Stereoisomers of *N*-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide: Synthesis, Stereochemistry, Analgesic Activity, and Opioid Receptor Binding Characteristics[1]

Zhi-Xian Wang, You-Cheng Zhu,* Wen-Qiao Jin, Xin-Jian Chen, Jie Chen, Ru-Yun Ji, and Zhi-Qiang Chi

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, Peoples Republic of China

Received March 1, 1995®

N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide (ohmefentanyl, **1**) is an extremely potent analgesic agent with high affinity and selectivity for opioid μ receptors. There are three chiral carbons in **1**, so eight optically active isomers are possible. Respective reaction of optically active 3-methyl-*N*-phenyl-4-piperidinamines (**5a–d**) with (*R*)- or (*S*)-styrene oxide produced eight optically active intermediates which were subsequently converted to eight optically active isomers of **1** (**1a–h**). The absolute configurations of **1a–h** were determined by X-ray analysis of (3*R*,4*S*,2′*R*)-(−)-*cis*-**1a** and (3*R*,4*R*,2′*S*)-(−)-*trans*-**1g**. The analgesic activity (mice, ip, hot plate) revealed their extreme stereodifferences; the ED_{50} values of (3*R*,4*S*,2′*R*)-(−)-*cis*-**1a** and (3*R*,4*S*,2′*S*)-(+)-*cis*-**1b**, which are the most potent isomers among eight isomers, were 0.004 65 (2990 times that of morphine) and 0.001 06 mg/kg (13 100 times that of morphine), respectively, while the corresponding antipodes **1d,c** were the least potent compounds among the eight isomers. In agreement with pharmacological results, both **1a,b** also had the highest receptor affinity and selectivity for the opioid μ receptor. The ratio of K_i(DPDPE)/K_i(DAMGO) was 22 800 for **1a** and 22 500 for **1b**. All isomers except **1c,d** strongly inhibited the electrically evoked smooth muscle contraction of GPI and MVD but not that of RVD, and the inhibitory effects could be reversed by naloxone, which indicated that these compounds were potent μ agonists in GPI and MVD. There was a good linear correlation between the analgesic potencies (ED_{50}) and the receptor affinities (K_i(DAMGO)) or inhibitory effects (IC_{50}) to GPI and MVD. These results suggested that the analgesic effects of ohmefentanyl are mediated by interaction between the agents and opioid μ receptors in the central nervous system and that the 3*R*,4*S* configuration at the piperidine 3- and 4-carbon atoms and the *S* configuration at the phenylethyl 2-carbon atom are beneficial for analgesic potency and inhibitory effects in GPI and MVD and the same for an *S* or *R* configuration at the phenylethyl 2-carbon atom besides the 3*R*,4*S* configuration for receptor μ affinity and selectivity.

Pharmacological studies *in vitro* and *in vivo* indicated the existence of at least three classes of opioid receptors, namely, μ, δ, and κ, which differ in their affinity with various opioid ligands and in their distribution in the nervous system. Recently, these three opioid receptors have been successfully cloned and characterized. These receptors, which are members of the family of G-protein-coupled receptors, have a high degree of amino acid sequence similarity with *ca.* 50% of the residues being identical.[2] It can be predicted that the cloned receptors will facilitate the studies on physiological roles of endogenous opioids and also stimulate the development of new clinically useful opioids with more specific actions and limited side effects. In an attempt to characterize and understand the different opioid receptors and their endogenous ligands, opioid receptor subtype-selective agonists and antagonists as pharmacological probes have particularly been instrumental. Some compounds as sufficiently type-selective ligands have been used for characterizing the physiological significance of these opioid receptors.[3]

