Structure−Antitussive Activity Relationships of Naltrindole Derivatives. Identification of Novel and Potent Antitussive Agents

Satoshi Sakami,[†] Masayuki Maeda,[†] Koji Kawai,[†] Takumi Aoki,[†] Kuniaki Kawamura,[†] Hideaki Fujii,[†,§] Ko Hasebe,[†] Mayumi Nakajima,[†] Takashi Endo,[†] Shinya Ueno,[†] Tsuyoshi Ito,[†] Junzo Kamei,[‡] and Hiroshi Nagase*[,†,§]

Pharmaceutical Research Laboratories, Toray Industries, Inc., 6-10-1 Tebiro, Kamakura, Kanagawa 248-8555, Japan, and Department of Pathophysiology & Therapeutics, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-chome, Shinagawa-ku, Tokyo 142-8501, Japan

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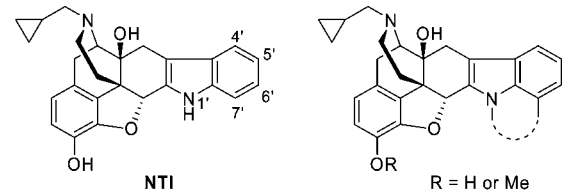
We have previously reported antitussive effects of naltrindole (NTI), a typical δ opioid receptor antagonist, in a rat model. The ED_{50} values of NTI by intraperitoneal and peroral injections were 104 μg/kg and 1840 μg/kg, respectively, comparable to those of codeine. Codeine, one of the most reliable centrally acting antitussive drugs, has μ agonist activity and thus the same side effects as morphine, e.g., constipation, dependency, and respiratory depression. Because NTI is a δ opioid antagonist, its derivatives have potential as highly potent antitussives, free from the μ opioid agonist side effects. We attempted to optimize the NTI derivatives to develop novel antitussive agents. On the basis of the studies of structure−antitussive activity relationships of alkyl substituted NTI derivatives, we designed NTI derivatives with extra ring fused structures. As a clinical candidate, we identified a highly potent new compound, (5*R*,9*R*,13*S*,14*S*)-17-cyclopropylmethyl-6,7-didehydro-4,5-epoxy-5′,6′-dihydro-3-methoxy-4′*H*-pyrrolo[3,2,1-*ij*]quinolino[2′,1′:6,7]morphinan-14-ol (**5b**) methanesulfonate (TRK-850) which was effective even by oral administration (ED_{50} 6.40 μg/kg).

Introduction

Three types of opioid receptors (μ, δ, and κ) are now well established based not only on pharmacological studies but also on molecular biological investigations,[1–3] and a number of nonpeptide opioid receptor ligands have been developed as either drug candidates or pharmacological tools.[4–6] The antitussive effects of the μ and κ opioid agonists have also been well recognized.[7–9] In addition, the δ opioid receptor may counteract the antitussive processes that are mediated by the μ and κ opioid receptor.[10,11] On the basis of these observations, we previously reported that naltrindole (NTI)[a] (Chart 1), a typical δ opioid antagonist,[12–14] exerted a marked and long-lasting antitussive effect in mice and rats.[15] We also demonstrated that NTI suppressed the cough reflex mainly by functioning as a δ opioid antagonist and that the antitussive effect of NTI resulted from the antagonism of the δ opioid receptor-mediated internal μ and κ opioid inhibitory system for the antitussive process.[15,16] These results directly supported the feasibility of the development of δ opioid antagonists as antitussives.

In contrast to our research results, SmithKline Beecham has described a δ opioid agonist with an antitussive effect.[17] This discrepancy can be explained hypothetically by the observation that the δ_1 and δ_2 pharmacological subtype receptors[18] affect the cough response through opposing mechanisms. The δ_2 receptor agonist may suppress the cough reflex in the same manner as the μ or κ receptor agonists do, whereas the δ_1 agonist inhibits the cough suppressive effects of the μ or κ receptor agonists.[19] Alternatively, the discrepancy in the pharmacological effects attributed to the opioid receptor subtypes, as described above, may in fact arise from activation of opioid receptor heterodimers.[20,21]

Chart 1. Structures of NTI and Its Derivatives with an Extra Fused Ring



NTI was designed and synthesized based on a message-address concept based on the endogenous δ opioid peptide enkephalin, with the structure (Tyr-Gly-Gly-Phe-X).[12] The morphinan structure, the pyrrole ring, and the benzene ring of NTI mimic ^{1}Tyr-^{2}Gly as a message, ^{3}Gly as a spacer, and ^{4}Phe as an address, respectively. We proposed that indolomorphinan is a good starting structure to synthesize new and potent antitussive products by conserving both the δ antagonist and antitussive activities of NTI. Therefore, we investigated the structure−antitussive activity relationships among the NTI derivatives to define their structural determinants in order to design novel and improved analogues.

Rationale

Centrally acting drugs must penetrate the intractable membrane barriers, such as the blood-brain barrier, in order to be effective in vivo. The physicochemical requisites for the compounds capable of passing through this barrier are low molecular weight, high *n*-octanol/water distribution coefficient characteristics, low numbers of hydrogen bond donors, and so on.[22–24] Although systemically injected NTI itself fully suppresses the cough reflex, we considered that the augmentation of the hydrophobic properties of NTI would enable a compound

* To whom correspondence should be addressed. Phone: +84-3-5791-6372. Fax: +84-3-3442-5707. E-mail: nagaseh@pharm.kitasato-u.ac.jp.
† Toray Industries, Inc.
‡ Hoshi University.
§ Current affiliation is School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan.

[a] Abbreviations: CHO, Chinese hamster ovary; DAMGO, [D-Ala^2, MePhe^4, Gly-ol^6]enkephalin; DPDPE, [D-Phe^{2,5}]-enkephalin; HEK, human embryonic kidney; MVD, mouse vas deferens; NTI, naltrindole.

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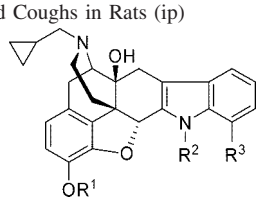
Table 1. Antitussive Effects of Alkyl Substituted NTI Derivatives on the Capsaicin-Induced Coughs in Rats (ip)



compound	R^1	R^2	R^3	antitussive activity, ED_{50} (μg/kg)[a,b]	
3a (NTI)	H	H	H	104	(20.3−553)
				1830	(890−3820)[c]
3b	Me	H	H	63.9	(29.4−139)
3c	H	Me	H	204	(41.8−999)
3d	Me	*n*-Pr	H	14.8	(4.09−53.8)
3e	Me	Me	7′-Me	6.35	(2.05−19.6)
3f	Me	Et	7′-Me	1.79	(0.67−4.89)

[a] ED_{50} values; the dose which reduces the number of coughs to 50% vs control, are expressed as mean ($N = 8$). Figures in parentheses indicate 95% confidence limits. [b] Each compound was administered ip route unless otherwise noted. [c] Administered perorally.

to more easily pass through the blood−brain barrier and thus potentiate its activity in vivo. We therefore prepared a series of compounds with these features and focused on NTI derivatives with hydrophobic substituents to identify the structural requirements as potent antitussive agents.

The antitussive activity of these compounds **3a−f** revealed interesting structure−activity relationships (Table 1). Simultaneous alkyl substitution at the 3-hydroxy group, the 1′ position, and the 7′ position of NTI significantly improved the antitussive activity (**3e,f**). Among these NTI derivatives, compound **3f** showed excellent antitussive activity, however, its δ receptor antagonist activity was decreased in an opioid receptor antagonism test using mouse vas deferens (MVD) preparations (vide infra).

