New μ-Opioid Receptor Agonists with Phenoxyacetic Acid Moiety

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New μ-opioid receptor (MOR) agonists containing 4-hydroxypiperidine, piperidine and piperazine moieties were synthesized and evaluated to find a peripheral opioid analgesic. Among the synthesized compounds, [2-[1-[3-(*N*,*N*-dimethylcarbamoyl)-3,3-diphenylpropyl]-4-hydroxypiperidin-4-yl]phenoxy]acetic acid (8: SS620) having phenoxyacetic acid and 4-hydroxypiperidine moieties showed the highest agonist potency on the MOR in an isolated guinea-pig ileum preparation, and it also had selectivity to the human MOR expressed in Chinese hamster ovary (CHO)-K1 cells compared with the same types of δ- and κ-opioid receptors (DOR and KOR). In addition, compound 8 showed a 10 times more potent MOR agonist activity than loperamide. Furthermore, compound 8 showed a peripheral analgesic activity *in vivo* screening on rat.

Key words μ-opioid receptor agonist; peripheral opioid analgesic; phenoxyacetic acid; 4-hydroxypiperidine

Loperamide[1] is a μ-opioid receptor (MOR) agonist and it does not easily pass through the blood-brain barrier (BBB). Therefore, it is now mainly used as an antidiarrheal. But recently, it has been reported that when loperamide was administered to a burn on a rat as a peripheal percutaneous cream, it was effective and could not easily pass through the BBB so the manifestion of tolerance did not occur.[2] This report indicates that the peripheral MOR agonists are useful analgesics in the peripheral, especially against inflammatory tissues.

In our previous paper,[3] we reported that though our final objective was the synthesis of MOR agonists having peripheral analgesic activity, we intended to synthesize the compounds having more potent activities in two *in vitro* tests mentioned in the above summary as the first step and then we found compound **A** accomplishing our purpose (Fig. 1).

On the other hand, we had another project. Namely, we intended to synthesize the new MOR agonists having unique structures different from the structure of loperamide. Therefore, we first screened many compounds in our chemical library and then found a seed compound. But, after optimizing the structure, finally we only found the new lead compound **7a**(I) which is one of loperamide derivatives. Even so, we continually optimized the structure of **7a**(I) in consideration of the structure of **A** according to the general structure as shown in Chart 1. Then, as a result, we finally found compound **8** having a 10 times more potent MOR agonist activity than loperamide and a potent peripheral analgesic activity *in vivo*. In this paper, we describe the synthesis of the compounds and their agonist activities on MOR in the guinea-pig ileum. We also describe the affinities to the human MOR, δ-opioid receptor (DOR) and κ-opioid receptor (KOR) expressed in CHO cells of the selected compound **8**, and the peripheral analgesic activity of compound **8** in the Randal–Selitto method on rat as an *in vivo* test.

Chemistry

The 4-hydroxypiperidine derivatives were synthesized as

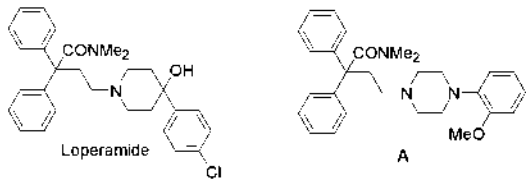

Fig. 1

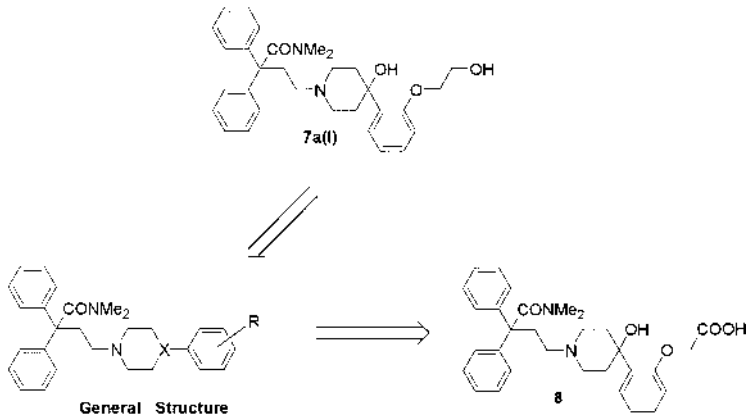

Chart 1

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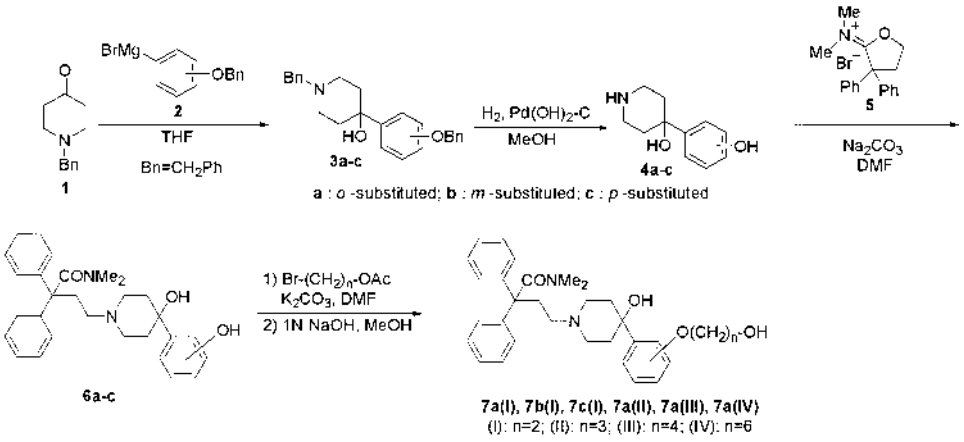

