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Design and synthesis of a metabolically stable and potent antitussive agent, a novel δ opioid receptor antagonist, TRK-851

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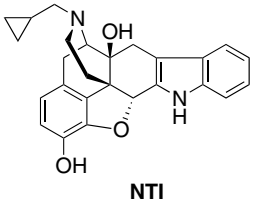
ABSTRACT

We have previously reported on antitussive effect of (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 (**1b**) methanesulfonate (TRK-850), a selective δ opioid receptor antagonist which markedly reduced the number of coughs in a rat cough model. We designed TRK-850 based on naltrindole (NTI), a typical δ opioid receptor antagonist, to improve its permeability through the blood–brain barrier by introducing hydrophobic moieties to NTI. The ED_{50} values of NTI and compound **1b** by intraperitoneal injections were 104 μg/kg and 2.07 μg/kg, respectively. This increased antitussive potency probably resulted from the improved brain exposure of >compound **1b**. However, **1b** was extremely unstable toward metabolism by cytochrome P450. In this study, we designed and synthesized compound **1b** derivatives to improve the metabolic instability, which resulted in affording highly potent and metabolically stable oral antitussive agent (5*R*,9*R*,13*S*,14*S*)-17-cyclopropylmethyl-6,7-didehydro-4,5-epoxy-8′-fluoro-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino[2′,1′:6,7]morphinan-3,14-diol (**1c**) methanesulfonate (TRK-851).

1. 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 non-peptide 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] Based on these observations, we previously reported that naltrindole (NTI) (Fig. 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.



NTI

Figure 1. Chemical structure of NTI.

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 partition coefficient (log*P*) characteristics, low number of hydrogen bond donors, and so on.[17–19] As described in the previous paper, we investigated NTI derivatives as antitussives and identified more potent analogue **1b** (TRK-850) (Fig. 2), which markedly reduced the number of coughs even by an oral administration in a rat model (ED_{50} 6.40 μg/kg).[20] We designed compound **1b** so as to improve permeability through the blood–brain barrier by introducing hydrophobic moieties to NTI. The ED_{50} values of NTI and compound **1b** by intraperitoneal (ip) injections were 104 μg/kg and 2.07 μg/kg, respectively. We supposed that this increased antitussive potency might result from the im-

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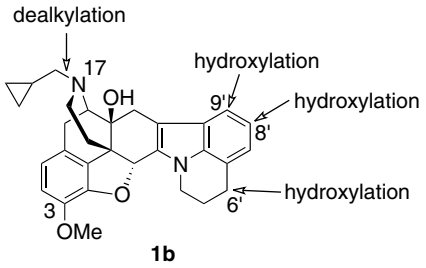


Figure 2. Chemical structure of compound **1b** and its main metabolic pathways. The metabolic positions of **1b** were determined by isolation and identification of main metabolites collected from the blood plasma of the rats to which compound **1b** was orally administered.

proved brain exposure of compound **1b** as a consequence of the increased hydrophobicity compared with that of NTI, which was supported by calculated log*P* (C log*P*) values.[21] We also identified compound **1a**, 3-OH analogue of compound **1b**, as a selective δ opioid antagonist which exhibited somewhat lower antitussive activity (ED_{50} 8.58 μg/kg, ip) compared with that of compound **1b**.[20]

Unfortunately, a defect was found for compound **1b** during an ADME study. After an oral administration of tritiated **1b** to a rat, only a small parent peak was observed in the HPLC [^{3}H]-detection, indicating an extremely high metabolic rate by cytochrome P450, which means **1b** is an inadequate compound for an oral administration. The metabolic pathways of compound **1b** were investigated in detail by the isolation of the main metabolites collected from the blood plasma of the rats to which **1b** was orally administered. We identified four of the primary metabolites: the 17-decyclopropylmethyl, 9′-hydroxy, 8′-hydroxy, and 6′-hydroxy analogues (Fig. 2). Although dealkylation at the 3-methoxy group has been reported as a possible metabolism process of the morphinan compounds,[22] no such kind of metabolite was isolated in this study. In general, oxidative metabolism may be prevented by the following strategies: (a) incorporation of blocking groups at the labile site, (b) modifying around the labile site with bulky substituents which prohibit the metabolic enzyme from approaching its substrate through steric hindrance, (c) reducing lipophilicity of the compound, and (d) introduction of electron-withdrawing groups at the substrate site where the catabolic enzyme attacks.[23–25] In this study, we synthesized analogues of compound **1b** according to these general considerations, and evaluated their metabolic stability in the presence of human liver microsomes. We also investigated structure–antitussive activity relationships of these compounds.

2. Chemistry

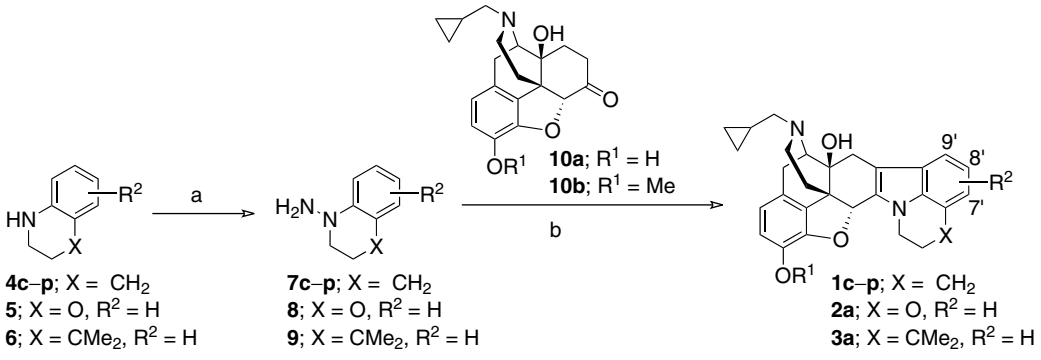
Compound **1b** analogues (**1c**–**p**, **2a**, and **3a**) were synthesized using the condition of the Fischer indole synthesis from naltrexone (**10a**) or 3-*O*-methylnaltrexone (**10b**) with aryl hydrazines **7**–**9**, which were derived from the corresponding cyclic aryl amines **4**–**6** (Scheme 1). Demethylation of the methoxy group of compound **1p** gave phenol **1u**, and methylation of **1c**, **1f**, **1k**, **1n**, **2a**, and **3a** was conducted to give compounds **1q**, **1r**, **1s**, **1t**, **2b**, and **3b**, respectively.

Each of the cyclic aryl amines **4**–**6** was synthesized from a commercially available compound (Scheme 2). Compounds **4c**, **4h**, **4j**, **4l**, **4o**, and **4p** were synthesized by reductive Beckmann rearrangement reaction[26] of oximes **12** which were derived from indanones **11**.

6-Trifluoromethoxy-1,2,3,4-tetrahydroquinoline (**4d**) was synthesized from aniline **13**, which was treated with acrylonitrile to give nitrile derivative **14**. Hydrolysis under basic conditions of the nitrile **14** and polyphosphoric acid-catalyzed cyclization were conducted to give oxo-compound **15**, which was reduced by $LiAlH_4$ in the presence of $AlCl_3$[27] to afford compound **4d**.

6-Trifluoromethyl-1,2,3,4-tetrahydroquinoline (**4e**) was prepared from 6-trifluoromethylquinoline-4-ol (**16**). Treatment of **16** with benzyl chloride followed by reduction of the afforded enone by lithium tri-*sec*-butylborohydride gave oxo-compound **17**. Subsequent reduction of compound **17** by $LiAlH_4/AlCl_3$,[27] followed by removal of the benzylic and hydroxy groups provided the intermediate **4e**.

Compounds **4g, 4i**, **4k**, and **4n** were prepared by PtO_2-catalyzed hydrogenation of the corresponding quinoline derivatives **18** in the presence of hydrogen chloride.

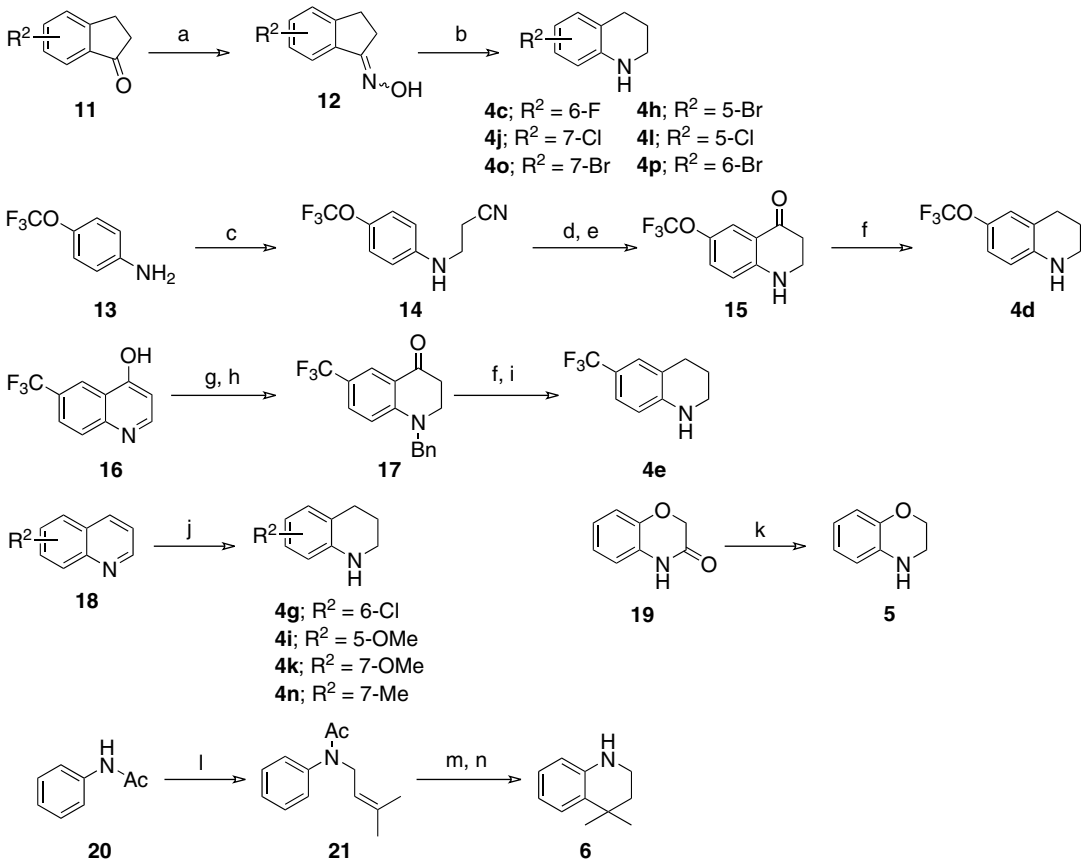


	X	R^1	R^2
c → **1c**	CH_2	H	8′-F
c → **1q**	CH_2	Me	8′-F
1d	CH_2	H	8′-OCF_3
1e	CH_2	H	8′-CF_3
c → **1f**	CH_2	H	9′-CF_3
c → **1r**	CH_2	Me	9′-CF_3
1g	CH_2	H	8′-Cl
1h	CH_2	H	7′-Br

	X	R^1	R^2
1i	CH_2	H	7′-OMe
1j	CH_2	H	9′-Cl
c → **1k**	CH_2	H	9′-OMe
c → **1s**	CH_2	Me	9′-OMe
1l	CH_2	H	7′-Cl
1m	CH_2	H	8′-OMe
c → **1n**	CH_2	H	9′-Me
c → **1t**	CH_2	Me	9′-Me

	X	R^1	R^2
1o	CH_2	H	9′-Br
d → **1p**	CH_2	Me	8′-Br
d → **1u**	CH_2	H	8′-Br
c → **2a**	O	H	H
c → **2b**	O	Me	H
c → **3a**	CMe_2	H	H
c → **3b**	CMe_2	Me	H

Scheme 1. Synthesis of NTI derivatives with an extra fused ring. 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; (d) BBr_3, CH_2Cl_2, 0 °C.