On the basis of these results, we hypothesized that substitution at the 1′ and 7′ positions with hydrophobic substituents would result in improved permeability through the intractable membrane barriers, and as for the hydrophobic substituents, compact functionalities might be superior for avoiding a decrease of δ opioid receptor antagonist activity. This working hypothesis led us to design new types of NTI analogues. We considered that connection of the 1′ with 7′ position by an alkyl chain may possibly satisfy this hypothesis. Consequently, we synthesized additional ring-fused derivatives (**4−6**) (Chart 1), which have hydrophobic structures with minimum steric hindrance.

Chemistry

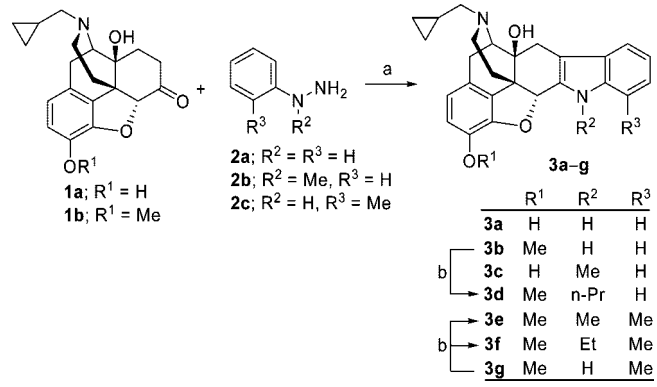
Substituted NTI derivatives were synthesized as described in Scheme 1. Compounds **3a−c**[13,25] and **3g** were obtained under the conditions of the Fischer indole synthesis from naltrexone (**1a**) or 3-*O*-methylnaltrexone (**1b**) and the corresponding arylhydrazines **2a−c**.[12] The indole nitrogens of **3b** and **3g** were alkylated with appropriate alkylating agents to give **3d** and **3e** or **3f**, respectively.

The additional ring-fused derivatives **4−6** were also synthesized using the Fischer indole synthesis from naltrexone (**1a**) or 3-*O*-methylnaltrexone (**1b**) with arylhydrazines **9a−c**, which were derived from the corresponding cyclic aryl amines **8a−c** (Scheme 2). The cyclohexene ring-fused derivative **7** was also prepared from hydrazine **10** using the same conditions (Chart 2).[26] *O*-Methylation of **6a** was conducted to give compound **6b**.

Results and Discussion

The antitussive effect was evaluated in in vivo studies using the rat capsaicin-induced cough model as described in our previous paper.[15] Test compounds were administered by ip or po routes, and the difference in the cough number for 3 min between the pre- and postdrug injection was examined.

Scheme 1. Synthesis of Alkyl Substituted NTI Derivatives[a]



	R^1	R^2	R^3
3a	H	H	H
3b	Me	H	H
3c	H	Me	H
3d	Me	n-Pr	H
3e	Me	Me	Me
3f	Me	Et	Me
3g	Me	H	Me

[a] Reagents and conditions: (a) $MeSO_3H$, EtOH, reflux; (b) R^2OTs, *n*-Bu_4NHSO_4, NaOH, water, benzene, 50 °C.

Opioid receptor antagonistic activities were evaluated using electrically stimulated mouse vas deferens (MVD) preparations.[27] Morphine, [D-Phe^{2,5}]-enkephalin (DPDPE), and U-50,488H were used as μ, δ, and κ agonists, respectively. The antagonist potencies are expressed as pA_2 values.

Opioid receptor binding affinities were determined by displacement of radioligands from guinea pig brain membranes.[28] [^{3}H][D-Ala^2,MePhe^4,Gly-ol^6]enkephalin ([^{3}H]DAMGO), [^{3}H]-NTI, and [^{3}H]U-69,593 were used as μ, δ, and κ radioligands, respectively.

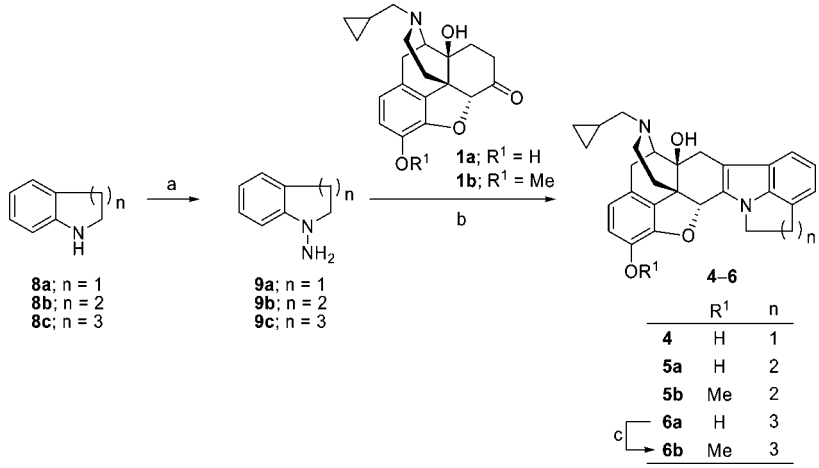
Opioid receptor agonist activities were evaluated by [^{35}S]GTPγS binding assay, which were carried out using membrane preparations from transfected CHO (δ and μ) or HEK-293 (κ) cells that constitutively expressed the respective human opioid receptor type.[29−32]

Substituted analogues of NTI were compared to the unsubstituted parent compound (Table 1). NTI (**3a**) showed a remarkable antitussive activity (ED_{50} 104 μg/kg, 95% confidence limits 20.3−533 μg/kg) when administered by ip route, but, in contrast, an oral administration of the compound resulted in significantly lower antitussive activity (ED_{50} 1830 μg/kg, 95% confidence limits 980−3820 μg/kg). This difference in antitussive activity between ip and po administration suggested that NTI would be poorly absorbed perorally.

Transformation of the 3-hydroxy group of NTI to the methyl ether (**3b**) slightly increased the antitussive activity, but the effect of this transformation was modest. In contrast, incorporation of a methyl group at the 1′ position of NTI decreased antitussive activity (**3c**). However, simultaneous alkyl substitution at both the 3-hydroxy group and the 1′ position drastically improved the antitussive activity (**3d**). Moreover, introduction of a third alkyl substituent at the 7′ position further enhanced the potency of compounds **3e** and **3f** by factors of 16 and 58, respectively, as compared to the reference compound. From these results, we presumed that the substitution of alkyl groups around the indole substructure increased the hydrophobicity of these compounds, which was supported by calculated logP values,[33] and improved their permeability through the blood−brain barrier and, as a consequence, the antitussive activities were improved.

Among these NTI derivatives, compound **3f** showed excellent antitussive activity, but its δ opioid receptor antagonist activity was decreased in the MVD test; its pA_2 values of δ, κ, and μ receptors were 6.69, 6.58, and 6.67, respectively. Because NTI is a highly

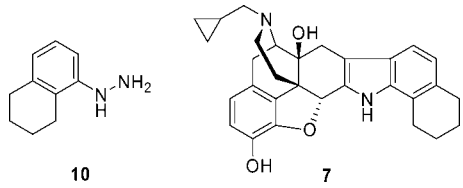
Scheme 2. Synthesis of NTI Derivatives with an Extra Fused Ring[a]



	R^1	n
4	H	1
5a	H	2
5b	Me	2
6a	H	3
6b	Me	3

[a] Reagents and conditions: (a) $NaNO_2$, HCl, EtOH, water, 0 °C, then $Na_2S_2O_4$, NaOH, reflux; (b) $MeSO_3H$, EtOH, reflux; (c) MeI, K_2CO_3, DMF, rt.