Chart 2

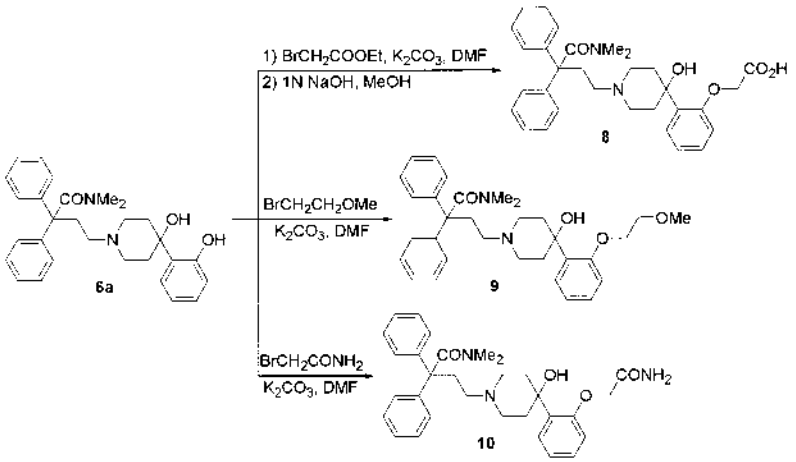

Chart 3

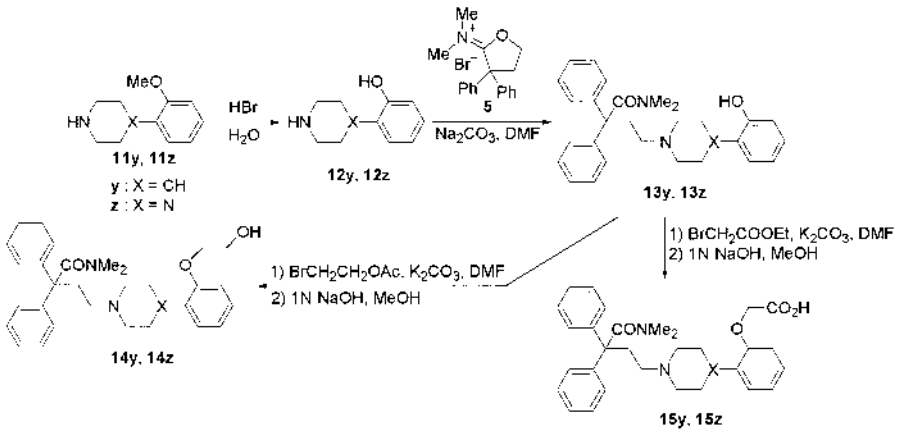

Chart 4

shown in Charts 2 and 3. The 1-benzyl-4-hydroxy-4-(benzyloxyphenyl)piperidine derivatives (**3a**—**c**) were synthesized from 1-benzyl-4-piperidinone (**1**) and benzyloxyphenylmagnesium bromide (**2**) according to the scheme shown in Chart 2. The 4-hydroxy-4-(hydroxyphenyl)piperidine derivatives (**4a**—**c**) were prepared according to the procedure described in our another previous paper.[4] The *N*-substituted derivatives (**6a**—**c**) were obtained by the reaction of **4a**—**c** with dimethyl(tetrahydro-3,3-diphenyl-2,2-furylidene) ammonium bromide (**5**), which was prepared according to the procedure of Stokbroekx *et al.*[1] The phenoxyalkylalcohol derivatives [**7a**(I)—**7c**(I), **7a**(II)—**7a**(IV)] were obtained from the reaction of **6** with ω-bromoalkyl acetate followed by alkaline hydrolysis at room temperature as shown in Chart 2.

The *O*-acetic acid (**8**), the *O*-methoxylethyl (**9**) and the *O*-acetamide (**10**) derivatives were synthesized as shown in

Table 1. Yields, Properties (Melting Points) and Mass Spectral Data for the Final Products

Compound	Yield (%)	Property [mp (°C)]	Formula	MS m/z M^+	HR-MS m/z M^+ Calcd (Found)
7a(I)	64	Colorless solid (179—180)	$C_{31}H_{38}N_2O_4$	502	502.2831 (502.2825)
7a(II)	63	Colorless solid (154—155)	$C_{32}H_{40}N_2O_4$	516	516.2988 (516.2960)
7a(III)	25	Colorless solid (152—153)	$C_{33}H_{42}N_2O_4$	530	530.3145 (530.3118)
7a(IV)	50	Yellowish viscous liquid	$C_{35}H_{46}N_2O_4$	558	558.3458 (558.3433)
7b(I)	55	Yellowish viscous liquid	$C_{31}H_{38}N_2O_4$	503 (M^++H)	503.2910 (503.2940)
7c(I)	46	Yellowish viscous liquid	$C_{31}H_{38}N_2O_4$	502	502.2832 (502.2831)
8	69	Colorless solid (162—164)	$C_{32}H_{35}N_3O$	FAB: 517 (M^++H)	Not measured
9	53	Yellowish viscous liquid	$C_{32}H_{40}N_2O_4$	516	516.2988 (516.2970)
10	33	Colorless solid (133—134)	$C_{31}H_{37}N_3O_4$	515	515.2784 (515.2767)
14y	22	Yellowish viscous liquid	$C_{31}H_{38}N_2O_3$	486	486.2883 (486.2862)
14z	51	Yellowish viscous liquid	$C_{30}H_{37}N_3O_3$	487	487.2834 (487.2825)
15y	54	Colorless solid (201—202)	$C_{29}H_{35}N_3O$	FAB: 501 (M^++H)	Not measured
15z	74	Colorless solid (125—126)	$C_{30}H_{35}N_3O_4$	501	501.2627 (501.2644)