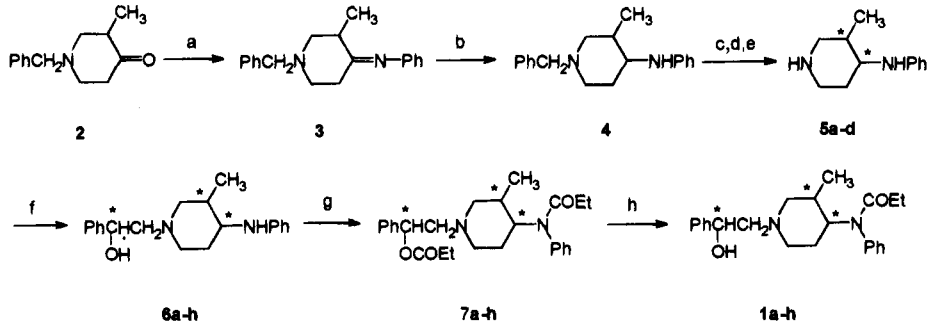
Fentanyl was first synthesized in the early 1960s.[4] Its prominent analgesic potency, opioid μ receptor selectivity, and characteristic structure attracted a great deal of attention.[5] Introduction of a methyl group into the 3-position of the piperidine ring could markedly increase the activity.[6] The potency and selectivity for the μ opioid receptor would further be enhanced by replacement of the hydrogen with a hydroxyl group at the 1β-position.[6c,7] Compound *N*-[1-(2-hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide (ohmefentanyl, F7302, **1**) is particularly interesting because it is a highly potent and selective agonist for the μ opioid receptor. The analgesic potency of *cis*-A-**1**[8] is *ca.* 28 times more than that of fentanyl and 6300 times more than that of morphine in mice (hot plate, ip).[9] Receptor binding assays demonstrated that [^{3}H]-ohmefentanyl bound to opioid μ receptors in mouse and rat brains with high affinity and high selectivity.[7,10] The similar results were given by isolated tissue bioassays[11] and autoradiography analysis.[12] Goldstein pointed out that **1** was more μ-selective than sulfentanil, about the same as DAMGO,[13] but **1** was more stable and easily crossed the blood–brain barrier. For these reasons, **1** has been widely used in studies of opioid receptor properties.[14,15]

There are three chiral carbons in **1**, so eight optically active isomers are possible. In our earlier studies, four diastereoisomeric pairs (*cis*-A-**1**, *cis*-B-**1**, *trans*-A-**1**, and *trans*-B-**1**) of **1** were separated by a two-step fractional crystallization, and it was found that the two more potent isomers were both of the *cis* form and that *cis*-A-**1** was 5.3 times more potent than *cis*-B-**1** for analgesic effects.[8,9] These stereodifferences in analgesic potency

® Abstract published in *Advance ACS Abstracts*, August 1, 1995.

0022-2623/95/1838-3652$09.00/0

Scheme 1[a]



[a] Reagents: (a) $PhNH_2$/toluene/acetic acid; (b) $NaBH_4$/MeOH; (c) 10% Pd–C/H_2; (d) fractional crystallization of fumarate and oxalate; (e) tartaric acid resolution; (f) (*R*)-(+)- or (*S*)-(−)-styrene oxide; (g) EtCOCl/toluene; (h) K_2CO_3/90% aqueous methanol.

partially reflected their binding affinities to opioid receptors. Therefore, preparation of optical isomers of ohmefentanyl (**1**) could provide enantiomers of very different affinities, which, in principle, would be extremely useful in receptor identification studies. In the present paper, we will describe the synthesis of eight stereoisomers of ohmefentanyl, the determination of their absolute configuration, and the evaluation of their analgesic activities and receptor binding characteristics.

Chemistry

Scheme 1 outlines the synthetic route of stereoisomers of ohmefentanyl. Synthesis of 1-benzyl-3-methyl-*N*-phenyl-4-piperidinamine (**4**) from *N*-benzyl-3-methylpiperidin-4-one (**2**) followed literature procedures.[6c] Debenzylation of **4** gave the mixture of *cis*- and *trans*-**5**. Treatment of the mixture of base **5** with fumaric acid in *i*-PrOH/MeOH gave pure *cis*-**5** furmarate (determined by ^{1}H NMR analysis of free base) after recrystallization three times with the same solvent. The mother liquid was evaporated, and the residue was converted into the free base and then turned into the oxalate in ether, which gave pure *trans*-**5** oxalate after recrystallization twice with $Me_2CO/H_2O/Et_2O$.[16,17] The resolution of racemic *cis*- and *trans*-**5** was successfully realized via fractional crystallization of their tartrates by Janssen's[6b] and Rice's[17,18] methods. The absolute configurations of **5a–d** are (+)-*cis*-**5** (**5a**) as 3*R*,4*S*, (−)-*cis*-**5** (**5b**) as 3*S*,4*R*, (+)-*trans*-**5** (**5c**) as 3*S*,4*S*, and (−)-*trans*-**5** (**5d**) as 3*R*,4*R*.