Chart 2. Structures of Compound **7** and Its Starting Material **10**



selective and potent δ receptor antagonist, we speculated that the bulkiness around the indole substructure may decrease the δ receptor binding affinity, resulting in a decrease in δ receptor antagonist activity. To examine this speculation, we evaluated opioid receptor binding affinity of selected compounds (Table 3). Binding affinity data of **3a**[34] and **3b**[25] were also cited from references for the purpose of comparison. Coop et al. reported that introduction of a methyl group to the 3-hydroxy group of **3a** increased δ selectivity in binding assays (Table 3, **3a**−**b**).[25] Compound **3f**, which possesses additional alkyl substituents around the indole substructure of compound **3b**, showed a significant decrease in δ receptor binding affinity and somewhat of a decrease in δ selectivity in comparison with compounds **3a** and **3b**. The antitussive activity of **3f** may be induced by its active metabolites (for example 3-OH analogue) rather than from **3f** itself.

On the basis of these observations, we hypothesized that compounds possessing a compact hydrophobic moiety around the indole substructure would show high antitussive activity without loss of δ receptor selectivity. As the connection of the 1′ position with the 7′ position by an alkyl chain is a possible strategy to satisfy this hypothesis, we synthesized the additional ring-fused derivatives **4**−**6** (Scheme 2).

Table 2 shows the results obtained from the evaluation of NTI analogues possessing an extra fused ring structure. Compounds with an alkylene bridge connecting the 1′ position and the 7′ position (**4**, **5a**−**b**, **6a**−**b**) dramatically improved antitussive activity compared with NTI. Particularly, the 3-methoxy analogue **5b** showed extremely high antitussive activity (its ED_{50} was 30-fold lower than that of NTI). As shown in Figure 1, compound **5b** decreased the number of coughs in a dose-dependent manner. This potency increment requires further investigation for these compounds, but the enhanced permeability through the blood−brain barrier of these compounds, which is assumed to result from increased hydrophobicity,[33] could be counted as a key factor. Compound **7**, another type of extra ring fused NTI derivative possessing a butylene bridge between the 6′ and 7′ positions, also exhibited more potent antitussive activity than NTI.

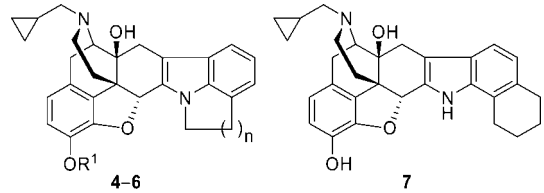
The NTI derivatives with an extra fused ring (**4**, **5a**−**b**, **6a**) exhibited high δ antagonist potency and high δ receptor selectivity (Table 2). The 5- and 6-membered ring fused derivatives **4** and **5a** showed especially high δ antagonist potency. Transformation of the 3-hydroxy group of compound **5a** into a methoxy group (**5b**) somewhat decreased antagonist potency, but the overall δ antagonist potency remained high. Compound **6b**, with a 7-membered fused ring and a methoxy group at the 3 position, exhibited poor antagonist potency. Despite this drawback, compound **6b** retained high antitussive activity. The antitussive activity of **6b** may be induced by its active metabolites rather than from **6b** itself.

Compounds **4**, **5a**−**b**, and **6a** also showed higher δ receptor binding affinity than compound **3f** by opioid receptor binding affinity assay (Table 3). In the case of 3-OMe derivatives, compound **5b** (propylene bridge) showed higher δ receptor binding affinity than compound **6b** (butylene bridge). With respect to 3-OH analogues, the order of δ receptor binding affinities was **4** (ethylene bridge) > **5a** (propylene bridge) > **6a** (butylene bridge). These results strongly suggested that δ receptor binding affinities would be correlated with bulkiness around the indole substructure and that compact fused rings would be important for exhibiting higher δ receptor binding affinity.

We examined the opioid receptor agonist activities of selected compounds at a high compound concentration of 1 μM using [^{35}S]GTPγS binding assay (Table 3). All of the tested compounds were inactive toward μ receptor, whereas some compounds exhibited weak δ or κ receptor agonist activities. The 3-OMe analogues showed increased δ receptor agonist activities compared with the parent compound NTI or the corresponding 3-OH derivatives, but their potencies were still weak (**3f**, **5b**, **6b**). NTI exhibited weak κ receptor agonist activity and compounds **3f**, **5a**, **6a**, and **7** revealed the same or slightly higher κ agonist potency than that of NTI.

Among these compounds, the 6-membered fused-ring derivative **5b**, whose methanesulfonic acid salt is TRK-850, showed excellent pharmacological properties. We evaluated the antitussive effect of **5b** by oral administration. As shown in Table 2, **5b** exhibited highly potent antitussive effect even by oral administration, with an ED_{50} value of 6.40 μg/kg (95% confidence limit: 1.54−26.5 μg/kg), which is ∼280 times smaller than that of peroral NTI. Increased hydrophobicity of compound **5b**, which might improve its permeability

Table 2. Antitussive Effect[a] and Opioid Antagonist Activity[b] of NTI and Its Analogues with an Extra Fused Ring Structure



				antagonist activity			
compd	R^1	*n*	antitussive activity[c,d,e] ED_{50} (μg/kg)	agonist	dose ratio[e,f]	conc (nM)[g]	pA_2[e,h]
4	H	1	9.14(4.23−19.8)	DPDPE (δ)	50 (38−67)	30	9.2 (9.1−9.3)
				morphine (μ)	2.8 (2.1−3.7)		7.8 (7.5−8.0)
				U-50,488H (κ)	4.5 (3.5−5.9)		8.1 (7.9−8.2)
5a	H	2	8.58(2.33−31.7)	DPDPE (δ)	38.5 (29.4−52.6)	30	9.10 (8.98−9.24)
				morphine (μ)	3.39 (2.19−5.33)		7.90 (7.60−8.16)
				U-50,488H (κ)	2.08 (1.32−3.59)		7.56 (7.03−7.94)
5b	Me	2	3.43(0.93−12.6)	DPDPE (δ)	17.0 (6.76−34.5)	100	8.20 (7.76−8.52)
			6.40(1.54−26.5)[i]	morphine (μ)	0.92 (0.61−1.37)		*j* (*j*−6.75)
				U-50,488H (κ)	2.48 (1.87−3.24)		7.17 (6.94−7.35)
6a	H	3	12.3(3.92−38.3)	DPDPE (δ)	4.18 (2.44−7.69)	10	8.50 (8.16−8.83)
				morphine (μ)	1.55 (1.16−2.10)		7.74 (7.22−8.04)
				U-50,488H (κ)	1.19 (0.83−1.75)		7.29 (*j*−7.87)
6b	Me	3	9.68(1.98−47.4)	DPDPE (δ)	1.03 (0.77−1.39)	10	6.53 (*j*−7.58)
				morphine (μ)	0.76 (0.58−0.98)		*j* (*j*−6.94)
				U-50,488H (κ)	1.58 (0.85−3.33)		7.77 (*j*−8.37)
7			39.5(12.8−121)	DPDPE (δ)	5.28 (4.02−6.80)	100	7.63 (7.48−7.76)
				morphine (μ)	0.3 (0.20−0.43)		*j* (*j*−*j*)
				U-50,488H (κ)	1.06 (0.75−1.50)		5.78 (*j*−6.70)

[a] Evaluated using rat capsaicin-induced cough model. [b] Evaluated using electrically stimulated mouse vas deferens (MVD) preparations. [c] Each compound was administered ip route unless otherwise noted. [d] ED_{50} values; the dose which reduces the number of coughs to 50% vs control, are expressed as mean ($N = 8$). [e] Figures in parentheses indicate 95% confidence limits. [f] Dose ratio, the ratio of agonist concentrations that elicit equal responses in the absence and presence of the antagonist at increasing concentrations, are expressed as mean ($N = 4$). [g] Concentration of competitive antagonist. [h] $pA_2 = -\log\{[B]/(\text{dose ratio} - 1)\}$. [B]: concentration of competitive antagonist (nM). [i] Administered perorally. [j] Not calculated.