Scheme 2. Preparation of cyclic aryl amines **4**–**6**. Reagents and conditions: (a) $HONH_2$·HCl, MeOH, reflux; (b) *i*-Bu_2AlH, CH_2Cl_2, rt; (c) acrylonitrile, $Cu(OAc)_2$, 110 °C, (d) NaOH, water, reflux; (e) Polyphosphoric acid, H_3PO_4, P_2O_5, 100 °C; (f) $LiAlH_4$, $AlCl_3$, THF, Et_2O, rt; (g) *t*-BuOK, BnCl, THF, reflux; (h) lithium tri-*sec*-butylborohydride, THF, rt; (i) Pd/C, conc. HCl, MeOH, rt; (j) PtO_2, conc. HCl, H_2, MeOH or EtOH; (k) BH_3·THF, THF, reflux; (l) 1-chloro-3-methyl-2-butene, NaOH, K_2CO_3, *n*-Bu_4NBr, toluene, 75 °C; (m) $AlCl_3$, PhCl, 110 °C; (n) aq. HCl, toluene, reflux.

Benzoxadine **5** was synthesized by borane reduction of the corresponding amide compound **19**.

The dimethylated compound **6** was prepared by introduction of an alkenyl chain to acetanilide **20**[28] to give compound **21**, followed by cyclization and then removal of the acetyl group under acidic conditions.

3. Results and discussion

Metabolic rates were determined by HPLC analyses by quantifying the remaining parent peak after mixing the compounds with human liver microsomal enzymes, and the data were expressed as elimination rate constants (Ke).

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, sc, or po routes, and the difference in the cough number for 3 minutes between the pre- and post-drug injection was examined.

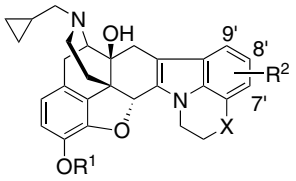
Opioid receptor antagonistic activities were evaluated using electrically stimulated mouse vas deferens (MVD) preparations.[29] Morphine, [D-Phe^{2,5}]-enkephalin (DPDPE), and U-50,488H were used as μ, δ, and κ agonists, respectively. The antagonist potencies are expressed as pA_2 values.

The relative metabolic rates compared to that of compound **1b** (Ke ratio) and their antitussive effects are listed in Table 1. The most effective derivatization for lowering the metabolic rate was the demethylation of the 3-methoxy group. The conversion of the 3-methoxy group of **1b** to a hydroxyl group of **1a** improved metabolic stability dramatically. Other 3-hydroxy analogues were also metabolically more stable than the corresponding 3-methoxy analogues (**1c**, **1q**; **1u**, **1p**; **1f**, **1r**; **1k**, **1s**; **1n**, **1t**; **2a**, **2b**). We assumed that these results derived from the decreasing lipophilicity of these compounds, which was supported by calculated log*P* values.[21] Replacement of the 6′-methylene group of compound **1b**, which is one of the metabolic sites (Fig. 2), by an oxygen or a dimethylmethylene group also improved metabolic stability (**2b**, **3b**). As for the effect of substituents, introduction of electron-withdrawing substituents to the indole moiety tended to suppress the metabolism (**1c**–**h**, **1u**). On the other hand, electron-donating substituents somewhat enhanced the metabolism (**1k**, **1m, 1n**). These observations suggested that the electron density around the indole moiety correlated with the cytochrome P450 oxidative metabolic rate.

3-Hydroxy analogues exhibited lower antitussive potency than 3-methoxy analogues (**1a**, **1b**; **1c**, **1q**). Mostly, incorporation of a substituent at the indole moiety resulted in lower potency compared with that of compound **1a**, but some analogues still exhibited high antitussive activity (**1c, 1u, 1f, 1j, 1m**, **1q**). The bulkiness of substituents at the 8′-position tended to reduce potency. Fluorine, which was the smallest substituent with the greatest electron-withdrawing capacity, could minimize this loss of potency (**1c**).

Among these analogues, compound **1c** showed a highly potent antitussive activity and 20 times lower metabolic rate compared with that of compound **1b**. Since compound **1c**, whose methanesulfonic acid salt is TRK-851, exhibited excellent pharmacological and metabolic profiles, we evaluated the antitussive effect of this compound by an oral administration. Figure 3 shows that peroral administration of compound **1c** decreased the number of coughs

Table 1
Metabolic rates[a] and antitussive effects on the capsaicin-induced coughs in rats (sc)[b] for compound **1b** derivatives



Compound	X	R^1	R^2	Metabolic rate		Antitussive activity	
				Ke (1/min) [c]	1/(Ke ratio)[d]	ED_{50} (μg/kg)[e]	
1b	CH_2	Me	H	0.341	1	2.07	(0.55–7.76)[f]
1c (TRK-851)	CH_2	H	8′-F	0.0174	19.6	1.7	(0.4–7.3)
						15.7	(5.31–46.4)[f]
1d	CH_2	H	8′-OCF_3	0.00909	37.5	>1000	
1u	CH_2	H	8′-Br	0.0151	22.6	5.05	(2.58–9.86)
1e	CH_2	H	8′-CF_3	0.0162	21.0	>1000	
1f	CH_2	H	9′-CF_3	0.0167	20.4	0.355	(0.137–0.919)
1g	CH_2	H	8′-Cl	0.0177	19.2	78.4	(11.5–538)[f]
1h	CH_2	H	7′-Br	0.0218	15.6	27.4	(8.81–85.1)
1a	CH_2	H	H	0.0249	13.7	0.2	(0.06–0.6)
						8.58	(2.33–31.7) [f]
1i	CH_2	H	7′-OMe	0.0262	13.0	54.2	(18.6–158)
1j	CH_2	H	9′-Cl	0.0270	12.6	3.5	(1.4–8.7)
1k	CH_2	H	9′-OMe	0.0285	12.0	8.67	(1.02–73.6)
1l	CH_2	H	7′-Cl	0.0290	11.7	13.8	(0.62–310)
1m	CH_2	H	8′-OMe	0.0331	10.3	2	(0.4–9.4)
2a	O	H	H	0.0334	10.2	14	(0.9–225.5)
1n	CH_2	H	9′-Me	0.0339	10.1	g	
1o	CH_2	H	9′-Br	0.0371	9.20	94.6	(5.11–389)
3b	CMe_2	Me	H	0.0618	5.51	12.8	(1.2–132.4)
2b	O	Me	H	0.0627	5.43	g	
3a	CMe_2	H	H	0.0758	4.50	g	
1r	CH_2	Me	9′-CF_3	0.137	2.49	g	
1s	CH_2	Me	9′-OMe	0.200	1.70	g	
1p	CH_2	Me	8′-Br	0.264	1.29	g	
1t	CH_2	Me	9′-Me	0.357	0.95	g	
1q	CH_2	Me	8′-F	0.388	0.88	4.73	(1.32–16.9)[f]

[a] Experiments were performed in the incubation mixture of human liver microsomes and the compounds.
[b] Compounds were administered by subcutaneous route unless otherwise noted.
[c] Ke value, an elimination rate constant, was calculated from a time course of compound elimination.
[d] Ke ratio = (Ke of each compound)/(Ke of compound **1b**).
[e] 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.
[f] Compounds were administered by intraperitoneal route.
[g] Not determined.

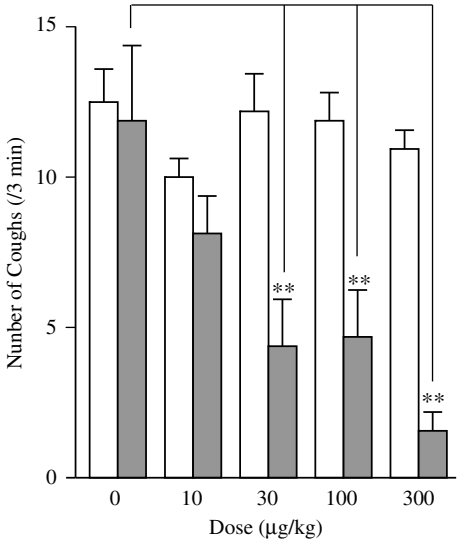


Figure 3. Antitussive effects of compound **1c** on the capsaicin-induced coughs in rats (po). The numbers of coughs for 3 min were counted in the same rats before (white bars) and 30 min after (gray bars) oral administration of compound **1c**. The ED_{50} value (the dose which reduces the number of coughs to 50% vs. control) of the inhibitory effects on coughs was calculated as 35.5 μg/kg (95% confidence limits: 16.8–72.9 μg/kg). $^{**}p < 0.01$ compared with control. Values are expressed as means ± SD ($n = 8$).

in a dose-dependent manner, with an ED_{50} value of 35.5 μg/kg (95% confidence limit: 16.8–72.9 μg/kg).

Finally, we tested the opioid receptor selectivity of compound **1c** using MVD preparations. Although **1c** inhibited the electrically stimulated contraction of MVD at an extremely high concentration ($IC_{50} = 8.8$ μM), it showed no inhibition at the low concentration (10 nM) used for the evaluation of its antagonistic action. As shown in Table 2, compound **1c** strongly antagonized the activity of DPDPE (δ), but not the effects of morphine (μ) or U-50,488H (κ). Based on the pA_2 values for the δ receptor calculated from this result, we concluded that compound **1c** was a selective antagonist

Table 2
Receptor selectivity of compound **1c** in Mouse Vas Deferens (MVD) tests

Antagonist	Agonist	Dose ratio[a,b]	pA_2[b,c]
1c (10 nM)[d]	DPDPE (δ)	7.87 (5.03–12.2)	8.84 (8.61–9.05)
	U-50,488H (κ)	1.03 (0.856–1.23)	6.48 (*e*–*e*)
	Morphine (μ)	0.98 (0.612–1.58)	e

[a] 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 ($n = 4$).
[b] Figures in parentheses indicate 95% confidence limits.
[c] $pA_2 = -\log([B]/(\text{dose ratio} - 1))$. [B]: concentration of competitive antagonist (nM).
[d] Concentration of the antagonist.
[e] Not calculated.

for the δ opioid receptor, and its selectivity for the δ opioid receptor was more than 100 times (more than 2 in pA_2) higher than that for the μ or κ opioid receptor. As compound **1c** exhibited antitussive effects at marked low dose (ED_{50} 1.7 μg/kg, sc) in the rat cough model and it showed extremely weak opioid agonist activities, the antitussive effects of **1c** would be derived from its selective antagonism of δ receptor, not from its weak opioid agonist effects.

4. Conclusions

In the present study, both a preservation of antitussive activity and an improvement of metabolic stability were achieved by modifying the structure of compound **1b**. We determined that a strong candidate compound must have low enough hydrophobicity to prevent a high rate of metabolism but also have sufficient hydrophobicity to obtain a potent antitussive activity. In addition, we examined compact indole substituents which block metabolic reaction to preserve the antitussive activity. Modification of compound **1b** including demethylation of the 3-methoxy group and introduction of 8′-fluorine was fully compatible with the control of suitable hydrophobicity and steric requirements. We finally identified the candidate compound **1c** methanesulfonate, TRK-851 for further clinical evaluation, which exhibits selective δ receptor antagonism and high antitussive activity even by an oral administration route without the associated metabolic problems.

5. Experimental

5.1. Chemistry

Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) data were taken on VALIAN GEMINI-300 (300 MHz) or JEOL GX-400 (400 MHz) spectrometers and reported in δ (ppm) downfield from tetramethylsilane (TMS). Infrared spectra (IR) were determined on a JASCO FT/IR-5000. 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; KYOTO ELECTRONICS AT-118 for chlorine; and YOKOGAWA IC-7000 for fluorine, bromine, and 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).