Respective alkylation of **5a,b** with (*R*)-(+)- or (*S*)-(−)-styrene oxide in ethanol at 50 °C afforded **6a–d** in high regioselectivity, while **5c** reacted with (*S*)-(+)-styrene oxide in ethanol at room temperature or at 50 °C to give two products, one of them as the desired compound **6e** and the other as a structural isomer, (3*S*,4*S*,2′*S*)-*trans*-3-methyl-*N*-(2-hydroxy-2-phenylethyl)-*N*-phenyl-4-piperidinamine (**8**), confirmed by ^{1}H NMR analysis (Figure 1). Causing the isomerism was the fact that the 3-methyl and 4-aniline groups in the *trans*-**5** molecule are on different sides of the piperidine ring, so comparing to *cis*-**5** the intramolecular steric hindrance from the 3-methyl in *trans*-**5** is smaller. On the other hand, under the condition of solution, *trans*-**5** and styrene oxide molecules had an extended conformation and a large space. These factors might lead the styrene oxide molecule to attack the 4-N atom. A modified method was to add the optically active styrene oxide dropwise to a melted *trans*-**5** at 100–110 °C under stirring in a period of 3–4 h. Then the neat mixture was continually reacted at this temperature for an additional 1 h to afford the desired compound **6** with high yield and high regioselectivity. The four *trans* compounds, **6e–h**, were prepared using the improved method.

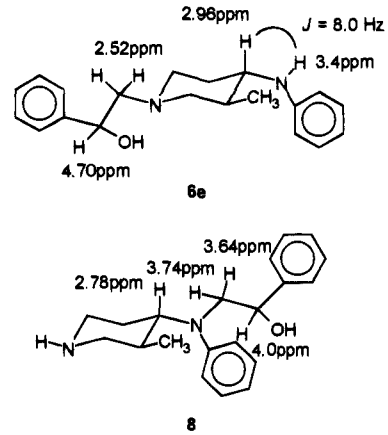


Figure 1. ^{1}H NMR of **6e** and **8**.

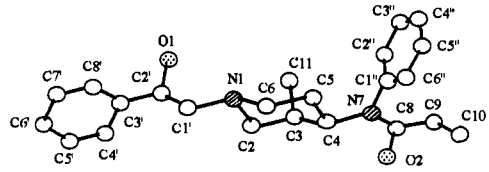
Optically active **6a–h** were acylated with propionyl chloride to give the corresponding ohmefentanyl propionate esters **7a–h** followed by direct treatment of the esters **7a–h** with K_2CO_3 powder in 90% aqueous methanol to give the title compound optically active ohmefentanyls **1a–h**. The overall yield was *ca.* 65%, calculated from optically active compounds **5a–d**.

In **1a–h** molecules, the absolute configurations of the piperidine ring 3- and 4-carbons corresponded with those of starting materials **5a–d** and the configuration of the 2′-position corresponded with that of the corresponding chiral styrene oxide. This stereospecific epoxide opening had previously provided a facile method of asymmetric synthesis of (*R*)-(−)-ubine.[19] These results were confirmed by single-crystal X-ray studies.

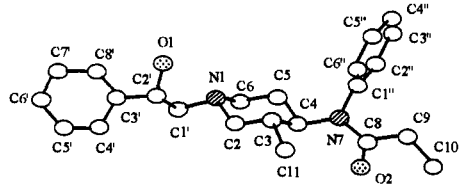
During this study, the preparation of the four *cis* stereoisomers of **1** by a different route was reported.[20] In this route, alkylation of **5a,b** with 2-bromoacetophenone followed by acylation and reduction afforded two diastereoisomeric mixtures (**1a,b**, **1c,d**), which were separated by fractional crystallization. The melting point and optical rotation values for the four isomers prepared by this route agree closely with our values (Table 1).