Table 2. ^{1}H-NMR and IR Spectral Data for the Final Products

Compound	^{1}H-NMR ($CDCl_3$) δ	IR (KBr) cm^{-1}
7a(I)	1.65—1.78 (2H, m), 2.00—3.50 (17H, m), 3.60—4.40 (1H, br), 3.96 (2H, t, J=4.4 Hz), 4.07 (2H, t, J=4.4 Hz), 6.87 (1H, dd, J=8.3, 1.0 Hz), 6.93 (1H, dt, J=8.3, 1.0 Hz), 7.20 (1H, dt, J=8.3, 1.5 Hz), 7.23—7.50 (11H, m)	3366 1617
7a(II)	1.80—1.95 (2H, m), 1.95—2.60 (14H, m), 2.60—2.78 (2H, m), 2.82—3.15 (3H, br), 3.55 (1H, s), 3.84 (2H, t, J=5.9 Hz), 4.16 (2H, t, J=5.9 Hz), 6.85—6.95 (2H, m), 7.19 (1H, t, J=7.8 Hz), 7.24—7.45 (11H, m)	3554 3376 1622
7a(III)	1.60—2.03 (6H, m), 2.03—2.57 (12H, m), 2.57—2.80 (2H, m), 2.80—3.20 (3H, br), 3.55 (1H, s), 3.69 (2H, t, J=6.1 Hz), 4.05 (2H, t, J=6.1 Hz), 6.80—6.98 (2H, m), 7.09—7.53 (12H, m)	3522 3143 1637
7a(IV)	1.36—1.56 (4H, m), 1.56—1.66 (2H, m), 1.72—1.90 (2H, m), 1.90—2.20 (7H, m), 2.20—2.60 (7H, m), 2.60—2.76 (2H, m), 2.76—3.15 (3H, br), 3.66 (2H, t, J=6.4 Hz), 4.03 (2H, t, J=6.4 Hz), 4.10 (1H, s), 6.83—6.95 (2H, m), 7.12—7.30 (3H, m), 7.30—7.48 (9H, m)	(neat) 3441 1633
7b(I)	1.43—1.77 (2H, m), 1.77—3.32 (17H, m), 3.78—4.40 (1H, br), 3.90 (2H, t, J=4.4 Hz), 4.04 (2H, t, J=4.4 Hz), 6.75 (1H, dd, J=8.1, 2.2 Hz), 7.03 (1H, d, J=7.8 Hz), 7.06 (1H, s), 7.12—7.65 (11H, m)	(neat) 3404 1633
7c(I)	1.55—1.68 (2H, m), 1.68—1.77 (1H, br), 1.94—2.08 (2H, m), 2.08—2.18 (2H, m), 2.18—2.43 (5H, m), 2.43—2.54 (2H, m), 2.59—2.77 (2H, m), 2.86—3.08 (3H, br), 3.91 (2H, t, J=4.4 Hz), 4.03 (2H, t, J=4.4 Hz), 6.82 (2H, d, J=7.8 Hz), 7.28 (2H, d, J=7.8 Hz), 7.30—7.48 (10H, m)	(neat) 3386 1621
8	1.45—1.65 (2H, m), 2.00—3.20 (18H, m), 4.34 (2H, s), 6.77 (1H, d, J=7.8 Hz), 6.86 (1H, t, J=7.3 Hz), 7.06—7.15 (1H, m), 7.23—7.52 (11H, m) [DMSO-d_6]	3446 1615
9	1.80—2.83 (15H, m), 2.83—3.15 (3H, br), 3.40 (3H, s), 3.66—3.77 (2H, m), 4.05—4.10 (2H, m), 4.28—4.42 (1H, br), 6.87 (1H, d, J=7.8 Hz), 6.92 (1H, dt, J=7.8, 1.0 Hz), 7.12—7.30 (4H, m), 7.30—7.50 (8H, m)	(neat) 3520 1643
10	1.48—1.60 (2H, m), 1.60—1.80 (1H, br), 1.88—2.03 (2H, m), 2.07—2.23 (2H, m), 2.26—2.37 (3H, br), 2.37—2.47 (2H, m), 2.62—2.80 (4H, m), 2.93—3.10 (3H, br), 4.54 (2H, s), 6.40—6.60 (1H, br), 6.82 (1H, d, J=8.3Hz), 7.01 (1H, t, J=7.2 Hz), 7.20—7.34 (4H, m), 7.34—7.46 (7H, m), 7.54 (1H, dd, J=7.2, 1.7 Hz), 8.28—8.50 (1H, br)	3377 1684 1622
14y	1.60—1.75 (2H, m), 1.85—2.00 (2H, m), 2.00—2.10 (2H, m), 2.33 (3H, s), 2.40—2.50 (2H, m), 2.65—2.75 (1H, m), 2.85—3.15 (7H, m), 3.39 (1H, s), 3.90—4.00 (2H, m), 4.02 (1H, t, J=4.0 Hz), 6.82 (1H, d, J=9.0 Hz), 6.88 (1H, dt, J=8.3, 1.0 Hz), 7.10—7.20 (1H, m), 7.20—7.50 (1H, m)	(neat) 3382 1633
14z	2.06—2.16 (2H, m), 2.23—2.40 (3H, br), 2.41—2.61 (6H, m), 2.88—3.10 (7H, m), 3.54—3.63 (2H, m), 4.13 (2H, t, J=4.6 Hz), 4.77—4.90 (1H, m), 6.93—7.04 (4H, m), 7.22—7.30 (2H, m), 7.32—7.45 (8H, m)	(neat) 3388 1633
15y	1.72—2.03 (4H, m), 2.28 (3H, s), 2.35—2.69 (7H, m), 2.95—3.08 (1H, m), 2.97(3H, s), 3.21—3.40 (1H, m), 4.40 (2H, s), 6.63—6.95 (4 H, m), 7.24—7.42 (10H, m)	3448 1624
15z	2.29 (3H, s), 2.52—2.71 (4H, m), 2.90—3.19 (7H, m), 3.21—3.38 (4H, m), 4.53 (2H, s), 6.80—6.99 (4H, m), 7.22—7.44 (10H, m)	1624