5.1.1. 6-Fluoro-1,2,3,4-tetrahydroquinoline (4c) hydrochloride

To a solution of 5-fluoro-1-indanone (**11c**) (10.0 g, 66.6 mmol) in MeOH (200 mL) was added hydroxylamine hydrochloride (20.0 g, 288 mmol). The suspension was refluxed for 1 h, and then the reaction mixture was cooled to room temperature. The mixture was evaporated, and saturated aqueous $NaHCO_3$ (350 mL) was added. The mixture was extracted with $CHCl_3$ (3× 300 mL), and the combined organic layers were dried over Na_2SO_4 and concentrated to give 5-fluoro-1-indanone oxime (**12c**) (12.5 g), which was mixture of *cis* and *trans* forms of oxime, as a yellow powder.

The oxime **12c** (12.5 g) was dissolved in CH_2Cl_2 (500 mL) and was cooled to 0 °C on an ice bath. To the solution was added *i*-Bu_2AlH (0.95 M solution in hexane, 500 mL) dropwise for 1 h, so maintaining the reaction temperature below 10 °C. After the addition, the mixture was allowed to warm up to room temperature and stirred for 3 h. NaF (56.0 g, 1.33 mol) and H_2O (18 mL) were added to the resulting mixture at 0 °C, and the mixture was stirred for 30 min at the same temperature. Insoluble was removed by filtration through a pad of Celite, and the cake was washed with $CHCl_3$ (1.3 L). The resulting solution was concentrated in vacuo to give an oily crude material (14.2 g), which was dissolved in AcOEt (400 mL) and was then treated with concentrated HCl (10 mL). The precipitate produced was collected by filtration and washed with AcOEt, and dried in vacuo to afford the title compound **4c**·HCl (9.05 g, 71% in 2 steps) as a white powder. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.95–2.05 (2H, m), 2.35 (s, 3H), 2.76 (2H, dd, J = 6.6, 6.6 Hz), 3.36 (2H, dd, J = 5.6, 5.6 Hz), 7.01 (1H, d, J = 9.0 Hz), 7.05–7.15 (2H, m), 10.09 (3H, br s).

5.1.2. 5-Bromo-1,2,3,4-tetrahydroquinoline (4h) hydrochloride

Using a procedure similar to that used for compound **4c**·HCl, the title compound **4h**·HCl was obtained from 4-bromo-1-indanone (**11h**) (84% in 2 steps). ^{1}H NMR data of compound **4h** were identical to that of the literature.[30]

5.1.3. 7-Chloro-1,2,3,4-tetrahydroquinoline (4j)

Using a procedure similar to that used for compound **4c**·HCl, the title compound **4j** was obtained from 6-chloro-1-indanone (**11j**) (52% in 2 steps). Purification of free base material by silica gel column chromatography (benzene) was performed instead of precipitate formation of HCl salt. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.85–1.95 (2H, m), 2.70 (2H, dd, J = 6.3, 6.3 Hz), 3.28 (2H, dd, J = 5.6, 5.6 Hz), 3.83 (1H, br s), 6.43 (1H, d, J = 1.8 Hz), 6.54 (1H, dd, J = 1.8, 7.8 Hz), 6.83 (1H, d, J = 7.8 Hz).

5.1.4. 5-Chloro-1,2,3,4-tetrahydroquinoline (4l)

Using a procedure similar to that used for compound **4j**, the title compound **4l** was obtained from 4-chloro-1-indanone (**11l**) (59% in 2 steps). ^{1}H NMR data of compound **4l** were identical to that of the literature.[31]

5.1.5. 7-Bromo-1,2,3,4-tetrahydroquinoline (4o)

Using a procedure similar to that used for compound **4j**, the title compound **4o** was obtained from 6-bromo-1-indanone (**11o**) (78% in 2 steps). ^{1}H NMR data of compound **4o** were identical to that of the literature.[30]

5.1.6. 6-Bromo-1,2,3,4-tetrahydroquinoline (4p) hydrochloride

Using a procedure similar to that used for compound **4c**·HCl, the title compound **4p**·HCl was obtained from 5-bromo-1-indanone (**11p**) (69% in 2 steps). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.85–1.93 (2H, m), 2.73–2.78 (2H, m), 3.24–3.28 (2H, m), 6.88 (1H, d, J = 8.2 Hz), 7.26–7.31 (2H, m), 8.54 (2H, br s).

5.1.7. 3-(4-(Trifluoromethoxy)phenylamino)propanenitrile (14)

To a mixture of 4-trifluoromethoxyaniline (**13**) (5.00 g, 28.2 mmol) and acrylonitrile (1.80 g, 33.9 mmol) was added anhydrous copper (II) acetate (60 mg, 0.33 mmol), and the mixture was stirred at 110 °C. After 3 h, the mixture was cooled to room temperature and concentrated. The crude material was purified by silica gel column chromatography ($CHCl_3$) to give the title compound **14** (3.28 g, 50%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.63–2.67 (2H, m), 3.49–3.54 (2H, m), 4.60 (1H, br s), 6.57–6.61 (2H, m), 7.05–7.10 (2H, m).

5.1.8. 6-(Trifluoromethoxy)-2,3-dihydroquinolin-4(1*H*)-one (15)

To an aqueous solution of NaOH (2.5 N, 30 mL) was added the compound **14** (1.68 g, 7.30 mmol), and the mixture was refluxed for 3 h. The reaction mixture was cooled to room temperature, and to this was added 1 N HCl (75 mL) at 0 °C. The mixture was extracted with Et_2O (2× 70 mL), dried over $MgSO_4$, and concentrated to give 3-(4-trifluoromethoxyphenylamino)propanoic acid

(1.60 g). This compound was used in the next step without purification.

The acid (1.60 g, 6.42 mmol) was mixed with polyphosphoric acid (80 g), H_3PO_4 (10 mL) and P_2O_5 (10 g), and the mixture was warmed up to 100 °C. After stirring for 18 h, the reaction mixture was cooled to room temperature and poured into a mixture of aqueous NaOH solution (3 N, 280 mL) and crushed ice (250 g). The resulting mixture was basified by addition of saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$ (2× 150 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to give the title compound **15** as a yellow powder (940 mg). This compound was used in the next step without purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.69–2.74 (2H, m), 3.57–3.63 (2H, m), 4.48 (1H, br s), 6.68 (1H, d, J = 8.8 Hz), 7.14–7.18 (1H, m), 7.70 (1H, d, J = 1.9 Hz).

5.1.9. 6-Trifluoromethoxy-1,2,3,4-tetrahydroquinoline (4d)

To a suspension of $LiAlH_4$ (462 mg, 12.2 mmol) in THF (20 mL) and Et_2O (50 mL) was added $AlCl_3$ (800 mg, 6.00 mmol) and stirred at room temperature for 10 min. To this were added a solution of 6-(trifluoromethoxy)-2,3-dihydroquinolin-4(1*H*)-one (**15**) (940 mg, 4.07 mmol) in THF (20 mL) and $AlCl_3$ (800 mg, 6.00 mmol) again, and the mixture was stirred at room temperature. After 4 h, the reaction mixture was quenched by addition of H_2O (150 mL) and saturated aqueous $NaHCO_3$ solution (100 mL) was added. The mixture was extracted with Et_2O (2× 100 mL), and combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes/AcOEt, 5:1) to afford the title compound **4d** (238 mg, 15% from **14**). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.83–1.95 (2H, m), 2.75 (2H, dd, J = 6.3, 6.3 Hz), 3.29 (2H, dd, J = 5.4, 5.4 Hz), 3.68 (1H, br s), 6.38–6.45 (1H, m), 7.20–7.40 (2H, m).

5.1.10. 1-Benzyl-6-trifluoromethyl-2,3-dihydroquinolin-4(1*H*)-one (17)

To a suspension of 6-trifluoromethylquinolin-4-ol (**16**) (1.00 g, 4.69 mmol) in THF (40 mL) was added *t*-BuOK (648 mg, 5.20 mmol) and the mixture was stirred at room temperature for 30 min, followed by addition of benzyl chloride (0.60 mL, 5.2 mmol). The mixture was refluxed for 1.5 h, cooled to room temperature, and filtered through a pad of Celite. The resulting solution was concentrated and purified by silica gel column chromatography ($CHCl_3$/MeOH, 97:3) to give 1-benzyl-6-trifluoromethylquinolin-4(1*H*)-one (1.00 g, 70%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 5.35 (2H, s), 6.41 (1H, d, J = 7.4 Hz), 7.10–7.20 (2H, m), 7.33–7.50 (4H, m), 7.68 (1H, d, J = 8.0 Hz), 7.70–7.78 (1H, m), 8.77 (1H, s).

The benzyl compound (1.00 g, 3.28 mmol) was dissolved in THF (20 mL) followed by addition of LiB(*sec*-Bu)$_3$H (1.0 M solution in THF, 3.60 mL) at 0 °C. The mixture was stirred at room temperature for 24 h and the reaction mixture was quenched by slow addition of H_2O (10 mL). To this were added $CHCl_3$ (50 mL) and H_2O (30 mL), the organic layer was separated, and aqueous layer was extracted with $CHCl_3$ (2× 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. The resulting residue was purified by silica gel column chromatography (benzene) to give the title compound **17** (310 mg, 31%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.79 (2H, m), 3.68 (2H, m), 4.64 (2H, s), 6.76 (1H, d, J = 9.1 Hz), 7.17 (1H, d, J = 7.4 Hz), 7.22–7.40 (4H, m), 7.49 (1H, dd, J = 2.5, 6.6 Hz), 8.19 (1H, d, J = 1.9 Hz).

5.1.11. 6-Trifluoromethyl-1,2,3,4-tetrahydroquinoline (4e)

$LiAlH_4$ (39 mg, 1.03 mmol) was suspended in Et_2O (10 mL) and THF (5 mL), and $AlCl_3$ (120 mg, 9.00 mmol) was added. After 10 min of stirring at room temperature, to the suspension was added the compound **17** (310 mg, 1.02 mmol) as an Et_2O (5 mL) solution, and then $AlCl_3$ (100 mg, 7.51 mmol) was added at room temperature. The mixture was stirred at the same temperature for 4 h, and the reaction mixture was quenched by addition of H_2O (50 mL). Saturated aqueous $NaHCO_3$ solution (20 mL) and Et_2O (50 mL) were added, and the organic layer was separated, dried over $MgSO_4$, and concentrated to give 1-benzyl-6-trifluoromethyl-1,2,3,4-tetrahydroquinoline (260 mg).

The benzyl compound obtained above (260 mg) was dissolved in MeOH (20 mL), and Pd on charcoal (10%, 30 mg) was added. The mixture was stirred at room temperature under H_2 (1 atm), and after 4 h, the mixture was filtered through a pad of Celite. The resulting solution was concentrated to give the title compound **4e** (200 mg) as a light brown oil, which was used in the next step without purification.

5.1.12. 6-Chloro-1,2,3,4-tetrahydroquinoline (4g)

To a solution of 6-chloroquinoline (**18g**) (4.00 g, 24.4 mmol) in EtOH (30 mL) were added concentrated HCl (0.1 mL) and PtO_2 (300 mg) under an Ar atmosphere, and the mixture was stirred at room temperature under H_2 (1 atm). After 8 h, the mixture was filtered to remove the catalyst, and the resulting solution was concentrated. The residue was dissolved in $CHCl_3$ (100 mL) and basified by addition of saturated aqueous $NaHCO_3$ (50 mL). The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (2× 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give the title compound **4g** (4.1 g, 100%). ^{1}H NMR data of compound **4g** were identical to that of the literature.[32]

5.1.13. 5-Methoxy-1,2,3,4-tetrahydroquinoline (4i) hydrochloride

To a solution of 5-methoxyquinoline (**18i**) (840 mg, 5.28 mmol) in MeOH (20 mL) were added concentrated HCl (0.1 mL) and PtO_2 (50 mg) under an Ar atmosphere, and the mixture was stirred at room temperature under H_2 (1 atm). After 27 h, the mixture was filtered to remove the catalyst, and the resulting solution was concentrated. To remove remaining H_2O, AcOEt (50 mL) was added and the mixture was concentrated. MeOH (10 mL) and 10% HCl methanol solution (2 mL) were added to the residue, and the mixture was concentrated in vacuo to give the title compound **4i**·HCl as a pale yellow solid (1.03 g, 93%). ^{1}H NMR (D_2O, 300 MHz) δ: 2.14 (2H, m), 2.77 (2H, m), 3.51 (2H, m), 3.90 (3H, s), 6.93 (1H, d, J = 7.4 Hz), 7.10 (1H, d, J = 8.0 Hz), 7.38 (1H, m).