X-ray Crystallographic Study

A *cis* form isomer, **1a**, and a *trans* form one, **1g**, were selected for X-ray crystallographic study (Figure 2). Since the absolute configurations of the intermediates **5a–d** have been previously established and the trans-

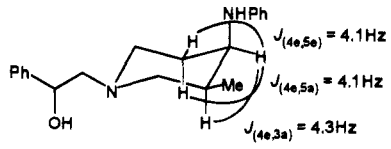


(3R,4S,2'R)-cis-1a

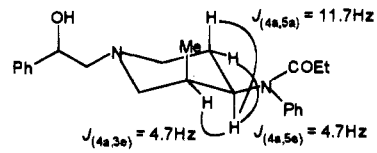


(3R,4R,2'S)-trans-1g

Figure 2. X-ray crystal structures of (3*R*,4*S*,2'*R*)-(−)-*cis*-ohmfentanyl (**1a**) and (3*R*,4*R*,2'*S*)-(−)-*trans*-ohmefentanyl (**1g**).



6a



1a

Figure 3. Splitting patterns of the 4-proton on the piperidine rings of (3*R*,4*S*,2'*R*)-*cis*-**6** (**6a**) and (3*R*,4*S*,2'*S*)-*cis*-ohmefentanyl (**1a**).

formations from intermediates **5a**−**d** to final products had no effect on the configurations of the piperidine 3- and 4-carbons,[6b,16-18] the absolute configuration of **1a** was confirmed as 3*R*,4*S*,2'*R* and that of **1g** as 3*R*,4*R*,2'*S*. This X-ray determination served to rigorously define the absolute configurations of the all eight stereoisomers of **1** (Table 1), and the results are identical with our previous suggestion.

In the **1a** molecule, the piperidine ring has a chair conformation with an equatorial 4-*N*-phenylpropanamide group and an axial 3-methyl group. This was confirmed by the ^{1}H NMR analysis of **1a**.[21] The 4-proton on the piperidine ring resonates as a dt peak, centered at δ 4.22, consisting of a doublet ($J_{(4a,5a)}$ = 11.7 Hz) of triplets ($J_{(4a,5e)} = J_{(4a,3e)}$ = 4.7 Hz). However, in the intermediate *cis*-**5** molecule, the 4-aniline group is axial and the 3-methyl group is equatorial.[17] ^{1}H NMR analysis also showed that the piperidine ring in compound *cis*-**6** has the same conformation as that in *cis*-**5** (Figure 3). These facts show that the conformation of the piperidine ring might be twisted when *cis*-**6** is acylated with propionyl chloride.

On the other hand, the stereoscopic view of **1g** showed that the piperidine ring has a chair conformation and that the 3-methyl group and the 4-*N*-phenylpropanamide group were all equatorial. This conformation is identical with that from ^{1}H NMR spectra analysis ($J_{(4a,3a)}$ = 11.0 Hz, $J_{(4a,5a)}$ = 11.7 Hz)[21] and the conformation in the *trans*-**5** molecule from X-ray studies.[17]

In addition, X-ray studies showed that there is an intramolecular hydrogen bond at O(1)-H···N(1) (2.748 Å) in the **1a** molecule, while **1g** has an intermolecular hydrogen bond at O(1)-H(A molecule)···O(2)(B molecule) (2.818 Å) in addition to an intramolecular hydrogen bond at O(1)-H···N(1) (2.818 Å).

Biological Results and Discussion

Analgesic Activity. The analgesic activity was assessed in mice by the hot plate method after ip administration of the compounds to be tested. All compounds showed a typical morphine-like analgesic action, and the ED_{50} values are given in Table 1. Among the eight isomers of ohmefentanyl, (3*R*,4*S*,2'*S*)-(+)-*cis*-**1** (**1b**) was found to be 13 110 times more potent than morphine with ED_{50} = 0.001 06 mg/kg, while the ED_{50} value of its antipode (3*S*,4*R*,2'*R*)-*cis*-**1** (**1c**) was over 10 mg/kg (0/10). The order of analgesic potency is **1b** > **1a** > **1g** > **1e** > **1h** ≈ **1f** ≫ **1d** > **1c**, which indicated that the 3*R*,4*S* configuration at the piperidine 3- and 4-carbons and the *S* configuration at the phenylethyl 2-carbon in **1** were beneficial to analgesic activity. The result of the piperidine ring configuration is also identical with that observed in stereoisomers of 3-methylfentanyl and its analogues; among them, all of the most potent isomers were found to have a 3*R*,4*S* configuration (Table 1).[16-18]