Table 3. Analytical Data for **7a**(I), **7a**(II), **7a**(III), **8**, **10**, **15y** and **15z**

Compound	Solvent for recrystallization	Formula	*Anal.* Calcd (Found)		
			C	H	N
7a(I)	Toluene	$C_{31}H_{38}N_2O_4$	74.07 (73.84)	7.62 (7.77)	5.57 (5.49)
7a(II)	Toluene	$C_{32}H_{40}N_2O_4$	74.39 (74.11)	7.80 (7.85)	5.42 (5.23)
7a(III)	Toluene	$C_{33}H_{42}N_2O_4$	74.69 (74.55)	7.98 (8.08)	5.28 (5.16)
8	Acetone–water	$C_{31}H_{36}N_2O_5 \cdot 0.25H_2O$	71.45 (71.22)	7.06 (7.27)	5.37 (5.32)
10	Ethyl acetate–petroleum ether	$C_{31}H_{37}N_3O_4 \cdot 1.4H_2O$	68.84 (69.03)	7.41 (7.52)	7.77 (7.80)
15y	Methylene chloride–*n*-hexane	$C_{31}H_{36}N_2O_4 \cdot 2.1H_2O$	69.15 (69.03)	7.52 (7.29)	5.20 (5.09)
15z	Acetone–ethanol	$C_{30}H_{35}N_3O_4 \cdot 0.4H_2O$	70.81 (70.76)	7.09 (7.15)	8.26 (8.21)

Chart 3. The *O*-acetic acid derivative (**8**) was synthesized by the reaction of **6a** with ethyl bromoacetate followed by alkaline hydrolysis. Compounds **9** and **10** were prepared by the reaction of **6a** with 2-methoxyethyl bromide and 2-bromoacetamide, respectively.

The piperidine derivatives (**14y**, **15y**) were synthesized from 4-(2-hydroxyphenyl)piperidine (**12y**), which was prepared according to the procedure described in the previous paper,[4] as shown in Chart 4. The reaction of 4-(2-hydroxyphenyl)piperidine (**12y**) with **5** gave the butanamide (**13y**), which was allowed to react with 2-bromoethyl acetate or ethyl bromoacetate followed by alkaline hydrolysis to give the 2-phenoxyethanol (**14y**) or the phenoxyacetic acid (**15y**), respectively.

The piperazine derivatives (**14z**, **15z**), were synthesized from the commercially available 1-(2-methoxyphenyl)piperazine (**11z**) as shown in Chart 4. 1-(2-Methoxyphenyl)piperazine (**11z**) was demethylated with aqueous hydrobromic acid to 1-(2-hydroxyphenyl)piperazine (**12z**) followed by the same procedure as described for the synthesis of **14y** or **15y** to give the 2-phenoxyethanol (**14z**) or the 2-phenoxyacetic acid (**15z**).

Agonist Activities, Binding Assays and Analgesic Effects

Each sample was converted to the HCl salt to increase it's solubility in water for the assays mentioned below and then converted into an amorphous powder.

As the first screening, we examined the potencies of the samples relative to that of [D-Ala^2, *N*-MePhe^4, Gly^5-ol]-enkephalin (DAMGO), which is highly selective MOR agonist in the guinea-pig ileum. Next, we checked the human MOR, DOR and KOR binding assays to confirm the selectivity of the chosen sample. Finally, we examined the peripheral analgesic effect of the sample using the Randal–Selitto method on rat as an *in vivo* test.

First, when the substitution position of the 2-hydroxyethoxy moiety on the phenyl group of **7a**(I) was changed from *ortho* to *meta* or *para*, the *meta*-substituted compound **7b**(I) showed an extremely lower MOR agonist potency than **7a**(I) while *para*-substituted compound **7c**(I) did not show any agonist activity (Table 4A).

We then examined the length of the carbon chain between the phenoxy moiety and the terminal hydroxy group of **7a**(I). The MOR agonist potencies of **7a**(II), **7a**(III) and **7a**(IV) having the 2-hydroxyethoxy moiety of **7a**(I) replaced with the 3-hydroxypropyloxy moiety, 4-hydroxybutyloxy moiety and 6-hydroxyhexyloxy moiety, respectively, were evaluated. Among them, **7a**(II) and **7a**(III) showed lower MOR agonist potencies than **7a**(I), while **7a**(IV) did not show any agonist activity (Table 4A).

Therefore, considering the length of the carbon chain of **7a**(I), the MOR agonist potencies of **8**, **9** and **10** having the 2-hydroxyethoxy moiety of **7a**(I) replaced with carboxymethoxy moiety, the 2-methoxyethoxy moiety and the carbamoylmethoxy moiety were evaluated. Among them, **8** showed a higher MOR agonist potency than **7a**(I) and the potency of **8** was 47 times higher than that of DAMGO. Compounds **9** and **10** had lower MOR agonist potencies than **8** (Table 4A).