5.1.14. 7-Methoxy-1,2,3,4-tetrahydroquinoline (4k) hydrochloride

To a solution of 7-methoxyquinoline[33] (**18k**) (1.02 g, 6.41 mmol) in MeOH (30 mL) was added PtO_2 (90 mg) under an Ar atmosphere, and the mixture was stirred at room temperature under H_2 (1 atm). After 16 h, the mixture was filtered through a pad of Celite to remove the catalyst, and the resulting solution was concentrated. The residue was dissolved in AcOEt (50 mL) and concentrated HCl (0.50 mL) was added, and the suspension was concentrated. To remove remaining H_2O, AcOEt (50 mL) was added again and the suspension was concentrated. The resulting material was suspended in AcOEt (50 mL) and the precipitate was collected by filtration to give the title compound **4k**·HCl (1.25 g, 97%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.94–1.98 (2H, m), 2.71–2.75 (2H, m), 3.30 (2H, t, J = 5.5 Hz), 3.74 (3H, s), 6.68–6.73 (1H, m), 6.79–6.85 (1H, m), 7.14 (1H, d, J = 8.5 Hz), 10.60 (2H, br s).

5.1.15. 7-Methyl-1,2,3,4-tetrahydroquinoline (4n) hydrochloride

Using a procedure similar to that used for the compound **4k**·HCl, the title compound **4n**·HCl was obtained from 7-methylquinoline (**18n**) (99%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.94–2.02 (2H, m), 2.29 (3H, s), 2.72–2.79 (2H, m), 3.29–3.33 (2H, m), 7.08

(1H, m), 7.09 (1H, d, J = 8.0 Hz), 7.16 (1H, d, J = 8.0 Hz), 10.96 (2H, br s).

5.1.16. 3,4-Dihydro-2*H*-benzo[*b*][1,4]oxazine (5) hydrochloride

To a solution of 2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one (**19**) (2.00 g, 13.4 mmol) in THF (70 mL) was added BH_3·THF (1.0 M solution in THF, 33 mL, 33 mmol) and the mixture was refluxed for 2 h. The reaction mixture was cooled to room temperature and quenched by addition of concentrated HCl (3 mL). After stirring for 30 min, the resulting mixture was poured into a mixture of $CHCl_3$ (100 mL) and saturated aqueous $NaHCO_3$ solution (100 mL), and the organic layer was separated. The aqueous layer was extracted with $CHCl_3$ (100 mL), and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The resulting residue was dissolved in AcOEt (80 mL) and concentrated HCl (1.3 mL) was added to precipitate a HCl salt. The precipitate produced was collected by filtration and washed with AcOEt, and dried in vacuo to afford the title compound **5**·HCl (1.93 g, 84%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.44–3.48 (2H, m), 4.29–4.32 (2H, m), 6.89-6.94 (2H, m), 7.02–7.08 (2H, m), 10.35 (2H, br s).

5.1.17. *N*-(3-Methylbut-2-enyl)acetanilide (21)

To a solution of acetanilide (**20**) (2.00 g, 14.8 mmol) in toluene (20 mL) were added K_2CO_3 (2.24 g, 16.2 mmol), NaOH (652 mg, 16.3 mmol), n-Bu_4NBr (106 mg, 0.400 mmol), and 1-chloro-3-methyl-2-buten (1.86 g, 17.8 mmol), and the mixture was heated to 75 °C and stirred vigorously. After 2 h, the resulting suspension was cooled to room temperature and H_2O (100 mL) was added, and the mixture was extracted with toluene. The organic layer was washed with 6 N HCl (50 mL) and brine (2× 50 mL), and concentrated to give the title compound **21** as yellow oil (2.93 g, 98%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.43 (3H, m), 1.67 (3H, m), 1.83 (3H, s), 4.28 (2H, d, J = 7.1 Hz), 5.22–5.27 (1H, m), 7.14 (2H, d, J = 6.9 Hz), 7.32–7.42 (3H, m).

5.1.18. 4,4-Dimethyl-1,2,3,4-tetrahydroquinoline (6) hydrochloride

To a solution of $AlCl_3$ (2.87 g, 21.5 mmol) in chlorobenzene (6 mL) was added the compound **21** (2.00 g, 9.84 mmol) and the mixture was heated to 110 °C. After stirring for 1 h, the mixture was cooled to room temperature and H_2O (7 mL) was added. The resulting mixture was extracted with toluene (50 mL), and the organic layer was washed with 6 N HCl (50 mL) to afford a toluene solution containing *N*-acetyl-4,4-dimethyl-1,2,3,4-tetrahydroquinoline. To this mixture was added 6 N HCl (7 mL), and the resulting heterogeneous solution was refluxed with vigorous stirring. After 16 h, the mixture was cooled to room temperature and poured into a mixture of 30% NaOH solution (30 mL) and toluene (50 mL), and the organic layer was separated and washed with brine (50 mL) and concentrated. The residue was dissolved in AcOEt (50 mL) followed by treatment with concentrated HCl (0.8 mL), and the resulting suspension was concentrated to give crude material. To this was added AcOEt (50 mL), and the precipitate was collected by filtration and dried in vacuo to give the title compound **6**·HCl as a white solid (1.12 g, 58% in 2 steps). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.30 (6H, s), 1.87–1.91 (2H, m), 3.33–3.37 (2H, m), 7.14–7.18 (1H, m), 7.22–7.28 (2H, m), 7.30–7.54 (1H, m), 10.78 (2H, br s).

5.1.19. General method for the amination of the cyclic aryl amines. Method A: 1-Amino-6-fluoro-1,2,3,4-tetrahydroquinoline (7c) methanesulfonate

To a solution of 6-fluoro-1,2,3,4-tetrahydroquinoline (**4c**) hydrochloride (9.05 g, 48.2 mmol) in ethanol (50 mL) was added a solution of $NaNO_2$ (4.00 g, 5.80 mmol) in H_2O (20 mL), and the mixture was cooled to 0 °C on an ice bath. The resulting solution was stirred vigorously, while concentrated HCl (5.0 mL) was added dropwise at 0 °C. After checking complete consumption of the substrate material by TLC analysis, a solution of NaOH (20.0 g, 500 mmol) in H_2O (45 mL) and $Na_2S_2O_4$ (75%, 33.5 g, 144 mmol) was added at 0 °C. The suspension was refluxed at 90 °C for 2 h, and then the reaction mixture was cooled to room temperature. To this mixture was added H_2O (400 mL) and extracted with toluene (400 mL). The organic phase was filtered and concentrated to give oily material. The crude product was dissolved in AcOEt (350 mL), and methanesulfonic acid (4.63 g, 48.2 mmol) in AcOEt (100 mL) was added dropwise. The precipitate produced was collected by filtration and washed with AcOEt, and dried *in vacuo* to afford the title compound **7c**·$MeSO_3H$ (9.25 g, 73%) as a white solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.95–2.05 (2H, m), 2.35 (s, 3H), 2.76 (2H, dd, J = 6.6, 6.6 Hz), 3.36 (2H, dd, J = 5.6, 5.6 Hz), 7.01 (1H, d, J = 9.0 Hz), 7.05–7.15 (2H, m), 10.09 (3H, br s).

5.1.20. General method for the indole synthesis. Method B: (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-8′-fluoro-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino[2′,1′:6,7]-morphinan-3,14-diol (1c) methanesulfonate (TRK-851)

To a suspension of naltrexone (**10a**) hydrochloride (693 mg, 1.83 mmol) in EtOH (10 mL) were added **7c**·$MeSO_3H$ (527 mg, 2.01 mmol) and methanesulfonic acid (0.59 mg, 9.15 mmol). The solution was stirred at 100 °C for 8 h. After the reaction mixture was cooled to room temperature, the mixture was poured into saturated aqueous $NaHCO_3$ (50 mL). The mixture was extracted with AcOEt (2× 50 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated to give a pale brown amorphous solid. This crude material was purified by silica gel column chromatography (1% MeOH in $CHCl_3$) to give the compound **1c** as a white solid (756 mg, 87%). Monomethanesulfonate was prepared by addition of methanesulfonic acid to the compound solution in MeOH. The solid obtained by concentration was suspended in AcOEt, and filtered to give the title compound **1c**·$MeSO_3H$ as a white solid. Mp 223 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.39–0.54 (2H, m), 0.58–0.77 (2H, m), 1.09 (1H, m), 1.84 (1H, d, J = 12.6 Hz), 2.08–2.24 (2H, m), 2.31 (4.2H, s), 2.53–2.78 (3H, m), 2.89–2.98 (4H, m), 3.11 (1H, m), 3.19–3.28 (1H, m), 3.35–3.47 (2H, m), 4.06 (1H, m), 4.12–4.21 (1H, m), 4.26–4.35 (1H, m), 5.90 (1H, s), 6.32 (1H, br s), 6.58–6.65 (2H, m), 6.78 (1H, m), 7.19 (1H, m), 8.94 (1.4H, br s), 9.21 (1H, br s). IR (KBr, cm^{-1}): 3400, 1626, 1493, 1460, 1437, 1193, 1046, 557. MS (FAB) m/z $(M+H)^+$ = 473. Anal. Calcd for $C_{29}H_{29}FN_2O_3$·1.4 $MeSO_3H$·0.4 H_2O: C, 59.44; H, 5.81; N, 4.56; F, 3.09; S, 7.31. Found: C, 59.29; H, 5.93; N, 4.84; F, 3.01; S, 7.22.

5.1.21. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-8′-trifluoromethoxy-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]-quinolino[2′,1′:6,7]morphinan-3,14-diol (1d) methanesulfonate

Using the method A, 1-amino-6-trifluoromethoxy-1,2,3,4-tetrahydroquinoline (**7d**) was obtained from 6-trifluoromethoxy-1,2,3,4-tetrahydroquinoline (**4d**), and using the method B, the title compound **1d**·$MeSO_3H$ was obtained from the compound **7d** and naltrexone (**10a**) hydrochloride (35% in 2 steps). Mp 235 °C (dec). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.41–0.53 (2H, m), 0.60–0.66 (1H, m), 0.70–0.76 (1H, m), 1.05–1.13 (1H, m), 1.85 (1H, d, J = 11.5 Hz), 2.12–2.26 (2H, m), 2.29 (3.54H, s), 2.56 (1H, d, J = 15.8 Hz), 2.59–2.63 (2H, m), 2.68–2.77 (1H, m), 2.91–3.01 (3H, m), 3.12 (1H, d, J = 10.7 Hz), 3.24 (1H, dd, J = 12.7, 6.8 Hz), 3.40 (2H, m), 4.05 (1H, d, J = 6.3 Hz), 4.16–4.22 (1H, m), 4.31–4.36 (1H, m), 5.92 (1H, s), 6.31 (1H, s), 6.59–6.64 (2H, m), 6.90 (1H, s), 7.15 (1H, s), 8.94 (1.18H, br s), 9.22 (1H, s). IR (KBr, cm^{-1}): 3420, 2935, 1637, 1632, 1507, 1494, 1457, 1435, 1374, 1259, 1213, 1158, 1115, 1044, 913, 868, 798, 783. MS (free base, EI) m/z $(M)^+$ = 538. Anal. Calcd for $C_{30}H_{29}F_3N_2O_4$·1.18$MeSO_3H$·0.5H_2O·0.1Et_2O: C, 56.75; H, 5.39; N, 4.19; F, 8.53; S, 5.66. Found: C, 56.96; H, 5.24; N, 4.36; F, 8.21; S, 5.52.