Table 3. Opioid Receptor Binding Affinities[a] and Agonist Activities[b] of NTI Analogues

	binding affinity					[^{35}S]GTPr.S binding[c]		
compd	K_i (nM)			selectivity		% response[d] at 1 μM[e]		
	[^{3}H]NTI (δ)	[^{3}H]DAMGO (μ)	[^{3}H]U-69,593 (κ)	μ/δ	κ/δ	δ	μ	κ
3a	0.135	12.7	7.44	94	55	−3	5	12
3a[f]	0.217	27	30.4	124	140			
3b[g]	6.57	2510	3911	382	595	*h*	*h*	*h*
3f	218	>3670	4650	>17	21	47	1	21
4	0.295	23.2	7.99	79	27	26	0	3
5a	0.673	60.6	15.1	90	22	−2	1	26
5b	79.0	>3670	3290	>46	42	47	−1	8
6a	2.26	113	40.0	50	18	20	−1	32
6b	274	>3670	3343	>13	12	45	−1	11
7	6.32	200	52.0	32	8.2	19	−1	33

[a] Evaluated by displacement of radioligands from guinea pig brain membranes. Data are expressed as mean ($N = 2$). [b] Evaluated using [^{35}S]GTPγS binding assay. Data are expressed as mean ($N = 2$). [c] Carried out using membrane preparations from transfected CHO (δ and μ) or HEK-293 (κ) cells that constitutively expressed the respective human opioid receptor type. [d] Response relative to DPDPE (δ), DAMGO (μ), or U-69,593 (κ) response. EC_{50} values of DPDPE, DAMGO, and U-69,593 were 16, 1.7, and 11 nM, respectively. [e] Concentration of each tested compound. [f] Binding affinity data were from ref 34. [g] Binding affinity data were from ref 25. [h] Not tested.

through the blood−brain barrier, could result in potent antitussive effects at extremely low doses (3.43 μg/kg, ip; 6.40 μg/kg, po). As compound **5b** also showed weak or no opioid agonist activities, especially for μ and κ opioid receptors, δ opioid receptor antagonist activity of **5b** would mainly induce its antitussive effect. However, it cannot be denied that the antitussive effects may be derived from weak opioid receptor agonist activities.

We also evaluated the effect of (−) TAN-67 (selective δ_1 receptor agonist)[35,36] on cough reflex using the rat capsaicin-induced cough model. Administration of (−) TAN-67 (10 mg/kg, sc) markedly increased the number of coughs.[37] The results also suggested that antitussive activity induced by compound **5b** would be derived from the δ receptor antagonist activity.

Conclusions

In the present study, we designed NTI derivatives possessing hydrophobic substituents based on the hypothesis that increased hydrophobicity might improve their permeabilities through the blood−brain barrier and thereby increase their antitussive activities compared with that of NTI itself. Although introduction of some alkyl groups into NTI skeleton increased the antitussive activities of these derivatives, it simultaneously decreased the δ opioid receptor antagonist activity. Introduction of an extra fused ring, a compact size moiety, at the indole ring

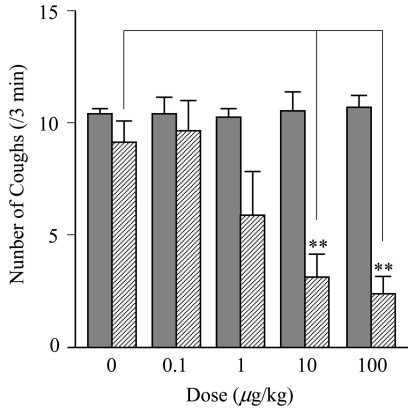


Figure 1. Antitussive Effects of **5b** on the Capsaicin-Induced Coughs in Rats (ip). The numbers of coughs for 3 min were counted in the same rats before 4.5 h (closed bars) and 30 min after (hatched bars) intraperitoneal administration of **5b**. The ED_{50} value (the dose which reduces the number of coughs to 50% vs control) of the inhibitory effects on coughs was calculated as 3.43 μg/kg (95% confidence limits: 0.93−12.6 μg/kg). **: $p < 0.01$ compared with control. Values are expressed as mean ± SD ($N = 8$).

in NTI was fully compatible with the control of suitable hydrophobicity and steric requirements. We finally identified the candidate compound **5b** for further clinical evaluation because it exhibited selective δ receptor antagonism and high antitussive activity even by an oral administration route.

Experimental Section

Chemistry: General. Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) data were taken on Varian GEMINI-300 (300 MHz), JEOL AL-400 (400 MHz), or JEOL GX-400 (400 MHz) spectrometers and reported in δ (ppm) downfield from tetramethylsilane (TMS). Mass spectra (MS) were obtained on a JEOL JES-D-300, JEOL JMS-D-303, or VG ZAB-HF instruments by applying an electric ionization method (EI) or a fast atom bombardment ionization method (FAB). Elemental analyses were determined with a Heraeus CHN-ORAPID for carbon, hydrogen, and nitrogen and YOKOGAWA IC-7000 for sulfur. Elemental analyses were within 0.4% of the theoretical values. The progress of the reactions and purity of final products were determined on Merck Silica Gel Art. 5715. Column chromatography was carried out using Merck Silica Gel (70−230 mesh).

General Method for the Amination of Aryl Amines. Method A: 1-Amino-1,2,3,4-tetrahydroquinoline (9b) Methanesulfonate. To a solution of 1,2,3,4-tetrahydroquinoline (**8b**, 20.0 g, 150 mmol) in ethanol (120 mL) was added a solution of $NaNO_2$ (12.4 g, 180 mmol) in H_2O (40 mL), and the mixture was cooled to 0 °C on an ice bath. The resulting solution was stirred vigorously, while concentrated HCl (30 mL) was added dropwise at 0 °C. After checking complete consumption of substrate material by TLC analysis, a solution of NaOH (90.0 g, 2.25 mol) in H_2O (200 mL) and $Na_2S_2O_4$ (75%, 104.5 g, 450 mmol) was added at 0 °C. The suspension was refluxed at 80 °C for 2 h, and then the reaction mixture was cooled to room temperature. After addition of H_2O (1250 mL) to the reaction mixture followed by extraction with toluene (300 mL), the organic layer was dried over anhydrous Na_2SO_4 and concentrated to give crude material. The crude product was dissolved in EtOAc (800 mL), and $MeSO_3H$ (14.4 g, 150 mmol) in EtOAc (200 mL) was added dropwise. The precipitate product was collected by filtration and washed with EtOAc, and dried in vacuo to afford the title compound (33.6 g, 91%): mp 146−148 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.97−2.10 (2H, m), 2.35 (3H, s), 2.76 (2H, dd, $J = 6.6, 6.6$ Hz), 3.38 (2H, dd, $J = 5.5, 5.5$ Hz), 6.95 (1H, m), 7.04 (1H, d, $J = 8.2$ Hz), 7.11 (1H, d, $J = 7.4$ Hz), 7.19 (1H, m), 10.09 (3H, br s).