We also noted that the analgesic potency of **1a** is 2.7 times (molar potency) more potent than that of *cis*-B-**1** (a racemic pair of **1a**,**d**),[8] and **1b** is 2 times more potent than *cis*-A-**1** (a racemic pair of **1b**,**c**),[8] while their antipodes **1c** was inactive in a dose up to 10 mg/kg and **1d** had only low analgesic activity (Table 1). These facts indicated that the analgesic effects of *cis*-A-**1** and *cis*-B-**1** are mainly mediated by **1b**,**a**, respectively.

Receptor Binding Affinity. The stereoisomers of ohmefentanyl were evaluated for their binding affinities at the μ and δ binding sites (Table 2). The assays were conducted using reported procedures,[7,10] and the binding of [^{3}H][D-Ala^2-MePhe^4-Gly-ol^5]enkephalin ([^{3}H]DAMGO)[22] to mouse brain membranes (P_2 fraction) was used as the specific labels for μ sites, while [^{3}H][D-Pen^2,D-Pen^5]enkephalin ([^{3}H]DPDPE)[23] was used for δ sites. K_i values for the eight isomers displacing [^{3}H]DAMGO and [^{3}H]DPDPE binding were determined, and the ratio of K_i(DPDPE)/K_i(DAMGO) was calculated to express the selectivity for the μ or δ opioid receptor.

Binding assays showed that these compounds have higher affinities for μ sites than for δ sites, but the affinity and the selectivity are different. In terms of binding affinity at the μ sites, the order of potency is **1b** ≈ **1a** > **1e** ≈ **1g** > **1h** > **1f** ≫ **1d** > **1c**. **1b** was 4 times more potent than **1a** for analgesic activity, but for μ binding affinity, **1b** was only slightly more potent than **1a**. Their antipodes **1d**,**c** were the least potent compounds among the eight isomers. Among the four *trans* isomers, **1e**,**g** with the 2'*S* configuration were slightly more potent than **1h**,**f** with the 2'*R* configuration for binding to μ sites. Comparing binding affinity at μ sites, these eight isomers exhibited much lower binding affinity at δ sites, but the same order of potency was observed except for compound **1e**, which was found to show a much lower activity than **1f**−**h** at δ sites (>1000 nM). For this reason, the selectivity of **1e** was much higher than other *trans* isomers, which put it into

Table 1. Physicochemical Properties and Analgesic Potencies of **1a–h**

compd[a]	absol config	mp (°C)	$[\alpha]^{25}_D$ (deg) (MeOH)	analgesic[b] ED_{50} (mg/kg)	rel potency,[c] morphine = 1
(−)-*cis*-**1a**	(3*R*,4*S*,2′*R*)	135–137 (138–140)	−31.91 (*c* 0.47) −32.6 (*c* 1.32))[20]	0.004 65 (0.0031–0.0066)	2990
(+)-*cis*-**1b**	(3*R*,4*S*,2′*S*)	117–119 (121–122)	+19.79 (*c* 0.60) +22.3 (*c* 0.77))[20]	0.001 06 (0.00079–0.0013)	13 110
(−)-*cis*-**1c**	(3*S*,4*R*,2′*R*)	117–119 (121–122)	−20.54 (*c* 0.31) −22.6 (*c* 0.65))[20]	>10 (0/10)	
(+)-*cis*-**1d**	(3*S*,4*R*,2′*S*)	135–137 (140–141)	+33.15 (*c* 0.36) +33.7 (*c* 0.85))[20]	>10 (4/10)	
(+)-*trans*-**1e**	(3*S*,4*S*,2′*S*)	98–100	+62.24 (*c* 0.22)	0.0142 (0.011–0.017)	980
(+)-*trans*-**1f**	(3*S*,4*S*,2′*R*)	107–108	+0.78 (*c* 0.38)	0.0751 (0.064–0.087)	185
(−)-*trans*-**1g**	(3*R*,4*R*,2′*S*)	107–109	−0.49 (*c* 0.41)	0.0096 (0.006–0.015)	1450
(−)-*trans*-**1h**	(3*R*,4*R*,2′*R*)	98–100	−58.11 (*c* 0.33)	0.0710 (0.057–0.091)	196
(±)-*cis*-A-1[d]				0.0022	6320
(±)-*cis*-B-1[e] (HCl salt)				0.014	985
(+)-*cis*-3-MF[f] (oxalate salt)	(3*R*,4*S*)			0.007 67 (0.0062–0.0095)	1812
(−)-*cis*-3-MF[f] (oxalate salt)	(3*S*,4*R*)			0.910 (0.746–1.110)	15