5.1.22. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-8′-trifluoromethyl-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino[2′,1′:6,7]morphinan-3,14-diol (1e) methanesulfonate

Using the method A, 1-amino-6- trifluoromethyl-1,2,3,4-tetrahydroquinoline (**7e**) was obtained from 6-trifluoromethyl-1,2,3,4-tetrahydroquinoline (**4e**), and using the method B, the title compound **1e**·$MeSO_3H$ was obtained from the compound **7e** and naltrexone (**10a**) hydrochloride (22% from **17**). Mp 259 °C (dec). ^1H NMR (DMSO-d_6, 300 MHz) δ: 0.41–0.53 (2H, m), 0.60–0.65 (1H, m), 0.70–0.76 (1H, m), 1.06–1.14 (1H, m), 1.87 (1H, d, J = 13.3 Hz), 2.13–2.90 (2H, m), 2.29 (3.75H, s), 2.54–2.66 (2H, m), 2.69–2.78 (1H, m), 2.91–2.98 (1H, m), 3.00–3.05 (3H, m), 3.13 (1H, d, J = 10.8 Hz), 3.21–3.27 (1H, m), 3.30–3.40 (1H, m), 3.45 (1H, d, J = 19.8 Hz), 4.06 (1H, d, J = 6.4 Hz), 4.19–4.25 (1H, m), 4.34–4.40 (1H, m), 5.59 (1H, s), 6.33 (1H, s), 6.60–6.64 (2H, m), 7.19 (1H, s), 7.57 (1H, s), 8.95 (1.25H, br s), 9.22 (1H, s). IR (KBr, cm^{-1}):3421, 2953, 1637, 1620, 1507, 1459, 1433, 1398, 1327, 1276, 1240, 1206, 1113, 1046, 916, 872, 801, 783. MS (free base, EI) m/z $(M)^+$ = 522. Anal. Calcd for $C_{30}H_{29}F_3N_2O_3$·1.25$MeSO_3H$·0.35H_2O·0.5Et_2O: C, 58.21; H, 5.83; N, 4.08; F, 8.31; S, 5.84. Found: C, 58.53; H, 5.61; N, 4.41; F, 7.94; S, 5.65.

5.1.23. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-9′-trifluoromethyl-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]-quinolino[2′,1′:6,7]morphinan-3,14-diol (1f) methanesulfonate

Using the method A, 1-amino-6-trifluoromethyl-1,2,3,4-tetrahydroquinoline (**7f**) methanesulfonate was obtained from 7-trifluoromethyl-1,2,3,4-tetrahydroquinoline (**4f**) hydrochloride (79%). ^1H NMR (DMSO-d_6, 300 MHz) δ: 1.99–2.08 (2H, m), 2.33 (3H, s), 2.79–2.84 (2H, m), 3.40–3.44 (2H, m), 7.23 (1H, d, J = 8.8 Hz), 7.31 (1H, d, J = 8.0 Hz), 7.38 (1H, s), 9.99 (3H, br s). MS (free base, EI) m/z $(M)^+$ = 216.

Using the method B, the title compound **1f**·$MeSO_3H$ was obtained from the compound **7f**·$MeSO_3H$ and naltrexone (**10a**) hydrochloride (93%). Mp 253 °C (dec). ^1H NMR (DMSO-d_6, 400 MHz) δ: 0.40–0.57 (2H, m), 0.60–0.78 (2H, m), 1.08–1.19 (1H, m), 1.86 (1H, d, J = 12.9 Hz), 2.12–2.29 (2H, m), 2.31 (3.6H, s), 2.51–2.80 (3H, m), 2.85–2.94 (1H, m), 3.01–3.29 (5H, m), 3.40–3.49 (2H, m), 4.11 (1H, d, J = 5.2 Hz), 4.19–4.29 (1H, m), 4.35–4.44 (1H, m), 5.97 (1H, s), 6.37 (1H, br s), 6.61–6.65 (2H, m), 7.04 (1H, d, J = 7.1 Hz), 7.31 (1H, d, J = 7.7 Hz), 8.90 (1.2H, br s), 9.23 (1H, br). IR (KBr, cm^{-1}): 3400, 2928, 1620, 1510, 1460, 1437, 1388, 1330, 1303, 1195, 1118, 1044, 919, 859, 847, 801, 785. MS (free base, EI) m/z $(M)^+$ = 522. Anal. Calcd for $C_{30}H_{29}F_3N_2O_3$ ·1.2$MeSO_3H$·0.4H_2O: C, 58.09; H, 5.41; N, 4.34; F, 8.84; S, 5.96. Found: C, 58.02; H, 5.58; N, 4.37; F, 8.84; S, 6.14.

5.1.24. (5*R*,9*R*,13*S*,14*S*)-8′-Chloro-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 (1g) hydrochloride

Using the method A, 1-amino-6-chloro-1,2,3,4-tetrahydroquinoline (**7g**) methanesulfonate was obtained from 6-chloro-1,2,3,4-tetrahydroquinoline (**4g**) (57%). The title compound **1g**·HCl was obtained from the compound **7g**·$MeSO_3H$ and naltrexone (**10a**) hydrochloride (78%) using the method B. 10% HCl methanol solution was used instead of methanesulfonic acid in the salt formation process. Mp 198–202 °C (dec). ^1H NMR (DMSO-d_6, 400 MHz) δ: 0.53–0.56 (2H, m), 0.75–0.91 (2H, m), 1.11–1.15 (1H, m), 1.97 (1H, dd, J = 3.0, 13.5 Hz), 2.24–2.29 (2H, m), 2.73–2.80 (3H, m), 2.89–3.02 (4H, m), 3.17 (1H, dd, J = 4.4, 13.0 Hz), 3.30–3.43 (3H, m), 4.18–4.86 (3H, m), 4.86 (2H, br s), 5.84 (1H, s), 6.65–6.70 (2H, m), 6.85 (1H, s), 7.20 (1H, s), 7.32 (1H, s). IR (KBr, cm^{-1}): 3378, 2938, 1640, 1620, 1504, 1435, 1116, 1060, 1036, 915, 864, 801. MS (FAB) m/z $(M+H)^+$ = 489. Anal. Calcd for $C_{29}H_{29}ClN_2O_3$·HCl·0.8H_2O·0.1AcOEt: C, 64.36; H, 5.95; Cl, 12.92; N, 5.11. Found: C, 64.56; H, 6.19; Cl, 12.64; N, 5.21.

5.1.25. (5*R*,9*R*,13*S*,14*S*)-7′-Bromo-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 (1h) methanesulfonate

Using the method A, 1-amino-5-bromo-1,2,3,4-tetrahydroquinoline (**7h**) methanesulfonate was obtained from 5-bromo-1,2,3,4-tetrahydroquinoline (**4h**) hydrochloride (80%), and using the method B, the title compound **1h**·$MeSO_3H$ was obtained from the compound **7h**·$MeSO_3H$ and naltrexone (**10a**) hydrochloride (78%). Mp 245 °C (dec). ^1H NMR (DMSO-d_6, 300 MHz) δ: 0.38–0.57 (2H, m), 0.58–0.79 (2H, m), 1.03–1.17 (1H, m), 1.84 (1H, d, J = 12.3 Hz), 2.14–2.29 (2H, m), 2.30 (3.6H, s), 2.53–2.79 (3H, m), 2.82–3.00 (4H, m), 3.10–3.35 (3H, m), 3.42–3.50 (1H, m), 4.06 (1H, d, J = 6.3 Hz), 4.10–4.12 (1H, m), 4.23–4.37 (1H, m), 5.90 (1H, s), 6.35 (1H, br s), 6.59–6.65 (2H, m), 7.10 (1H, d, J = 8.5 Hz), 7.16 (1H, d, J = 8.5 Hz), 8.95 (1.2H, br s), 9.23 (1H, br s). IR (KBr, cm^{-1}): 3407, 2936, 1638, 1506, 1469, 1432, 1375, 1359, 1327, 1207, 1115, 1040, 947, 914, 856, 785. MS (FAB) m/z $(M+H)^+$ = 533. Anal. Calcd for $C_{29}H_{29}BrN_2O_3$·1.2$MeSO_3H$·0.4H_2O: C, 55.29; H, 5.32; Br, 12.18; N, 4.27. Found: C, 54.96; H, 5.33; Br, 12.56; N, 4.26.

5.1.26. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-7′-methoxy-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino [2′,1′:6,7]morphinan-3,14-diol (1i) methanesulfonate

Using the method A, 1-amino-5-methoxy-1,2,3,4-tetrahydroquinoline (**7i**) methanesulfonate was obtained from 5-methoxy-1,2,3,4-tetrahydroquinoline (**4i**) (61%). ^1H NMR (DMSO-d_6, 300 MHz) δ: 1.75–2.30 (2H, m), 2.32 (3H, s), 2.56 (2H, dd, J = 6.8, 6.8 Hz), 3.32 (2H, dd, J = 5.0, 5.0 Hz), 3.77 (3H, s), 6.66 (1H, d, J = 8.2 Hz), 6.68 (1H, d, J = 8.2 Hz), 7.18 (1H, dd, J = 8.2, 8.2 Hz), 10.06 (3H, br s).

Using the method B, the title compound **1i**·$MeSO_3H$ was obtained from the compound **7i**·$MeSO_3H$ and naltrexone (**10a**) hydrochloride (68%). Mp 230–239 °C (dec). ^1H NMR (DMSO-d_6, 300 MHz) δ: 0.39–0.54 (2H, m), 0.58–0.77 (2H, m), 1.08 (1H, m), 1.83 (1H, d, J = 12.4 Hz), 2.03–2.21 (2H, m), 2.29 (3H, s), 2.42–2.98 (7H, m), 3.10 (1H, m), 3.20–3.46 (3H, m), 3.77 (3H, s), 4.04–4.13 (2H, m), 4.19–4.27 (1H, m), 5.85 (1H, s), 6.31 (1H, br s), 6.60 (2H, m), 6.74 (1H, d, J = 8.8 Hz Hz), 7.14 (1H, d, J = 8.8 Hz), 8.92 (1H, br s), 9.19 (1H, br s). IR (KBr, cm^{-1}): 3400, 1628, 1508, 1247, 1195, 1116, 1052, 785, 561. MS (FAB) m/z $(M+H)^+$ = 485. Anal. Calcd for $C_{30}H_{32}N_2O_4$·$MeSO_3H$·0.8H_2O: C, 62.57; H, 6.37; N, 4.71; S, 5.39. Found: C, 62.37; H, 6.41; N, 4.86; S, 5.67.