General Method for the Indole Synthesis. Method B: (5*R*,9*R*,13*S*,14*S*)-17-cyclopropylmethyl-6,7-didehydro-4,5-epoxy-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino[2′,1′:6,7]morphinan-3,14-diol (5a) Methanesulfonate. To a suspension of naltrexone (**1a**) hydrochloride (847 mg, 2.20 mmol) in EtOH (20 mL) were added **9b**·$MeSO_3H$ (586 mg, 2.40 mmol) and $MeSO_3H$ (474 mg, 4.90 mmol). The solution was stirred at room temperature for 1 h and then refluxed for 7 h. After the reaction mixture was cooled to room temperature, the mixture was poured into saturated aqueous $NaHCO_3$ (30 mL). The mixture was extracted with $CHCl_3$ (30 mL × 3). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated to give yellow oil, which was purified by silica gel column chromatography ($CHCl_3$/MeOH/NH_4OH). Monomethanesulfonate was prepared by addition of $MeSO_3H$ to the compound solution in MeOH. The solid obtained by concentration was suspended in EtOAc and filtered to give the title compound **5a**·$MeSO_3H$ (545 mg, 45%) as a white powder: mp 210 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.39−0.54 (2H, m), 0.58−0.67 (1H, m), 0.68−0.78 (1H, m), 1.03−1.14 (1H, m), 1.84 (1H, br d, $J = 11.2$ Hz), 2.09−2.25 (2H, m), 2.30 (3.6H, s), 2.56 (1H, d, $J = 16.1$ Hz), 2.56−2.78 (2H, m), 2.78−3.00 (4H, m), 3.12 (1H, br d, $J = 10.3$ Hz), 3.26 (1H, dd, $J = 6.6, 19.6$ Hz), 3.34−3.43 (1H, m), 3.43 (1H, d, $J = 19.5$ Hz), 4.07 (1H, d, $J = 6.4$ Hz), 4.14−4.23 (1H, m), 4.26−4.35 (1H, m), 5.90 (1H, s), 6.31 (1H, br s), 6.60 (1H, d, $J = 8.3$ Hz), 6.62 (1H, d, $J = 8.3$ Hz), 6.84−6.93 (2H, m), 7.17 (1H, dd, $J = 2.2, 6.8$ Hz), 8.93 (1.2H, br s), 9.17 (1H, br s). FAB-MS *m*/*z* 455 $(M + 1)^+$. Anal. ($C_{29}H_{30}N_2O_3$·1.2CH_3SO_3H·0.3H_2O) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-indolo[2′,3′:6,7]morphinan-3,14-diol (3a) Methanesulfonate. To a suspension of naltrexone (**1a**) hydrochloride (150 g, 0.397 mol) in EtOH (2.5 L) were added phenylhydrazine (**2a**, 45.0 g, 0.417 mol) and $MeSO_3H$ (382 g, 3.98 mol). The solution was refluxed for 2 h and then cooled to room temperature. Precipitate was collected by filtration and washed with EtOH. The solid material was purified by recrystallization from MeOH to give the title compound **3a**·$MeSO_3H$ (147 g, 73%) as a white powder: mp 308−312 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.43 (1H, m), 0.49 (1H, m), 0.63 (1H, m), 0.73 (1H, m), 1.09 (1H, m), 1.82 (1H, br d, $J = 11.5$ Hz), 2.30 (3H, s), 2.54 (1H, d, $J = 16.1$ Hz), 2.61 (1H, dd, $J = 4.4, 13.2$ Hz), 2.66−2.77 (1H, m), 2.89−2.99 (1H, m), 2.95 (1H, d, $J = 15.9$ Hz), 3.11 (1H, d, $J = 11.0$ Hz), 3.26 (1H, dd, $J = 9.8, 16.6$ Hz), 3.34−3.47 (2H, m), 4.08 (1H, d, $J = 6.6$ Hz), 5.69 (1H, s), 6.33 (1H, br s), 6.59 (1H, d, $J = 8.2$ Hz), 6.63 (1H, d, $J = 8.2$ Hz), 6.97 (1H, dd, $J = 7.8, 7.8$ Hz), 7.11 (1H, dd, $J = 7.8, 8.3$ Hz), 7.35 (1H, d, $J = 7.8$ Hz), 7.36 (1H, d, $J = 8.3$ Hz), 8.93 (1H, br s), 9.21 (1H, br s), 11.32 (1H, s). FAB-MS *m*/*z* 415 $(M + 1)^+$. Anal. ($C_{26}H_{26}N_2O_3$·CH_3SO_3H·0.3H_2O) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-3-methoxy-indolo[2′,3′:6,7]morphinan-14-ol (3b) Methanesulfonate. Using the method B, the title compound **3b**·$MeSO_3H$ was obtained from phenylhydrazine (**2a**) and 3-*O*-methylnaltrexone (**1b**): mp >300 °C (dec). ^{1}H NMR (DMSO-d_6 with a drop of D_2O, 400 MHz): δ 0.42−0.55 (2H, m), 0.63 (1H, m), 0.74 (1H, m), 1.11 (1H, m), 1.84 (1H, br d, $J = 13.4$ Hz), 2.31 (3H, s), 2.50−2.73 (3H, m), 2.94−3.00 (3H, m), 3.13 (1H, m), 3.30−3.60 (3H, m), 3.68 (3H, s), 4.11 (1H, d, $J = 6.7$ Hz), 5.75 (1H, s), 6.73 (1H, d, $J = 8.4$), 6.81 (1H, d, $J = 8.4$ Hz), 6.97 (1H, dd, $J = 7.9, 7.9$ Hz), 7.11 (1H, dd, $J = 7.3, 8.3$ Hz), 7.35 (2H, d, $J = 9.2$ Hz). FAB-MS *m*/*z* 429 $(M + 1)^+$. Anal. ($C_{27}H_{28}N_2O_3$·CH_3SO_3H·0.2H_2O) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-1′-methyl-indolo[2′,3′:6,7]morphinan-3,14-diol (3c) Methanesulfonate. Using the method B, the title compound **3c**·$MeSO_3H$ was obtained from 1-methyl-1-phenylhydrazine (**2b**) and naltrexone (**1a**) hydrochloride (65%): mp >300 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.45 (1H, m), 0.50 (1H, m), 0.64 (1H, m), 0.73 (1H, m), 1.09 (1H, m), 1.86 (1H, br d, $J = 11.7$ Hz), 2.30 (3H, s), 2.56 (1H, d, $J = 16.1$ Hz), 2.63 (1H, dd, $J = 4.2, 12.9$ Hz), 2.73 (1H, m), 2.95 (1H, m), 2.97 (1H, d, $J = 16.1$ Hz), 3.07 (1H, br d, $J = 11.7$ Hz), 3.26 (1H, dd, $J = 6.8, 19.5$ Hz), 3.33−3.47 (2H, m), 3.85

