ compared with the placebo group, one-sided ANCOVA

high dose, low dose, and placebo groups, respectively. Mild to moderate ADRs were observed in 10 of the 226 patients, and the most common was insomnia (sleep disturbance), observed in 24 of the 226 patients. However, because all the ADRs were transient and readily resolved, nalfurafine hydrochloride could be considered a safe agent. This Phase III study clearly showed that oral nalfurafine hydrochloride effectively reduced uremic pruritus with few significant ADRs.

After the Phase III study, a 1-year open-label study showed that nalfurafine hydrochloride (5 μ g, p.o.) provided antipruritic effects for 211 patients on hemodialysis with itching that was resistant to currently available treatments. These results suggested that tolerance to the antipruritic effect of the agent was not observed, at least after 1 year of treatment. It is noteworthy that nalfurafine hydrochloride exhibited neither physical nor psychological dependency [68].

This novel drug was officially approved for clinical use in January 2009 by the Ministry of Health, Labour, and Welfare of Japan. This is the first pharmaceutical success ever achieved for a selective opioid κ agonist. All the other compounds derived from modifications of U-50,488H have been withdrawn or halted in early clinical phase development. Furthermore, nalfurafine hydrochloride is recognized as the first nonnarcotic opioid drug in history.

3.5 Concluding Remarks

The development of opioid drugs has been considered a high-risk endeavor. Indeed, it was expected to be particularly tough because the goal was to discover an opioid drug without addictive properties. Because opioids have potent analgesic, antitussive, antipruritic, and antiurinary incontinence effects, much more research is expected to be undertaken in order to produce many ideal drugs. The success of TRK-820 will contribute to the initiation of new and creative studies for discovering clinically applicable opioid compounds.

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