5.1.27. (5*R*,9*R*,13*S*,14*S*)-9′-Chloro-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 (1j) methanesulfonate

Using the method A, 1-amino-7-chloro-1,2,3,4-tetrahydroquinoline (**7j**) methanesulfonate was obtained from 7-chloro-1,2,3,4-tetrahydroquinoline (**4j**) (79%), and using the method B, the title compound **1j**·$MeSO_3H$ was obtained from the compound **7j**·$MeSO_3H$ and naltrexone (**10a**) hydrochloride (26%). Mp 245–255 °C (dec). ^1H NMR (DMSO-d_6, 300 MHz) δ: 0.39–0.54 (2H, m), 0.59–0.66 (1H, m), 0.70–0.77 (1H, m), 1.06–1.15 (1H, m), 1.85 (1H, d, J = 13.0 Hz), 2.08–2.24 (2H, m), 2.30 (3.6H, s), 2.57–2.64 (1H, m), 2.67–2.76 (2H, m), 2.86–2.93 (3H, m), 3.11 (1H, d, J = 11.5 Hz), 3.25–3.32 (1H, m), 3.35–3.45 (3H, m), 4.08 (1H, d, J = 6.6 Hz), 4.15–4.20 (1H, m), 4.30–4.35 (1H, m), 5.91 (1H, s), 6.35 (1H, br s), 6.60–6.65 (2H, m), 6.84 (1H, d, J = 7.7 Hz), 6.88 (1H, d, J = 7.5 Hz), 8.91 (1.2H, br s), 9.21 (1H, br s). IR (KBr, cm^{-1}): 3407, 2936, 1638, 1506, 1492, 1472, 1432, 1368, 1327, 1296, 1205, 1115, 1046, 915, 847, 799, 785. MS (FAB) m/z $(M+H)^+$ = 489. Anal. Calcd for $C_{29}H_{29}ClN_2O_3$·1.2$MeSO_3H$·0.5H_2O: C, 59.21; H, 5.81; Cl, 5.71; N, 4.51; S, 6.20. Found: C, 58.93; H, 5.85; Cl, 6.03; N, 4.53; S, 6.30.

5.1.28. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-9′-methoxy-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino-[2′,1′:6,7]morphinan-3,14-diol (1k) methanesulfonate

Using the method A, 1-amino-7-methoxy-1,2,3,4-tetrahydroquinoline (**7k**) methanesulfonate was obtained from 7-methoxy-1,2,3,4-tetrahydroquinoline (**4k**) hydrochloride (89%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.95–2.09 (2H, m), 2.34 (3H, s), 2.67 (2H, t, J = 6.6 Hz), 3.35 (2H, t, J = 5.5 Hz), 3.73 (3H, s), 6.55 (1H, dd, J = 2.5, 6.0 Hz), 6.67 (1H, d, J = 2.2 Hz), 7.01 (1H, d, J = 8.2 Hz), 10.09 (3H, br s).

Using the method B, the title compound **1k**·$MeSO_3H$ was obtained from the compound **7k**·$MeSO_3H$ and naltrexone (**10a**) (92%). Mp 245–255 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.41–0.55 (2H, m), 0.58–0.78 (2H, m), 1.04–1.16 (1H, m), 1.82 (1H, d, J = 11.5 Hz), 2.02–2.23 (2H, m), 2.32 (3.9H, s), 2.54–2.76 (3H, m), 2.82–2.95 (3H. m), 3.09 (1H, d, J = 12.4 Hz), 3.20–3.45 (4H, m), 3.75 (3H, s), 4.06 (1H, d, J = 6.0 Hz), 4.11–4.19 (1H, m), 4.21–4.30 (1H, m), 5.86 (1H, s), 6.25 (1H, br s), 6.33 (1H, d, J = 7.7 H), 6.59 (1H, d, J = 8.2 Hz), 6.63 (1H, d, J = 8.2 Hz), 6.75 (1H, d, J = 7.9 Hz), 8.90 (1.3H, br s), 9.20 (1H, br s). IR (KBr, cm^{-1}): 3400, 1620, 1514, 1462, 1433, 1373, 1296, 1257, 1178, 1116, 1048, 909, 845, 783. MS (free base, EI) m/z (M)$^+$ = 484. Anal. Calcd for $C_{30}H_{32}N_2O_3$·1.3$MeSO_3H$·0.2H_2O: C, 61.31; H, 6.18; N, 4.57; S, 6.80. Found: C, 61.15; H, 6.41; N, 4.82; S, 6.86.

5.1.29. (5*R*,9*R*,13*S*,14*S*)-7′-Chloro-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 (1l) methanesulfonate

Using the method A, 1-amino-5-chloro-1,2,3,4-tetrahydroquinoline (**7l**) methanesulfonate was obtained from 5-chloro-1,2,3,4-tetrahydroquinoline (**4l**) hydrochloride (90%). ^{1}H NMR (DMSO-d_6, 300 MHz) d: 2.00–2.12 (2H, m), 2.33 (3H, s), 2.73 (2H, dd, J = 6.6, 6.6 Hz), 3.38 (2H, dd, J = 5.4, 5.4 Hz), 7.01 (1H, d, J = 8.4 Hz), 7.10 (1H, d, J = 7.2 Hz), 7.24 (1H, m), 10.05 (3H, br s).

Using the method B, the title compound **1l**·$MeSO_3H$ was obtained from the compound **7l**·$MeSO_3H$ and naltrexone (**10a**) (62%). Mp 242 °C (dec). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.40–0.46 (1H, m), 0.47–0.53 (1H, m), 0.60–0.66 (1H, m), 0.70–0.76 (1H, m), 1.05–1.13 (1H, m), 1.83–1.87 (1H, m), 2.14–2.28 (2H, m), 2.30 (3.6H, s), 2.55 (1H, d, J = 16.1 Hz Hz), 2.59–2.64 (1H, m), 2.67–2.77 (1H, m), 2.88–3.00 (4H, m), 3.11 (1H, d, J = 11.6 Hz), 3.22–3.28 (1H, m), 3.36–3.46 (2H, m), 4.06 (1H, d, J = 6.4 Hz Hz), 4.12–4.18 (1H, m), 4.28–4.33 (1H, m), 5.90 (1H, s), 6.33 (1H, br s), 6.60 (1H, d, J = 8.1 Hz), 6.63 (1H, d, J = 8.1 Hz), 6.97 (1H, d, J = 8.5 Hz), 7.21 (1H, d, J = 8.4 Hz), 8.93 (1.2H, br s), 9.21 (1H, br s). IR (KBr, cm^{-1}):3858, 2942, 1639, 1505, 1469, 1432, 1378, 1324, 1203, 1115, 1044, 914, 861, 790. MS (free base, EI) m/z (M)$^+$ = 488. Anal. Calcd for $C_{29}H_{29}ClN_2O_3$·1.2 $MeSO_3H$·0.4H_2O: C, 59.31; H, 5.70; Cl, 5.80; N, 4.58; S, 6.29. Found: C, 59.30; H, 5.90; Cl, 5.96; N, 4.52; S, 6.35.

5.1.30. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-8′-methoxy-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino-[2′,1′:6,7]morphinan-3,14-diol (1m) methanesulfonate

Using the method A, 1-amino-6-methoxy-1,2,3,4-tetrahydroquinoline (**7m**) methanesulfonate was obtained from 6-methoxy-1,2,3,4-tetrahydroquinoline (**4m**) hydrochloride (82%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.94–2.01 (2H, m), 2.34 (3H, s), 2.51–2.77 (2H, m), 3.32 (2H, t, J = 5.5 Hz), 3.71 (3H, s), 6.71 (1H, d, J = 3.0 Hz), 6.80 (1H, dd, J = 3.0, 9.1 Hz), 7.05 (1H, d, J = 9.1 Hz), 9.96 (3H, br s).

Using the method B, the title compound **1m**·$MeSO_3H$ was obtained from the compound **7m**·$MeSO_3H$ and naltrexone (**10a**) (75%). Mp 218 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.40–0.53 (2H, m), 0.60–0.66 (1H, m), 0.69–0.77 (1H, m), 1.04–1.12 (1H, m), 1.83 (1H, d, J = 11.2 Hz), 2.08–2.22 (2H, m), 2.30 (3.9H, s), 2.50–2.76 (3H, m), 2.86–2.98 (4H, m), 3.11 (1H, d, J = 11.7 Hz), 3.17 (1H, dd, J = 6.8, 20.0 Hz), 3.33–3.47 (2H, m), 3.68 (3H, s), 4.05 (1H, d, J = 6.3 Hz), 4.11–4.17 (1H, m), 4.23–4.30 (1H, m), 5.86 (1H, s), 6.30 (1H, br s), 6.57 (1H, m), 6.61 (1H, d, J = 5.4 Hz), 6.65 (1H, d, J = 5.4 Hz), 6.66–6.68 (1H, m), 8.93 (1.3H, br s), 9.19 (1H, br s). IR (KBr, cm^{-1}): 3400, 2834, 1620, 1495, 1460, 1437, 1241, 1195, 1116, 1052, 926, 758. MS (FAB) m/z (M+H)$^+$ = 485. Anal. Calcd for $C_{30}H_{32}N_2O_4$·1.3$MeSO_3H$·0.6H_2O: C, 60.60; H, 6.24; N, 4.52; S, 6.27. Found: C, 60.60; H, 6.44; N, 4.70; S, 6.52.

5.1.31. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-9′-methyl-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino-[2′,1′:6,7]morphinan-3,14-diol (1n) methanesulfonate

Using the method A, 1-amino-7-methyl-1,2,3,4-tetrahydroquinoline (**7n**) methanesulfonate was obtained from 7-methyl-1,2,3,4-tetrahydroquinoline (**4n**) hydrochloride (86%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.96–2.04 (2H, m), 2.26 (3H, s), 2.35 (3H, s), 2.67-2.72 (2H, m), 3.35 (2H, t, J = 5.5 Hz), 6.78 (1H, dd, J = 0.8, 7.7 Hz), 6.89 (1H, s), 6.99 (1H, d, J = 7.7 Hz), 10.10 (3H, br s).

Using the method B, the title compound **1n**·$MeSO_3H$ was obtained from the compound **7n**·$MeSO_3H$ and naltrexone (**10a**) benzoate (90%). Mp 248 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.40–0.57 (2H, m), 0.58–0.79 (2H, m), 1.04–1.16 (1H, m), 1.84 (1H, d, J = 12.3 Hz), 2.07–2.22 (2H, m), 2.32 (3.3H, s), 2.46 (3H, s), 2.53–2.67 (3H, m), 2.78 (1H, d, J = 16.5 Hz), 2.84–2.97 (2H, m), 3.07–3.16 (1H, m), 3.21–3.32 (2H, m), 3.37–3.49 (2H, m), 4.06 (1H, d, J = 6.0 Hz), 4.12–4.33 (2H, m), 5.88 (1H, s), 6.30 (1H, br s), 6.58–6.65 (3H, m), 6.72 (1H, d, J = 7.1 Hz), 8.97 (1.1H, br s), 9.17 (1H, br s). IR (KBr, cm^{-1}): 3400, 2926, 1657, 1562, 1510, 1460, 1433, 1296, 1195, 1116, 1052, 849, 785. MS (FAB) m/z (M+H)$^+$ = 469. Anal. Calcd for $C_{30}H_{32}N_2O_3$·1.1$MeSO_3H$·0.7H_2O: C, 63.64; H, 6.49; N, 4.77; S, 6.01. Found: C, 63.51; H, 6.58; N, 4.06; S, 6.06.

5.1.32. (5*R*,9*R*,13*S*,14*S*)-9′-Bromo-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 (1o) methanesulfonate

Using the method A, 1-amino-7-bromo-1,2,3,4-tetrahydroquinoline (**7o**) methanesulfonate was obtained from 7-bromo-1,2,3,4-tetrahydroquinoline (**4o**) hydrochloride (75%), and using the method B, the title compound **1o**·$MeSO_3H$ was obtained from compound **7o**·$MeSO_3H$ and naltrexone (**1a**) hydrochloride (81%). Mp 250 °C (dec). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 0.39–0.57 (2H, m), 0.58–0.80 (2H, m), 1.03–1.19 (1H, m), 1.85 (1H, d, J = 11.5 Hz), 2.07–2.25 (2H, m), 2.31 (3.6H, s), 2.53–2.79 (3H, m), 2.82–2.98 (3H, m), 3.11 (1H, d, J = 12.1 Hz), 3.22–3.32 (1H, m), 3.35–3.48 (2H, m), 3.53 (1H, d, J = 16.5 Hz), 4.08 (1H, d, J = 6.3 Hz), 4.11–4.23 (1H, m), 4.27–4.40 (1H, m), 5.92 (1H, s), 6.37 (1H, br s), 6.60–6.66 (2H, m), 6.79 (1H, d, J = 7.7 Hz), 7.05 (1H, d, J = 7.7 Hz), 8.94 (1.2H, br s), 9.25 (1H, br s). IR (KBr, cm^{-1}): 3412, 2954, 1640, 1504, 1465, 1432, 1367, 1325, 1199, 1115, 1046, 913, 844, 782. MS (FAB) m/z (M+H)$^+$ = 533. Anal. Calcd for $C_{29}H_{29}BrN_2O_3$·1.2$MeSO_3H$·0.7H_2O: C, 54.84; H, 5.36; Br, 12.08; N, 4.24; S, 5.82. Found: C, 54.84; H, 5.52; Br, 12.02; N, 4.18; S, 5.94.