(3H, s), 4.09 (1H, d, J = 6.3 Hz), 5.92 (1H, s), 6.31 (1H, s), 6.60 (1H, d, J = 8.1 Hz), 6.63 (1H, d, J = 8.1 Hz), 7.02 (1H, dd, J = 7.8, 7.8 Hz), 7.19 (1H, dd, J = 8.3, 8.3 Hz), 7.38 (1H, d, J = 7.8 Hz), 7.47 (1H, d, J = 7.8 Hz), 8.94 (1H, br s), 9.21 (1H, s). FAB-MS m/z 429 $(M + 1)^+$. Anal. ($C_{27}H_{28}N_2O_3 \cdot CH_3SO_3H$) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-3-methoxy-1′-propyl-indolo[2′,3′:6,7]morphinan-14-ol (3d) Methanesulfonate. To a solution of compound **3b** (1.00 g, 2.33 mmol) in benzene (16 mL) were added a 50% aqueous NaOH (4.5 mL) and tetra-*n*-butylammonium hydrogensulfate (750 mg, 1.19 mmol). The resulting solution was stirred vigorously, and *n*-propyl *p*-toluenesulfonate (750 mg, 3.50 mmol) was added to the solution at room temperature. The suspension was stirred at 50 °C for 1.5 h and cooled to room temperature. To the reaction mixture was added H_2O (50 mL), and the mixture was extracted with EtOAc (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give crude material, which was purified by silica gel column chromatography (1% MeOH in $CHCl_3$). Methanesulfonate was prepared by addition of $MeSO_3H$ to the compound solution in MeOH. The solid obtained by concentration was suspended in EtOAc and filtered to give the title compound **3d**·$MeSO_3H$ (1.21 mg, 91%) as a white solid: mp 165−167 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.40−0.58 (2H, m), 0.59−0.69 (1H, m), 0.70−0.80 (1H, m), 0.99 (3H, t, J = 7.2 Hz), 1.04−1.18 (1H, m), 1.75−1.92 (3H, m), 2.31 (3H, s), 2.52−2.78 (3H, m), 2.91−3.04 (2H, m), 3.10−3.19 (1H, m), 3.23−3.42 (2H, m), 3.50 (1H, d, J = 19.6 Hz), 3.69 (3H, s), 4.11 (1H, d, J = 6.2 Hz), 4.22 (2H, dd, J = 2.5, 6.5 Hz), 5.95 (1H, s), 6.35 (1H, br s), 6.74 (1H, d, J = 8.4 Hz), 6.82 (1H, d, J = 8.4 Hz), 7.04 (1H, t, J = 7.2 Hz), 7.18(1H, t, J = 7.2 Hz), 7.37 (1H, d, J = 7.8 Hz), 7.47 (1H, d, J = 8.1 Hz), 8.98 (1H, s br). EIMS (free base) m/z 470 (M^+). Anal. ($C_{30}H_{34}N_2O_3 \cdot CH_3SO_3H \cdot 0.3H_2O$) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-3-methoxy-7′-methyl-indolo[2′,3′:6,7]morphinan-14-ol (3g). Using the method B, the title compound **3g** was obtained from *o*-tolylhydrazine (**2c**) hydrochloride and 3-*O*-methylnaltrexone (**1b**) (86%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.11−0.22 (2H, m), 0.52−0.62 (2H, m), 0.84−0.96 (1H, m), 1.76−1.86 (1H, m), 2.30 (1H, ddd, J = 3.4, 12.0, 12.2 Hz), 2.37−2.50 (3H, m), 2.45 (3H, s), 2.63 (1H, d, J = 15.6 Hz), 2.75 (1H, dd, J = 4.1, 11.5 Hz), 2.83 (1H, dd, J = 6.6, 18.5 Hz), 2.89 (1H, d, J = 15.9 Hz), 3.15 (1H, d, J = 18.5 Hz), 3.38 (1H, d, J = 6.3 Hz), 3.75 (3H, s), 5.70 (1H, s), 6.60 (1H, d, J = 8.3 Hz), 6.63 (1H, d, J = 8.3 Hz), 6.91−6.99 (2H, m), 7.22−7.29 (1H, m), 8.13 (1H, br s). EIMS (free base) m/z 442 (M^+).