[a] All compounds were analyzed for C, H, and N (values within ±3%); the test compounds were dissolved by addition of 1.0 equiv of aqueous HCl and further diluted. [b] Hot plate test (in mice, ip), 95% confidence limits or effective animal number shown in parentheses. [c] Morphine: ED_{50} = 13.9 mg/kg. [d] Data from refs 6c and 9. [e] Data from ref 9. [f] Data from ref 16; 3-MF = 3-methylfenatnyl.

Table 2. Opioid Receptor Binding Affinity and μ/δ Selectivity of **1a–h** (n = 3, $\bar{X} \pm$ SD)

compd[a]	K_i (nM) [^{3}H]DAMGO (μ)	K_i (nM) [^{3}H]DPDPE (δ)	μ/δ selectivity, $K_i(\delta)/K_i(\mu)$
1a	0.005 ± 0.004	114 ± 14	22 800
1b	0.004 ± 0.002	90 ± 10	22 500
1c	5.85 ± 0.21	>10 000	>1710
1d	2.89 ± 0.36	>10 000	>3460
1e	0.08 ± 0.07	>1000	>12 500
1f	0.15 ± 0.05	230 ± 16	1533
1g	0.06 ± 0.03	200 ± 13	3333
1h	0.13 ± 0.02	150 ± 10	1153

[a] See the corresponding footnote in Table 1.

Table 3. Inhibitory Effects (IC_{50}, nM) of **1a–h** in Three Different Isolated Tissues (n = 3, $\bar{X} \pm$ SD)

compd[a]	GPI	MVD	RVD
1a	0.42 ± 0.07	0.59 ± 0.16	>100
1b	0.08 ± 0.01	0.10 ± 0.03	>100
1c	>1000	>1000	>1000
1d	>1000	>1000	>1000
1e	0.92 ± 0.11	1.15 ± 0.09	>1000
1f	2.58 ± 0.22	4.87 ± 0.7	>1000
1g	0.82 ± 0.35	1.64 ± 0.23	>1000
1h	4.33 ± 0.55	4.87 ± 0.80	>1000

[a] See the corresponding footnote in Table 1.

the same selectivity level as the more potent compounds **1b,a**. Two more potent isomers, **1a,b**, were also found to be more selective compounds, the selectivities for μ versus δ binding sites are 22 800 for **1a** and 22 500 for **1b**. These facts indicated that the 3*R*,4*S* configuration at piperidine 3- and 4-carbons was beneficial to μ receptor affinity and selectivity, but the influence of the phenylethyl 2-carbon configuration for binding affinity was not as important as that for analgesic activity.

Rothman *et al.* had reported that the $K_i(\delta)/K_i(\mu)$ of (±)-*cis*-**1** (RTI-4614-4) is 26 909,[24] but their results[20] of recent studies on four stereoisomers of (±)-*cis*-**1** are in contrast to ours. They reported that the order of μ binding potency obtained using [^{3}H]DAMGO as ligand is **1c** > **1a** ≫ **1d** > **1b**. The two more active isomers, **1c,a**, had the *R* configuration at the phenylethyl 2-carbon and the most active isomer, **1c**, had the 3*S*,4*R* configuration at the piperidine ring, so they suggested that the configuration of the phenylethyl 2-carbon was more important than that of piperidine 3- and 4-carbons. We also noted their very recent publication on *cis*-ohmefentanyl stereoisomers,[25] in which an interesting contrast to their above binding data was provided by the EC_{50} values for displacement of specific equilibrium binding of [^{3}H]etorphine and analgesic ED_{50} values for the mouse analgesic assays. These assays both revealed a potency order of **1b** > **1a** > **1d** > **1c**, which corresponded to ours in the present work. It is very interesting and merits further investigation that there is a difference in binding data on isomers **1b,c** from two laboratories. We have repeated our experiment, included characterizing physicochemical properties and assays, to confirm our results.