Finally, we examined the activities of the piperidine derivatives and the piperazine derivatives of **7a**(I) and **8**. Namely, the MOR agonist potencies of **14y** and **14z** having the 4-hydroxypiperidine moiety of **7a**(I) replaced with the piperidine moiety and piperazine moiety, respectively, were evaluated, and the above potencies of **15y** and **15z** having the 4-hydroxypiperidine moiety of **8** replaced with piperidine moiety and piperazine moiety respectively were evaluated. Similar to the results of **7a**(I) and **8**, the potency of the phenoxyacetic acid type, **15y** or **15z**, was higher than that of the 2-phenoxyethyl alcohol type, **14y** or **14z** (Table 4B).

Here, we would like to review our piperazine derivatives. Compounds **14z** and **15z** have the methoxy moiety of our previous lead compound **A** replaced with the 2-hydroxyethoxy moiety and the carboxymethoxy moiety, respectively. Among them, **15z** showed 4 times more potent MOR agonist activity but **15y** showed almost the same MOR agonist potency compared to **A**. Then we could confirm that **15z** has the highest MOR agonist potency among our piperazine derivatives.

As mentioned above, the phenoxyacetic acid derivatives showed higher MOR agonist potencies than the 2-phenoxyethyl alcohol derivatives, and for the phenoxyacetic acid derivatives, the 4-hydroxypiperidine derivative **8** had the

Table 4A. Potencies of Test Compounds Relative to That of DAMGO in Isolated Guinea-Pig Ileum Preparation

Compound	*n*	Relative potency
7a(I)	1	13
7b(I)	1	<0.1
7c(I)	1	—
7a(II)	1	2.8
7a(III)	1	3.8
7a(IV)	1	—
8	1	47
9	1	4.0
10	1	3.9
14y	1	19
14z	1	10
15y	1	25
15z	1	38
DAMGO	14	1
Loperamide	1	4.0

The % inhibition of the stimulated muscle twitch produced by a compound was plotted against the log concentration (10^{-5}—10^{-11} M) of the compound to estimate the IC_{50} (concentration of the compound to produce 50% inhibition of the twitch). The relative potencies of the test compounds were calculated by the following formula: relative potency=DAMGO's IC_{50}/compound's IC_{50}. The test compounds were confirmed to be an opioid agonist by antagonism of naloxone (sufficiently at 10^{-8} M). Furthermore, the compounds showing over 4.0 in the value of relative potency were evaluated precisely (Table 4B).

Table 4B. Potencies of Test Compounds Relative to That of DAMGO in Isolated Guinea-Pig Ileum Preparation

Compound	*n*	Relative potency
7a(I)	6	13.3±1.5
8	4	54.4±6.6
9	5	6.64±1.7
14y	7	17.7±2.8
14z	5	11.4±1.9
15y	4	33.1±3.8
15z	6	24.2±4.3
DAMGO	41	1
Loperamide	4	5.43±0.68

Values are the means±S.E.M. of *n* observations.

Table 5. Binding Assays to Human Opiate μ-, δ- and κ-Receptors of Compound **8**

Compound	μ, IC_{50} (nM)[a]	δ, IC_{50} (nM)[b]	κ, IC_{50} (nM)[c]
8	4.2	360	>10000

Quantitative follow-up: IC_{50} assessed in three independent experiments over a range of 5—6 concentrations: total 34 tubes. *a*) This assay measures binding of [^{3}H]Diprenorphine (DPN) to human opiate μ-receptors. *b*) This assay measures binding of [^{3}H]Naltrindole to human opiate δ-receptors. *c*) This assay measures binding of [^{3}H]DPN to human opiate κ-receptors.

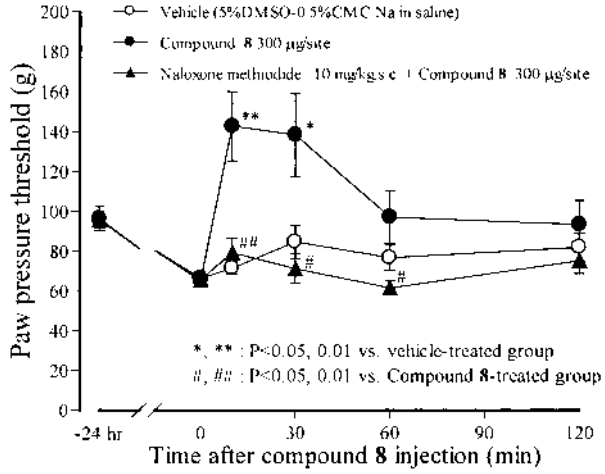

Fig. 2. Analgesic Effects of Compound **8** on the Freund's Complete Adjuvant (FCA) Induced Hyperalgesia in Rats

Compound **8** was injected by intraplantar route into the inflamed paw approximately 24 h after intraplantar injection of 150 ml of FCA. Naloxone methiodide (10 mg/kg) was subcutaneously administered 15 min before compound **8** injection. Pressure threshold on inflamed paw was measured before drug injection, 10, 30, 60 and 120 min after the injection. Each point represents the ±S.E. of 8 rats.

highest potency (Table 4B).

Next, we examined the affinities to the human MOR, DOR and KOR to confirm the selectivity of the most potent MOR agonist, **8** (Table 5). We then confirmed that it had the selectivity to the MOR because it showed lower affinities to the DOR and KOR than to the MOR.