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-3-methoxy-1′,7′-dimethyl-indolo[2′,3′:6,7]morphinan-14-ol (3e) Methanesulfonate. Using a procedure similar to the synthesis of compound **3d**·$MeSO_3H$, the title compound **3e**·$MeSO_3H$ was obtained from compound **3g** and methyl *p*-toluenesulfonate as a white solid (77%): mp 201−203 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.41−0.53 (2H, m), 0.60−0.67 (1H, m), 0.71−0.77 (1H, m), 1.08−1.11 (1H, m), 1.87 (1H, d, J = 12.7 Hz), 2.31 (3.45H, s), 2.54−2.73 (3H, m), 2.74 (3H, s), 2.92−2.98 (2H, m), 3.13 (1H, d, J = 11.2 Hz), 3.27−3.43 (2H, m), 3.49 (1H, d, J = 20.0 Hz), 3.70 (3H, s), 4.08 (3H, s), 4.10 (1H, d, J = 6.8 Hz), 5.97 (1H, s), 6.32 (1H, br s), 6.73 (1H, d, J = 8.3 Hz), 6.82 (1H, d, J = 8.3 Hz), 6.84−6.89 (2H, m), 7.15−7.19 (1H, m), 8.96 (1.15H, br s). EIMS (free base) m/z 456 (M^+). Anal. ($C_{29}H_{32}N_2O_3 \cdot 1.15CH_3SO_3H \cdot 0.2H_2O$) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-1′-ethyl-3-methoxy-7′-methyl-indolo[2′,3′:6,7]morphinan-14-ol (3f) Methanesulfonate. Using a procedure similar to the synthesis of compound **3d**·$MeSO_3H$, the title compound **3f**·$MeSO_3H$ was obtained from compound **3g** and ethyl *p*-toluenesulfonate (97%) as a white solid: mp 193−196 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.42−0.53 (2H, m), 0.61−0.67 (1H, m), 0.71−0.77 (1H, m), 1.06−1.15 (1H, m), 1.40 (3H, t, J = 7.3 Hz), 1.87 (1H, d, J = 11.7 Hz), 2.30 (3H, s), 2.54−2.74 (3H, m), 2.70 (3H, s), 2.93−3.01 (2H, m), 3.13 (1H, d, J = 11.2 Hz), 3.27−3.43 (2H, m), 3.49 (1H, d, J = 20.0 Hz), 3.70 (3H, s), 4.10 (1H, d, J = 6.1 Hz), 4.42−4.54 (2H, m), 5.95 (1H, s), 6.32 (1H, br s), 6.74 (1H, d, J = 8.3 Hz), 6.83 (1H, d, J = 8.3 Hz), 6.87−6.92 (2H, m), 7.16−7.20 (1H, m), 8.95 (1H, br s). EIMS (free base) m/z 470 (M^+). Anal. ($C_{30}H_{34}N_2O_3 \cdot CH_3SO_3H \cdot 0.6H_2O$) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-1′,2′-dihydropyrrolo[3,2,1-*hi*]indolo[4′,5′:6,7]morphinan-3,14-diol (4) Methanesulfonate. Using the method A, 1-aminoindoline (**9a**) methanesulfonate was obtained from indoline (**8a**) (36%), and using the method B, the title compound **4**·$MeSO_3H$ was obtained from the compound **9a**·$MeSO_3H$ and naltrexone (**1a**) hydrochloride (43%): mp 235 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.39−0.53 (2H, m), 0.58−0.67 (1H, m), 0.68−0.77 (1H, m), 1.03−1.13 (1H, m), 1.82 (1H, br d, J = 10.7 Hz), 2.29 (3H, s), 2.52−2.77 (2H, m), 2.60 (1H, d, J = 16.1 Hz), 2.92 (1H, br s), 2.95 (1H, d, J = 15.6 Hz), 3.10 (1H, br d, J = 11.7 Hz), 3.20−3.43 (2H, m), 3.42 (1H, d, J = 19.5 Hz), 3.76 (2H, t, J = 6.8 Hz), 4.04 (1H, d, J = 6.4 Hz), 4.45−4.54 (1H, m), 4.57−4.66 (1H, m), 5.81 (1H, s), 6.32 (1H, s), 6.60 (1H, d, J = 8.3 Hz), 6.62 (1H, d, J = 8.3 Hz), 6.83−6.90 (2H, m), 7.09 (1H, d, J = 6.8 Hz), 8.92 (1H, br s), 9.20 (1H, br s). FAB-MS m/z 441 $(M + 1)^+$. Anal. ($C_{28}H_{28}N_2O_3 \cdot CH_3SO_3H \cdot 0.4H_2O$) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-5′,6′-dihydro-3-methoxy-4′*H*-pyrrolo[3,2,1-*ij*]quinolino[2′,1′:6,7]morphinan-14-ol (5b) Methanesulfonate. Using the method B, the title compound **5b**·$MeSO_3H$ was obtained from 1-amino-1,2,3,4-tetrahydroquinoline (**9b**) methanesulfonate and 3-*O*-methylnaltrexone (**1b**) (64%): mp 185−205 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.40−0.55 (2H, m), 0.63 (1H, m), 0.74 (1H, m), 1.09 (1H, m), 1.86 (1H, d, J = 12.9 Hz), 2.08−2.25 (2H, m), 2.29 (3H, s), 2.52−2.77 (3H, m), 2.90−3.10 (4H, m), 3.12 (1H, d, J = 11.5 Hz), 3.26−3.45 (2H, m), 3.49 (1H, d, J = 19.8 Hz), 3.70 (3H, s), 4.06−4.17 (2H, m), 4.32 (1H, m), 5.97 (1H, s), 6.36 (1H, br s), 6.73 (1H, d, J = 8.3 Hz), 6.81 (1H, d, J = 8.3 Hz), 6.86−6.93 (2H, m), 7.17 (1H, dd, J = 2.2, 6.8 Hz), 8.97 (1H br s). EIMS (free base) m/z 468 $(M)^+$. Anal. ($C_{30}H_{32}N_2O_3 \cdot CH_3SO_3H \cdot 0.7H_2O$) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-4′,5′,6′,7′-tetradehydro-azepino[3,2,1-*hi*]indolo[2′,1′:6,7]morphinan-3,14-diol (6a) Methanesulfonate. Using the method A, 1-amino-2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepine (**9c**) hydrochloride was obtained from 2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepine (**8c**) (62%). In the salt formation process, HCl methanol solution was used instead of $MeSO_3H$. Using the method B, the title compound **6a**·$MeSO_3H$ was obtained from the compound **9c**·HCl and naltrexone (**1a**) hydrochloride (87%): mp 180 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.39−0.55 (2H, m), 0.58−0.79 (2H, m), 1.01−1.16 (1H, m), 1.84 (1H, d, J = 11.8 Hz), 1.92−2.04 (2H, m), 2.08−2.24 (2H, m), 2.30 (3H, s), 2.52−2.80 (3H, m), 2.88−2.99 (1H, m), 2.94 (1H, d, J = 16.2 Hz), 3.03−3.17 (3H, m), 3.13−3.50 (2H, m), 3.26 (1H, dd, J = 6.9, 19.8 Hz), 4.08 (1H, d, J = 6.0 Hz), 4.29 (2H, br t, J = 5.2 Hz), 5.90 (1H, s), 6.30 (1H, br s), 6.60 (1H, d, J = 8.2 Hz), 6.62 (1H, d, J = 8.2 Hz), 6.85−6.96 (2H, m), 7.17 (1H, dd, J = 1.8, 7.3 Hz), 8.93 (1H, br s), 9.22 (1H, br s). FAB-MS m/z 469 $(M + 1)^+$. Anal. ($C_{30}H_{32}N_2O_3 \cdot CH_3SO_3H \cdot 0.4H_2O$) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-3-methoxy-4′,5′,6′,7′-tetradehydro-azepino[3,2,1-*hi*]indolo[2′,1′:6,7]morphinan-14-ol (6b) Methanesulfonate. To a solution of compound **6a** (323 mg, 0.69 mmol) in DMF (10 mL) were added methyl iodide (50 μL, 0.80 mmol) and anhydrous K_2CO_3 (193 mg, 1.4 mmol), and the mixture was stirred at room temperature for 14 h. The reaction mixture was poured into H_2O (100 mL) and extracted with Et_2O (50 mL × 3). The combined organic layers were washed with H_2O (50 mL) and saturated aqueous NaCl (50 mL), dried over anhydrous Na_2SO_4, and concentrated. The resulting amorphous material was purified by silica gel column chromatography (1% MeOH in $CHCl_3$) to give the compound **6b** (259 mg, 78%). Monomethanesulfonate was prepared by addition of one equivalent of $MeSO_3H$ to the compound solution in MeOH. The solid obtained by concentration was suspended in Et_2O and filtered

to give the title compound **6b**·$MeSO_3H$ as a white solid: mp 190 °C (dec). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.39−0.56 (2H, m), 0.58−0.72 (2H, m), 1.01−1.16 (1H, m), 1.86 (1H, d, J = 11.3 Hz), 1.92−2.04 (2H, m), 2.08−2.19 (2H, m), 2.30 (3H, s), 2.53−2.79 (3H, m), 2.89−3.01 (1H, m), 2.95 (1H, d, J = 16.2 Hz), 3.03−3.18 (3H, m), 3.31 (1H, dd, J = 6.5, 19.9 Hz), 3.34−3.57 (2H, m), 3.70 (3H, s), 4.11 (1H, d, J = 6.0 Hz), 4.20−4.38 (2H, m), 5.96 (1H. s), 6.34 (1H, br s), 6.74 (1H, d, J = 8.2 Hz), 6.81 (1H, d, J = 8.2 Hz), 6.85−6.96 (2H, m), 7.18 (1H, dd, J = 1.6, 7.3 Hz), 8.97 (1H, br s). FAB-MS m/z 483 $(M + 1)^+$. Anal. ($C_{31}H_{34}N_2O_3$·CH_3SO_3H·1.5H_2O) C, H, N, S.