5.1.33. (5*R*,9*R*,13*S*,14*S*)-8′-Bromo-17-cyclopropylmethyl-6,7-didehydro-4,5-epoxy-3-methoxy-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino[2′,1′:6,7]morphinan-14-ol (1p) methanesulfonate

Using the method A, 1-amino-6-bromo-1,2,3,4-tetrahydroquinoline (**7p**) methanesulfonate was obtained from 6-bromo-1,2,3,4-tetrahydroquinoline (**4p**) hydrochloride (82%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.95–2.04 (2H, m), 2.34 (3H, s), 2.73–2.77 (2H, m), 3.35–3.39 (2H, m), 6.98 (1H, d, J = 8.8 Hz), 7.31 (1H, s), 7.37 (1H, dd, J = 2.5, 7.7 Hz), 10.03 (3H, br s).

Using the method B, the title compound **1p**·$MeSO_3H$ was obtained from the compound **7p**·$MeSO_3H$ and 3-*O*-methylnaltrexone (**10b**) (73%). Mp 220–222 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.41–0.51 (2H, m), 0.60–0.67 (1H, m), 0.70–0.77 (1H, m), 1.07–1.18 (1H, m), 1.87 (1H, d, J = 12.7 Hz), 2.08–2.26 (2H, m), 2.29 (3H, s),

2.52–2.76 (3H, m), 2.90–2.99 (4H, m), 3.13 (1H, d, J = 12.2 Hz), 3.24–3.31 (1H, m), 3.38–3.45 (1H, m), 3.50 (1H, d, J = 20.0 Hz), 3.53 (3H, s), 4.06–4.14 (2H, m), 4.30–4.36 (1H, m), 5.97 (1H, s), 6.36 (1H, s), 6.74 (1H, d, J = 8.3 Hz), 6.82 (1H, d, J = 8.3 Hz), 7.04 (1H, d, J = 1.5 Hz), 7.35 (1H, t, J = 1.5 Hz), 8.96 (1H, br s). IR (KBr, cm^{-1}): 3400, 2934, 1636, 1613, 1510, 1485, 1437, 1367, 1315, 1286, 1199, 1122, 1044, 978, 903, 864, 785. MS (FAB) m/z $(M+H)^+$ = 547. Anal. Calcd for $C_{30}H_{31}BrN_2O_3 \cdot MeSO_3H \cdot 0.3H_2O$: C, 57.37; H, 5.53; Br, 12.31; N, 4.32; S; 4.94. Found: C, 57.23; H, 5.63; Br, 12.42; N, 4.23; S; 5.04.

5.1.34. (5*R*,9*R*,13*S*,14*S*)-2′a-Aza-17-cyclopropylmethyl-6,7-didehydro-4,5-epoxy-3′,4′-dihydro-5′-oxa-acenaphthyleno[2′,1′:6,7]morphinan-3,14-diol (2a) methanesulfonate

Using the method A, 4-amino-3,4-dihydro-2*H*-benzo[*b*][1,4]-oxazine (**8**) methanesulfonate was obtained from 3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (**5**) hydrochroride (89%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.35 (3H, s), 3.51–3.54 (2H, m), 4.36–4.39 (2H, m), 6.84–6.87 (1H, m), 6.90–7.01 (2H, m), 7.14–7.17 (1H, m), 10.15 (3H, br s).

Using the method B, the title compound **2a**·$MeSO_3H$ was obtained from compound **8**·$MeSO_3H$ and naltrexone (**10a**) hydrochloride (97%). Mp 240 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.41–0.54 (2H, m), 0.57–0.79 (2H, m), 1.03–1.15 (1H, m), 1.86 (1H, d, J = 11.5 Hz), 2.31 (3.6H, s), 2.51–2.79 (3H, m), 2.91–3.00 (2H, m), 3.12 (1H, d, J = 11.8 Hz), 3.21–3.30 (1H, m), 3.34–3.48 (3H, m), 4.08 (1H, d, J = 6.9 Hz), 4.23–4.30 (1H, m), 4.44–4.49 (1H, m), 4.52–4.60 (1H, m), 5.90 (1H, s), 6.37 (1H, br s), 6.56–6.65 (3H, m), 6.83–6.89 (1H, m), 6.95 (1H, d, J = 7.9 Hz), 8.96 (1.2H, br s), 9.20 (1H, br). IR (KBr, cm^{-1}): 3400, 1636, 1584, 1506, 1460, 1435, 1383, 1325, 1270, 1245, 1197, 1116, 1060, 1009, 948, 913, 888, 872, 839, 785, 733. MS (free base, EI) m/z $(M)^+$ = 456. Anal. Calcd for $C_{28}H_{28}N_2O_4 \cdot 1.2MeSO_3H \cdot 0.1H_2O$: C, 61.14; H, 5.80; N, 4.88; S, 6.71. Found: C, 61.15; H, 5.97; N, 4.97; S, 6.41.

5.1.35. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-6′,6′-dimethyl-4,5-epoxy-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]-quinolino[2′,1′:6,7]morphinan-3,14-diol (3a) methanesulfonate

Using the method A, 1-amino-4,4-dimethyl-1,2,3,4-tetrahydroquinoline (**9**) methanesulfonate was obtained from 4,4-dimethyl-1,2,3,4-tetrahydroquinoline (**6**) hydrochloride (83%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 1.27 (6H, s), 1.85–1.89 (2H, m), 2.32 (3H, s), 3.38 (2H, t, J = 5.5 Hz), 6.91–7.01 (2H, m), 7.16–7.22 (1H, m), 7.34–7.37 (1H, m), 10.06 (3H, br s).

Using the method B, the title compound **3a**·$MeSO_3H$ was obtained from the compound **9**·$MeSO_3H$ and naltrexone (**10a**) hydrochloride (99%). Mp 242 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.40–0.55 (2H, m), 0.59–0.79 (2H, m), 1.02–1.13 (1H, m), 1.32 (3H, s), 1.34 (3H, s), 1.85 (1H, d, J = 11.3 Hz), 1.97–2.00 (2H, m), 2.30 (3.6H, s), 2.53-2.80 (3H, m), 2.88–2.98 (2H, m), 3.09–3.14 (1H, m), 3.21–3.48 (3H, m), 4.07 (1H, d, J = 6.9 Hz), 4.22–4.30 (2H, m), 5.91 (1H, s), 6.32 (1H, br s), 6.58–6.64 (2H, m), 6.92–6.97 (1H, m), 7.05 (1H, d, J = 6.6 Hz), 7.18 (1H, d, J = 7.7 Hz), 8.94 (1.2H, br s), 9.22 (1H, br s). IR (KBr, cm^{-1}): 3400, 2930, 1626, 1508, 1464, 1433, 1365, 1296, 1187, 1116, 1052, 1013, 948, 915, 857, 833, 875, 745. MS (free base, EI) m/z $(M)^+$ = 482. Anal. Calcd for $C_{31}H_{34}N_2O_3 \cdot 1.2MeSO_3H \cdot 0.2H_2O$: C, 64.29; H, 6.57; N, 4.66; S, 6.40. Found: C, 64.41; H, 6.61; N, 4.84; S, 6.23.

5.1.36. (5*R*,9*R*,13*S*,14*S*)-8′-Bromo-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 (1u) methanesulfonate

Compound **1p** (515 mg, 0.941 mmol) was dissolved in CH_2Cl_2 (20 mL) and the mixture was cooled to 0 °C on an ice bath. To the solution was added BBr_3 (1 M dichloromethane solution, 5.0 mL, 5.0 mmol), and the mixture was stirred at the same temperature for 40 min. The reaction mixture was quenched by addition of H_2O (20 mL), and the resulting mixture was poured into a mixture of $CHCl_3$ (80 mL) and saturated aqueous $NaHCO_3$ (80 mL). The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (2× 50 mL). Combined organic layers were dried over anhydrous Na_2SO_4 and concentrated. The resulting light brown amorphous material was purified by silica gel column chromatography (1–2% MeOH in $CHCl_3$) to give the compound **1u** (316 mg, 63%). 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 **1u**·$MeSO_3H$ as a white solid. Mp 234 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.42–0.52 (2H, m), 0.58–0.78 (2H, m), 1.02–1.17 (1H, m), 1.84 (1H, d, J = 12.1 Hz), 2.06–2.24 (2H, m), 2.31 (3.75H, s), 2.51–2.80 (3H, m), 2.90–2.99 (4H, m), 3.10–3.17 (1H, m), 3.22 (1H, dd, J = 19.4, 6.6 Hz), 3.35–3.48 (2H, m), 4.05 (1H, d, J = 6.9 Hz), 4.10–4.23 (1H, m), 4.25–4.37 (1H, m), 5.91 (1H, s), 6.34 (1H, br s), 6.59–6.67 (2H, m) 7.03 (1H, s), 7.36 (1H, s), 8.95 (1.25H, br s), 9.23 (1H, br). IR (KBr, cm^{-1}): 3400, 2928, 1618, 1508, 1485, 1462, 1433, 1367, 1328, 1195, 1116, 1054, 948, 915, 888, 866, 785. MS (free base, EI) m/z $(M)^+$ = 532. Anal. Calcd for $C_{29}H_{29}BrN_2O_3 \cdot 1.25MeSO_3H \cdot 0.1H_2O$: C, 55.44; H, 5.26; Br, 12.19; N, 4.27; S, 6.12. Found: C, 55.70; H, 5.49; Br, 11.85; N, 4.19; S, 5.94.

5.1.37. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-3-methoxy-9′-trifluoromethyl-5′,6′-dihydro-4′*H*-pyrrolo-[3,2,1-*ij*]quinolino[2′,1′:6,7]morphinan-14-ol (1r) methanesulfonate

To a solution of compound **1f** (500 mg, 0.957 mmol) in DMF (10 mL) were added iodomethane (77 μL, 1.2 mmol) and anhydrous K_2CO_3 (529 mg, 3.83 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was quenched by addition of H_2O (70 mL), and the mixture was extracted with Et_2O (2× 50 mL). 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 **1r** (304 mg, 59%). 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 **1r**·$MeSO_3H$ as a white solid. Mp 207–210 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.42–0.55 (2H, m), 0.61-0.80 (2H, m), 1.10–1.20 (1H, m), 1.88 (1H, d, J = 11.3 Hz), 2.20–2.28 (2H, m), 2.30 (3H, s), 2.52–2.78 (3H, m), 2.78–2.95 (1H, m), 3.00–3.19 (5H, m), 3.30 (1H, dd, J = 6.3, 20.0 Hz), 3.42–3.47 (1H, m), 3.70 (3H, s), 4.13 (1H, d, J = 6.3 Hz), 4.15–4.22 (1H, m), 4.39–4.47 (1H, m), 6.04 (1H, s), 6.42 (1H, br s), 6.76 (1H, d, J = 8.2 Hz), 6.83 (1H, d, J = 8.2 Hz), 7.04 (1H, d, J = 7.7 Hz), 7.31 (1H, d, J = 7.7 Hz), 8.94 (1H, br s). IR (KBr, cm^{-1}): 3400, 2936, 1638, 1510, 1437, 1388, 1330, 1299, 1195, 1118, 1052, 901, 843, 812, 785, 748. MS (free base, EI) m/z $(M)^+$ = 536. Anal. Calcd for $C_{31}H_{31}F_3N_2O_3 \cdot MeSO_3H \cdot 0.7H_2O$: C, 59.56; H, 5.69; F, 8.83; N, 4.34; S, 4.97. Found: C, 59.29; H, 5.93; F, 8.59; N, 4.55; S, 5.15.