Lastly, we examined the peripheral analgesic activity of compound **8** in the Randal–Selitto method on rat as an *in vivo* test. As shown in Fig. 2, compound **8** had peripheral analgesic activity because the effect was inhibited by naloxone methiodide of the peripheral opioid receptor antagonist.

Conclusion

As mentioned above, we found compound **8** (SS620) having a 10 times more potent MOR agonist activity than loperamide and a potent peripheral analgesic activity *in vivo*. Therefore, we selected compound **8** as one of the promising candidates for a peripheral analgesic of the MOR agonist and are now running various pharmacological tests to confirm its profile. Also, we are continuing to examine the precise structure-activity relationship, and some derivatives of compound **8** are now being synthesized and evaluated.

Experimental

Melting points were determined on a Yanaco micro melting point apparatus without correction. IR spectra were measured with a Perkin-Elmer 1600 FT-IR spectrometer. ^{1}H-NMR spectra were recorded on a JEOL JNM-EX400 FT-NMR spectrometer in $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6 using tetramethylsilane as the internal reference. The following abbreviations were used: s=singlet, d=doublet, dd=double doublet, dt=double triplet, t=triplet, q=quartet, m=multiplet and br=broad. FAB-MS, electron ionization mass spectrometry (EI-MS) or high-resolution mass spectra (HR-MS) were obtained using JEOL JMS-DX303 or JEOL JMS-AX500 mass spectrometer. TLC was performed by using Silica gel 60F_{254} (Merck). Column chromatography was performed with Silica gel 60 (70—230 mesh) (Merck). Sodium sulfate was employed as the drying agent. Palladium hydroxide [20 wt%, Pd (dry basis) on carbon, wet] and hydrogen chloride, 1.0 M solution in diethyl ether were obtained from Aldrich Chemical Company, Inc. The yields, physical and spectral data for **7a**(I)—**7a**(IV), **7b**(I), **7c**(I), **8**—**10**, **14y**, **14z**, **15y** and **15z** are shown in Tables 1 and 2. The analytical data for the solid compounds among the above compounds, **7a**(I), **7a**(II), **7a**(III), **8**, **10**, **15y** and **15z**, are shown in Table 3.

4-Hydroxy-4-(3-hydroxyphenyl)piperidine (4b)[4] Typical Procedure: A solution of 1-benzyl-4-[3-(benzyloxy)phenyl]-4-hydroxypiperidine (**3b**) (10.0 g, 26.8 mmol) in MeOH (100 ml) was hydrogenated in the presence of $Pd(OH)_2$ (3.0 g) under H_2 atmosphere (4 atm) at room temperature for 6 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give **4b** (4.00 g, 77%) as a colorless solid. mp 188—189 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.42—1.52 (2H, m), 1.66—1.80 (2H, m), 2.62—2.76 (2H, m), 2.84—2.97 (2H, m), 4.40—4.80 (1H, br), 6.54—6.60 (1H, m), 6.85 (1H, dd, J=7.8, 2.0 Hz), 6.89 (1H, t, J=2.0 Hz), 7.07 (1H, t, J=7.8 Hz), 8.00—10.00 (1H, br). EI-MS *m/z*: 193 (M^+). HR-MS *m/z*: 193.1097 (Calcd for $C_{11}H_{15}NO_2$: 193.1102). IR ν (KBr) cm^{-1}: 3410, 3265.

4-Hydroxy-4-(2-hydroxyphenyl)piperidine (**4a**): Colorless solid. mp 188—189 °C. Yield 95%. ^{1}H-NMR (DMSO-d_6) δ: 1.57—1.72 (2H, m), 1.94—2.09 (2H, m), 2.67—2.82 (2H, m), 2.87—3.02 (2H, m), 4.20—6.60 (3H, br), 6.68—6.80 (2H, m), 7.05 (1H, dt, J=7.6, 1.5 Hz), 7.22 (1H, dd, J=7.6, 1.5 Hz). EI-MS *m/z*: 193 (M^+). HR-MS *m/z*: 193.1090 (Calcd for $C_{11}H_{15}NO_2$: 193.1102). IR ν (KBr) cm^{-1}: 3410, 3263.

4-Hydroxy-4-(4-hydroxyphenyl)piperidine (**4c**): Yellowish solid. mp 206—207 °C. Yield 46%. ^{1}H-NMR (DMSO-d_6) δ: 1.50—1.70 (2H, m), 1.80—2.00 (2H, m), 2.80—3.15 (4H, m), 3.20—5.40 (3H, br), 6.71 (2H, d, J=8.3 Hz), 7.24 (1H, d, J=8.3 Hz). EI-MS *m/z*: 193 (M^+). HR-MS *m/z*: 193.1095 (Calcd for $C_{11}H_{15}NO_2$: 193.1102). IR ν (KBr) cm^{-1}: 3385, 3231.

The above compounds **4a** and **4c** were synthesized according to the synthetic procedure of **4b**.

***N*,*N*-Dimethyl-2,2-diphenyl-4-[4-hydroxy-4-(hydroxyphenyl)piperidin-1-yl]butanamide (6) (General Procedure A)** A mixture of **4** (2.5 mmol), dimethyl(tetrahydro-3,3-diphenyl-2,2-furylidene) ammonium bromide (**5**) (2.8 mmol), sodium carbonate (500 mg, 4.7 mmol) and DMF (25 ml) was stirred at 80 °C for 8 h. The reaction mixture was concentrated *in vacuo* and then H_2O was added into the residue. The mixture was extracted with $CHCl_3$, dried, concentrated *in vacuo*, purified by column chromatography on silica gel, eluting with 3% MeOH in $CHCl_3$ to give **6** as a solid or viscous liquid.