(5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-6′,7′,8′,9′-tetrahydro-1′*H*-benzo[*g*]indolo[2′,3′:6,7]morphinan-3,14-diol (7) Methanesulfonate. Using the method B, the title compound **7**·$MeSO_3H$ was obtained from (5,6,7,8-tetrahydronaphthalen-1-yl)hydrazine (**10**) and naltrexone (**1a**) hydrochloride (32%): mp 235 °C (dec). ^{1}H NMR ($CDCl_3$, 400 MHz, data for free base): δ 0.13−0.20 (2H, m), 0.53−0.61 (2H, m), 0.85−0.93 (1H, m), 1.77−1.89 (5H, m), 2.30 (1H, dt, J = 3.5, 12.5 Hz), 2.38−2.47 (2H, m), 2.46 (1H, dd, J = 6.4, 12.5 Hz), 2.60 (1H, dd, J = 1.1, 15.7 Hz), 2.67−2.86 (6H, m), 2.86 (1H, d, J = 15.6 Hz), 3.12 (1H, d, J = 18.3 Hz), 3.36 (1H, d, J = 6.6 Hz), 5.04 (2H, br s), 5.71 (1H, s), 6.53 (1H, d, J = 8.1 Hz), 6.60 (1H, d, J = 8.1 Hz), 6.74 (1H, d, J = 8.1 Hz), 7.14 (1H, d, J = 8.1 Hz), 8.06 (1H, s). EIMS (free base) m/z 468 $(M)^+$. Anal. ($C_{30}H_{32}N_2O_3$·CH_3SO_3H·0.6H_2O) C, H, N, S.

Antitussive Activity Assay. The number of coughs was counted by the method of body-plethysmograph[38] in conscious male Spraque−Dawley (SD) rats. To induce coughs, capsaicin solution in saline (60 μM) was nebulized by an ultrasonic nebulizer (OMURON NE-U12). The rats were exposed to the capsaicin aerosol for 3 min using a respirator 4.5 h before administration of tested compounds, and the number of coughs produced during the exposure period was counted as a control. Then 30 min (ip) or 60 min (po) after the administration, the rats were exposed to the capsaicin aerosol for 3 min again, and the number of coughs was counted. The percentage reduction relative to the number of control coughs was calculated. ED_{50} and 95% confidence limits were calculated using Statistics Library II statistical analysis software (Yukms Co., Ltd., Tokyo).

Opioid Receptor Antagonist Activity Assay. Each vas deferens isolated from male ddy strain mice was hung in a Magnus tube, which was maintained at 37 °C, filled with a Krebes Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.1 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose), and aerated with 5% CO_2 and 95% O_2. Electric stimulation was applied through upper and lower ring-shaped platinum electrodes (0.1 Hz, 5.0 mS) using Nihon Kohden SEN-7203 electric stimulation system and Nihon Kohden SEG-3104 amplifier. Tissue contraction was recorded on a polygraph using an isometric transducer (Nihon Kohden WT-687G).

Morphine, DPDPE, and U-50,488H were added in a cumulative manner to determine the IC_{50} values (concentration for 50% inhibition of contraction induced by electric stimulation). Next, a tested compound solution (see Table 2 for concentration of each compound) was added to the system beforehand, and 20 min later, morphine, DPDPE, and U-50,488H were added in a cumulative manner. According to the above procedure, the ratio of the IC_{50} values of morphine, DPDPE, and U-50,488H in the presence of the tested compound to that in its absence was determined. The pA_2 values were calculated from the equation, $pA_2 = -\log([\text{antagonist}]/(\text{dose ratio} - 1))$, where dose ratio represents the ratio of agonist concentrations that elicit equal responses in the absence and presence of the antagonist at increasing concentrations.[39]

Opioid Receptor Binding Assay. For membrane preparation, the brain was quickly removed from 4-week male Hartley guinea pigs (Japan SLC) and dissected forebrain and cerebellum and immediately frozen in liquid nitrogen. These tissues were homogenized using a Potter−Elvejham tissue grinder with a Teflon pestle in 10 vol/g wet weight of ice-cold 50 mM Tris−HCl buffer (pH 7.4). The homogenate was centrifuged at 12000g at 4 °C for 20 min, and the pellet was resuspended in 20 vol/g wet weight of ice-cold Tris buffer. After 1 h of incubation at 4 °C in order to remove endogenous opioid ligands, homogenate was centrifuged at 12000g at 4 °C for 20 min. The pellet was resuspended in 20 vol/g wet weight of ice-cold Tris buffer and centrifuged at 12000g at 4 °C for 20 min. The resultant pellet was resuspended in 2 vol/g wet weight of ice-cold Tris buffer and stored at −80 °C until use. Binding affinities for μ and δ receptors were determined by displacing [^{3}H]DAMGO (specific activity: 1850 GBq/mmol, ARC) and [^{3}H]NTI (specific activity: 2220 GBq/mmol, ARC) from guinea pig forebrain membrane binding sites, and binding affinities for κ receptors were measured by displacement of [^{3}H]U-69,593 (specific activity: 1541 GBq/mmol, PerkinElmer) from guinea pig cerebellum membrane binding sites.

The homogenated membrane fractions (280−500 mg of protein/assay) were incubated at 25 °C for 2 h in 50 mM Tris−HCl buffer with various concentrations of tested compounds and 0.5 nM [^{3}H]DAMGO, [^{3}H]NTI or 0.1 nM [^{3}H]U-69,593 in a total volume of 500 mL. Specific bindings were defined as the difference in bindings observed in the absence and presence of 1 mM nontritiated ligand in each experiment (μ: DAMGO, δ: NTI, κ: U-69,593). Incubations were terminated by collecting membranes on GF/B filters (Whatman) using a cell harvester (Brandel). The filters were transferred to scintillation vials. Then, 5 mL of Creasol II (Nacalai Tesque) was added to the vials. After 12 h equilibration period, radioactivity in the samples was determined in a liquid scintillation counter (Packard, liquid scintillation analyzer TRI-CARB 1900). Calculated IC_{50} values were converted into K_i values (equilibrium inhibition constants) according to the Cheng and Prusoff equation:[40] $K_i = IC_{50}/(1 + L/K_d)$, where L is the concentration of the tritiated ligands. The equilibrium dissociation constants Kd were determined by displacement of the tritiated ligands by the particular nontritiated ones and were compared to the Kd values resulting from the saturation binding experiments. All reactions were carried out in duplicate.

[^{35}S]GTPγS Binding Assay. [^{35}S]GTPγS Binding assays were performed by MDS Pharma Services-Taiwan Ltd. (Taipei, Taiwan). The membranes from CHO cells expressing human μ or κ opioid receptor were purchased from Perkin-Elmer. The CHO cells stably expressing human δ opioid receptor were grown in Ham's F12 medium containing 10% fetal bovine serum and 500 μg/mL hygromycin under 5% CO_2 at 37 °C. The cell monolayers were harvested by scraping from plates. The cells were homogenized with Teflon homogenizer and centrifuged at 1100g for 10 min at 4 °C. The combined supernatants were centrifuged at 110000g for 15 min at 4 °C. The final pellet was resuspend in incubation buffer and frozen at −80 °C. The membranes (0.1, 0.09, and 0.02 mg/mL protein for δ, κ, and μ opioid receptor, respectively) were preincubated with a tested compound in incubation buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, and 3 μM GDP) for 20 min at 30 °C and then scintillation proximity assay (SPA) beads (GE Amersham) were added. After another 60 min of preincubation, the reaction was initiated by addition of 0.3 nM [^{35}S]GTPγS and the mixture was incubated for 30 min. Samples were counted after 10 min centrifugation on the Trilux liquid scintillation counter. Tested compound-induced increase of [^{35}S]GTPγS binding was calculated by the percentage response relative to the 10 μM DPDPE, 3 μM U-69,593, and 10 μM DAMGO response for indicating possible δ, κ, and μ opioid receptor agonist activity, respectively.

Supporting Information Available: Analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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