5.1.38. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-3,9′-dimethoxy-4,5-epoxy-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino[2′,1′:6,7]morphinan-14-ol (1s) methanesulfonate

Using a procedure similar to that used for the compound **1r**·$MeSO_3H$, the title compound **1s**·$MeSO_3H$ was obtained from the compound **1k** (68%). Mp 220–222 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.40–0.55 (2H, m), 0.56–0.79 (2H, m), 1.03–1.17 (1H, m), 1.83 (1H, d, J = 10.7 Hz), 2.05–2.19 (2H, m), 2.29 (3H, s), 2.55–2.72 (3H, m), 2.81–2.95 (3H, m), 3.08 (1H, d, J = 9.9 Hz), 3.23 (1H, d, J = 17.0 Hz), 3.30–3.49 (3H, m), 3.69 (3H, s), 3.74 (3H, s), 4.01–4.09 (2H, m), 4.22–4.31 (1H, m), 5.91 (1H, s), 6.31 (1H, s), 6.33 (1H, d, J = 8.0 Hz), 6.71 (1H, d, J = 8.2 Hz), 6.74 (1H, d, J = 8.2 Hz), 6.79 (1H, d, J = 8.5 Hz), 8.92 (1H, br s). IR (KBr, cm^{-1}):

3400, 2912, 1636, 1514, 1437, 1375, 1286, 1257, 1158, 1122, 1050, 978, 953, 895, 843, 783. MS (free base, EI) *m/z* $(M)^+$ = 498. Anal. Calcd for $C_{31}H_{34}N_2O_4{\cdot}MeSO_3H{\cdot}0.4H_2O$: C, 63.85; H, 6.50; N, 4.65; S, 5.33. Found: C, 63.71; H, 6.54; N, 4.80; S, 5.31.

5.1.39. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-3-methoxy-9′-methyl-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]-quinolino[2′,1′:6,7]morphinan-14-ol (1t) methanesulfonate

Using a procedure similar to that used for the compound **1r**·$MeSO_3H$, the title compound **1t**·$MeSO_3H$ was obtained from the compound 1n (59%). Mp 204–207 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.42–0.55 (2H, m), 0.60–0.68 (1H, m), 0.70–0.78 (1H, m), 1.05–1.16 (1H, m), 1.86 (1H, d, *J* = 11.3 Hz), 2.05–2.19 (2H, m), 2.31 (3.6H, s), 2.46 (3H, s), 2.60–2.98 (5H, m), 3.12 (1H, d, *J* = 12.1 Hz), 3.25–3.45 (4H, m), 3.70 (3H, s), 4.05–4.15 (3H, m), 4.26–4.34 (1H, m), 5.94 (1H, s), 6.33 (1H, br s), 6.60 (1H, d, *J* = 7.4 Hz), 6.72 (1H, d, *J* = 7.4 Hz), 6.74 (1H, d, *J* = 8.5 Hz), 6.81 (1H, d, *J* = 8.5 Hz), 8.97 (1.2H, br s). IR (KBr, cm^{-1}): 3400, 2930, 1638, 1562, 1510, 1433, 1288, 1197, 1123, 785. MS (FAB) *m/z* $(M+H)^+$ = 483. Anal. Calcd for $C_{31}H_{34}N_2O_3{\cdot}1.2MeSO_3H{\cdot}0.7H_2O$: C, 63.34; H, 6.64; N, 4.59; S, 6.30. Found: C, 63.09; H, 6.75; N, 4.91; S, 6.42.

5.1.40. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-8′-fluoro-3-methoxy-5′,6′-dihydro-4′*H*-pyrrolo[3,2,1-*ij*]quinolino[2′,1′:6,7]morphinan-14-ol (1q) methanesulfonate

Using a procedure similar to that used for the compound **1r**·$MeSO_3H$, the title compound **1q**·$MeSO_3H$ was obtained from the compound **1c** (83%). Mp 185–196 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.40–0.54 (2H, m), 0.59–0.78 (2H, m), 1.09 (1H, m), 1.86 (1H, d, *J* = 11.8 Hz), 2.06–2.23 (2H, m), 2.31 (3.6H, s), 2.52–2.78 (3H, m), 2.91–3.00 (4H, m), 3.12 (1H, m), 3.24–3.53 (3H, m), 3.70 (3H, s), 4.05–4.15 (2H, m), 4.28–4.37 (1H, m), 5.96 (1H, s), 6.38 (1H, br s), 6.72–6.83 (3H, m), 6.93 (1H, dd, *J* = 10.2, 2.2 Hz), 8.97 (1.2H, br s). IR (KBr, cm^{-1}): 3356, 1628, 1508, 1441, 1209, 1193, 1125, 1052, 785, 561, 536. MS (FAB) *m/z* $(M+H)^+$ = 487. Anal. Calcd for $C_{30}H_{31}N_2O_3{\cdot}1.2MeSO_3H{\cdot}0.8H_2O$: C, 60.80; H, 6.12; F, 3.08; N, 4.55; S, 6.24. Found: C, 60.71; H, 6.06; F, 3.06; N, 4.60; S, 6.47.

5.1.41. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-4,5-epoxy-3′,4′-dihydro-3-methoxy-5′-oxa-2′a-aza-acenaphthyleno[2′,1′:6,7]morphinan-14-ol (2b) methanesulfonate

Using a procedure similar to that used for the compound **1r**·$MeSO_3H$, the title compound **2b**·$MeSO_3H$ was obtained from the compound **2a** (76%). Mp 214–216 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.41–0.56 (2H, m), 0.59–0.79 (2H, m), 1.03–1.15 (1H, m), 1.87 (1H, d, *J* = 11.3 Hz), 2.31 (3.45H, s), 2.53–2.78 (3H, m), 2.92–3.01 (2H, m), 3.09–3.18 (1H, m), 3.26–3.42 (2H, m), 3.50 (1H, d, *J* = 19.8 Hz), 3.70 (3H, s), 4.10 (1H, d, *J* = 6.3 Hz), 4.16–4.24 (1H, m), 4.42–4.51 (2H, m), 4.55–4.61 (1H, m), 5.96 (1H, s), 6.40 (1H, br s), 6.58 (1H, d, *J* = 7.4 Hz), 6.74 (1H, d, *J* = 8.2 Hz), 6.82 (1H, d, *J* = 8.2 Hz), 6.87 (1H, dd, *J* = 7.4, 7.4 Hz), 6.95 (1H, d, *J* = 7.4 Hz), 8.98 (1.15H, br s). IR (KBr, cm^{-1}): 3400, 1636, 1586, 1506, 1444, 1383, 1286, 1245, 1193, 1122, 1052, 1000, 944, 901, 835, 785, 733. MS (free base, EI) *m/z* $(M)^+$ = 470. Anal. Calcd for $C_{29}H_{30}N_2O_4{\cdot}1.15MeSO_3H{\cdot}0.7H_2O$: C, 61.00; H, 6.11; N, 4.72; S, 6.21. Found: C, 61.04; H, 6.10; N, 4.67; S, 6.14.

5.1.42. (5*R*,9*R*,13*S*,14*S*)-17-Cyclopropylmethyl-6,7-didehydro-3-dimethoxy-4,5-epoxy-5′,6′-dihydro-6′,6′-dimethyl-4′*H*-pyrrolo-[3,2,1-*ij*]quinolino[2′,1′:6,7]morphinan-14-ol (3b) methanesulfonate

Using a procedure similar to that used for the compound **1r**·$MeSO_3H$, the title compound **3b**·$MeSO_3H$ was obtained from the compound **3a** (76%). Mp 207–211 °C (dec). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.41–0.55 (2H, m), 0.60–0.80 (2H, m), 1.05–1.16 (1H, m), 1.31 (3H, s), 1.34 (3H, s), 1.87 (1H, d, *J* = 10.7 Hz), 1.96–2.01 (2H, m), 2.31 (3.45H, s), 2.53–2.78 (3H, m), 2.91–3.00 (2H, m), 3.12 (1H, d, *J* = 9.3 Hz), 3.26–3.42 (2H, m), 3.50 (1H, d, *J* = 19.8 Hz), 3.70 (3H, s), 4.10 (1H, d, *J* = 7.9 Hz), 4.17–4.23 (1H, m), 4.27–4.35 (1H, m), 5.98 (1H, s), 6.36 (1H, br s), 6.74 (1H, d, *J* = 8.2 Hz), 6.81 (1H, d, *J* = 8.5 Hz), 6.92–6.97 (1H, m), 7.06 (1H, d, *J* = 6.6 Hz), 7.18 (1H, d, *J* = 7.7 Hz), 8.98 (1.15H, br s). IR (KBr, cm^{-1}): 3400, 2920, 1636, 1611, 1508, 1454, 1363, 1286, 1195, 1123, 1054, 948, 893, 857, 785, 748. MS (free base, EI) *m/z* $(M)^+$ = 496. Anal. Calcd for $C_{32}H_{36}N_2O_3{\cdot}1.15MeSO_3H{\cdot}0.4H_2O$: C, 64.81; H, 6.79; N, 4.56; S, 6.00. Found: C, 64.50; H, 6.96; N, 4.57; S, 6.15.

5.2. Biological assays

5.2.1. Antitussive activity assay

The number of coughs was counted by the method of body-plethysmograph[34] in conscious male Spraque–Dawley (SD) rats. In order 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 test compounds, and the number of coughs produced during the exposure period was counted as a control. Thirty min (ip, sc) 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).

5.2.2. 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 test compound solution (10 nM) 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 test 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.[35]

5.2.3. Metabolic stability assay

Each test compound (1 μM) was incubated with pooled human liver microsomal protein (0.435 mg/mL), G-6-P (10 mM), G-6-PDH (2 U/mL), and magnesium chloride (8 mM) in 154 mM potassium phosphate buffer (pH 7.4) in a final incubation volume of 1 mL. Following a 5 min preincubation period at 37 °C, the reaction was initiated by addition of NADPH (20 μM). After an incubation at 37 °C for 0 to 30 min, the reaction mixture was quenched by addition of 0.1 M NaOH (100 μM). When the incubation period was 0 min, 0.1 N NaOH was added just after the preincubation without addition of NADPH. To the resulting mixture were added an internal standard compound (one of **1a** derivatives) as 10% DMSO solution (20 μg/mL, 100 μL) and Et_2O (5 mL), and the mixture was vortexed (1 min) and centrifuged (10 min, 3000 rpm, HITACHI 05PR-22). The ethereal

layer was transferred to a new tube containing 250 mM potassium phosphate buffer (400 μL), and the mixture was vortexed (1 min) and concentrated. Addition of DMSO (40 μL) to the resulting solution gave a tested sample, which was used in HPLC analysis.

Concentrations of each compound were determined by UV (280 nm) detection using HPLC (SHIMAZU LC-10A Liquid Chromatography System). An AM-312 column (YMC ODS-AM, 150 × 6.0 mm) was used for each compound at 40 °C. The mobile phase consisted of (A) 50 mM sodium dihydrogenphosphate and (B) MeOH and was delivered at a flow rate of 1.0 mL/min. Elution was achieved by a linear gradient of 50–100% B over 35 min, then held 100% B for 10 min.

References and notes

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