N,*N*-Dimethyl-2,2-diphenyl-4-[4-hydroxy-4-(2-hydroxyphenyl)piperidin-1-yl]butanamide (**6a**): Yellowish solid. mp 128—130 °C. Yield 76%. ^{1}H-NMR ($CDCl_3$) δ: 1.86—1.96 (2H, m), 1.96—2.20 (4H, m), 2.20—2.55 (7H, m), 2.63—2.80 (2H, m), 2.80—3.05 (3H, m), 6.73—6.83 (2H, m), 7.05 (1H, dd, J=7.8, 1.5 Hz), 7.12 (1H, dt, J=8.3, 1.5 Hz), 7.22—7.32 (2H, m), 7.32—7.43 (8H, m). EI-MS *m/z*: 458 (M^+). HR-MS *m/z*: 458.2599 (Calcd for $C_{29}H_{34}N_2O_3$: 458.2570). IR ν (KBr) cm^{-1}: 3237, 1637.

N,*N*-Dimethyl-2,2-diphenyl-4-[4-hydroxy-4-(3-hydroxyphenyl)piperidin-1-yl]butanamide (**6b**): Colorless solid. mp 168—169 °C. Yield 74%. ^{1}H-NMR ($CDCl_3$) δ: 1.50—1.70 (2H, m), 1.70—2.66 (12H, m), 2.66—2.90 (2H, m), 2.90—3.20 (3H, br), 6.71 (1H, d, J=7.8 Hz), 6.87 (1H, s), 6.97 (1H, d, J=7.8 Hz), 7.16 (1H, t, J=7.8 Hz), 7.25—7.34 (2H, m), 7.34—7.47 (8H, m). EI-MS *m/z*: 459 (M^+). HR-MS *m/z*: 459.2649 (Calcd for $C_{32}H_{40}N_2O_4$: 459.2648). IR ν (KBr) cm^{-1}: 3385, 1625.

N,*N*-Dimethyl-2,2-diphenyl-4-[4-hydroxy-4-(4-hydroxyphenyl)piperidin-1-yl]butanamide (**6c**): Yellowish solid. mp 154—155 °C. Yield 55%. ^{1}H-NMR (DMSO-d_6) δ: 1.63—1.80 (2H, m), 1.90—3.60 (16H, m), 4.82—5.15 (1H, br), 6.71 (2H, d, J=8.3 Hz), 7.20 (2H, d, J=8.3 Hz), 7.26—7.55 (10H, m), 9.21 (1H, s). EI-MS *m/z*: 440 (M^+-H_2O). HR-MS *m/z*: 440.2473 (Calcd for $C_{29}H_{34}N_2O_3$: 440.2464). IR ν (KBr) cm^{-1}: 3406, 1618.

***N*,*N*-Dimethyl-2,2-diphenyl-4-[4-(2-hydroxyphenyl)piperidin-1-yl]butanamide (13y)** Compound **13y** was synthesized from 4-(2-hydroxyphenyl)piperidine (**12y**)[4] and dimethyl(tetrahydro-3,3-diphenyl-2,2-furylidene)ammonium bromide (**5**) according to the general procedure A. Colorless solid. mp 221—222 °C. Yield 75%. ^{1}H-NMR ($CDCl_3$) δ: 1.77—1.88 (2H, m), 2.06—2.22 (2H, m), 2.31 (3H, s), 2.52—2.79 (6H, m), 2.90—3.04 (1H, m), 3.01 (3H, s), 3.24—3.37 (2H, m), 6.72—7.78 (1H, m), 6.88—6.97 (2H, m), 7.03—7.08 (1H, m), 7.26—7.43 (10H, m). EI-MS *m/z*: 442 (M^+).

HR-MS *m/z*: 442.2603 (Calcd for $C_{29}H_{34}N_2O_2$: 442.2620). IR ν (KBr) cm^{-1}: 3421, 1638.

***N*,*N*-Dimethyl-2,2-diphenyl-4-[4-(2-hydroxyphenyl)piperazin-1-yl]butanamide (13z)** Compound **13z** was synthesized from 1-(2-hydroxyphenyl)piperazine (**12z**) and dimethyl(tetrahydro-3,3-diphenyl-2,2-furylidene)ammonium bromide (**5**) according to the general procedure A. Yellowish viscous liquid. Yield 99%. ^{1}H-NMR ($CDCl_3$) δ: 2.09—2.17 (2H, m), 2.27—2.39 (3H, br), 2.43—2.58 (6H, m), 2.81 (4H, t, J=4.4 Hz), 2.93—3.05 (3H, br), 6.81 (1H, dt, J=7.8, 1.2 Hz), 6.89 (1H, dd, J=7.8, 1.2 Hz), 7.02 (1H, dt, J=7.8, 1.2 Hz), 7.11 (1H, dd, J=7.8, 1.2 Hz), 7.25—7.32 (2H, m), 7.34—7.46 (8H, m). EI-MS *m/z*: 443 (M^+). HR-MS *m/z*: 443.2555 (Calcd for $C_{28}H_{33}N_3O_2$: 443.2573). IR ν (neat) cm^{-1}: 3332, 1650.

1-(2-Hydroxyphenyl)piperazine (12z) A mixture of 1-(2-methoxyphenyl)piperazine (**11z**) (1.00 g, 5.20 mmol) and 48% hydrobromic acid (15.0 ml, 278 mmol) was stirred at 140 °C for 6 h. After cooling, the mixture was made alkaline with 3 N NaOH to pH 9. The resulting precipitate was collected by filtration, washed with water, air-dried at room temperature to give **12z** (500 mg, 54%) as an yellowish solid. mp 118—120 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.76—3.05 (8H, m), 4.40—6.20 (2H, br), 6.68—6.98 (4H, m). MS *m/z*: 178 (M^+). HR-MS *m/z*: 178.1071 (Calcd for $C_{10}H_{14}N_2O$: 178.1106). IR ν (KBr) cm^{-1}: 3300.

***N*,*N*-Dimethyl-2,2-diphenyl-4-[4-hydroxy-4-(2-alkoxyphenyl)-piperidin-1-yl]butanamide (9 or 10) (General Procedure B)** A mixture of **6a** (459 mg, 1.0 mmol), an alkyl bromide (1.5 mmol), potassium carbonate (300 mg, 2.2 mmol) and DMF (10 ml) was stirred at 40 °C for 4 h. The reaction mixture was concentrated *in vacuo* and then H_2O was added into the residue. The mixture was extracted with $CHCl_3$, dried, concentrated *in vacuo*, purified by column chromatography on silica gel, eluting with 5% MeOH in $CHCl_3$ to give **9** or **10** as a colorless solid or yellowish viscous liquid.

N,*N*-Dimethyl-2,2-diphenyl-4-[4-hydroxy-4-[2-(2-methoxyethoxy)-phenyl]piperidin-1-yl]-butanamide (**9**) and *N*,*N*-dimethyl-2,2-diphenyl-4-[4-hydroxy-4-[2-(carbamoylmethoxy)-phenyl]piperidin-1-yl]butanamide (**10**) were synthesized from **6a** according to the general procedure B.

4-Aryl-1-[3-(*N*,*N*-dimethylcarbamoyl)-3,3-diphenylpropyl]-4-hydroxypiperidine (7 or 8) (General Procedure C) A mixture of **6** (1.0 mmol), an alkyl bromide (1.2 mmol), potassium carbonate 300 mg (2.2 mmol) and *N*,*N*-dimethylformamide (DMF) (15 ml) was stirred at 40 °C for 4 h. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in a mixture of 1 N NaOH (10 ml), MeOH (10 ml) and 1,4-dioxane (10 ml), then stirred at room temperature for 1 h. The mixture was neutralized with dil. HCl to pH 7 at 0 °C. The mixture was extracted with $CHCl_3$, washed with H_2O, dried, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with 5% MeOH in $CHCl_3$ to give **7** or **8** as a colorless solid or yellowish viscous liquid.

Compounds **7a**(I)—**7a**(IV), **7b**(I), **7c**(I) and **8** were synthesized from **6** according to the general procedure C.

N,*N*-Dimethyl-2,2-diphenyl-4-[4-[2-(2-hydroxyethoxy)phenyl]piperidin-1-yl]butanamide (**14y**) and [2-[1-[3-(*N*,*N*-dimethylcarbamoyl)-3,3-diphenylpropyl]piperidin-4-yl]phenoxy]acetic acid (**15y**) were synthesized from **13y** according to the general procedure C.

N,*N*-Dimethyl-2,2-diphenyl-4-[4-[2-(2-hydroxyethoxy)phenyl]piperazin-1-yl]butanamide (**14z**) and [2-[4-[3-(*N*,*N*-dimethylcarbamoyl)-3,3-diphenylpropyl]piperazin-1-yl]phenoxy]acetic acid (**15z**) were synthesized from **13z** according to the general procedure C.

Preparation of HCl Salts (General Procedure D) A final compound (1.0 mmol) was dissolved in diethyl ether (60 ml). To the resulting solution was added 1.0 ml of 1.0 M hydrogen chloride solution in diethyl ether under ice cooling and stirring. The colorless precipitate thus formed was collected by filtration, washed with diethyl ether and dried *in vacuo* at room temperature.

Evaluation of MOR Agonist Activities *in Vitro*

Binding Assays to Human MORs Using [^{3}H]Diprenorphine (DPN)

Binding Assays to Human DORs Using [^{3}H]Naltrindole

Binding Assays to Human KORs Using [^{3}H]DPN

The assays mentioned above were performed according to the procedures described in our previous paper.[3)]

Animals The experiment was performed on male Sprague–Dawley rats weighing 110—150 g (Charles River, Japan). The animals were housed under 12-h light–dark cycle (lights on 7 : 00 a.m.), with room temperature maintained 23±3 °C, and humidity at 50±20%. Food and water were freely available.

Materials Naloxone methiodide and Freund's complete adjuvant (FCA) were obtained from Sigma. Naloxone methiodide was diluted in saline and injected (10 mg/kg) by subcutaneous route in a volume of 2 ml/kg body weight 15 min before compound treatment. Compound was diluted in 5% dimethylsulfoxide–0.5% carboxymethylcellulose sodium–saline and injected by intraplantar route in a volume of 50 μl/site.

Analgesic Assay A modification of the methods of Dehaven-Hudkins *et al.*[5)] was used, where hyperalgesia in response to inflammation was measured by determining the paw pressure threshold of inflamed paw using a pressure analgesia apparatus (Ugo Basile, Italy). Hyperalgesia was induced by subcutaneously injection of 150 μl of FCA into the right hind paw of rats. Compound was subcutaneously injected into the inflamed paw approximately 24 h after FCA injection. The experimenter was unaware of the compound used.

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