Isolated Tissue Bioassays. Three different isolated tissues, guinea pig ileum (GPI), mouse vas deferens (MVD), and rabbit vas deferens (RVD), were used to characterize stereoisomers of ohmefentanyl. The GPI assay is usually considered as being representative for μ receptor interactions. In the MVD assay, opioid effects are primarily mediated by δ receptors, but μ receptors also exist in this tissue. The RVD contains solely opioid κ receptors.

Our earlier studies showed that *cis*-A-**1** (a racemic pair of **1b,c**) only had very low action for κ receptors in *in vitro* and *in vivo* assays.[7,11] This nature was also observed in the stereoisomers of **1**. In RVD, a κ receptor system, all isomers showed no inhibitory effects at concentrations up to 100 (**1a,b**) and 1000 nM (the others), which indicated that **1a–h** might be inactive or have only very low action for opioid κ receptors.

Except **1c,d**, the other six isomers of ohmefentanyl strongly inhibited the electrically evoked smooth muscle contraction of GPI, and the inhibitory effects were able to be reversed by the opioid μ antagonist naloxone. The results suggested that these compounds were potent μ agonists in GPI. The inhibitory effects of **1a–h** in MVD were slightly weaker than those in GPI, but the order of potency corresponded to that of GPI, and the effects were also able to be reversed by naloxone. Our previous studies showed that the inhibitory effect of *cis*-A-**1** was mainly mediated by μ receptors in MVD assays.[11] This evidence suggested that the inhibitory effects of **1a–h** in MVD might be primarily mediated by μ receptors,

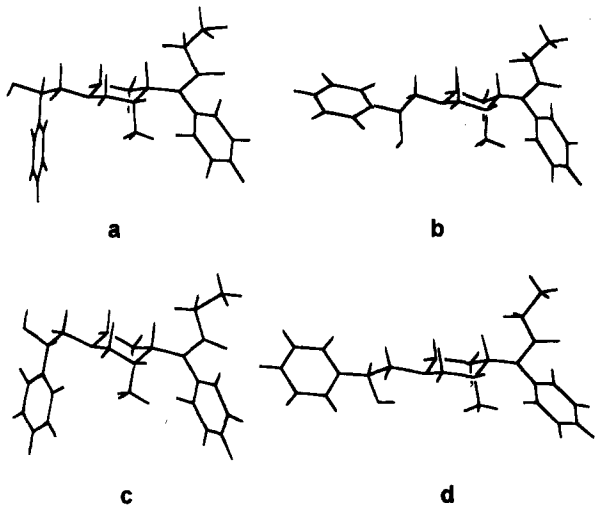


Figure 4. Low-energy conformations of **1a,b**: (a) **1a** (lowest, −5.6828 kcal/mol), (b) **1a** (second lowest, −4.4866 kcal/mol), (c) **1b** (lowest, −5.8500 kcal/mol), and (d) **1b** (second lowest, −4.7939 kcal/mol).

and this suggestion corresponded to the results recently reported by Brine *et al.*[25] A good parallel relationship between the inhibitory potencies of these compounds in GPI and MVD and in [^{3}H]DAMGO binding to μ receptors was found, which indicated that the inhibitory effects of **1a−h** in isolated tissues are interrelated to their μ receptor affinities. The two more active isomers, **1b,a**, both had the 3*R*,4*S* configuration at piperidine 3- and 4-carbons, and **1b** with the *S* configuration at the phenylethyl 2-carbon was found to be the most potent compound with IC_{50} values of 0.08 nM for GPI and 0.10 nM for MVD (5−6 times more potent than **1a** (2′*R* configuration)). Among the four *trans* isomers, **1e,f** with the 2′*S* configuration were found to be 3−5 times more active than **1f,h** with the 2′*R* configuration. These results indicated that not only the 3*R*,4*S* configuration at piperidine 3- and 4-carbons but also the *S* configuration at the phenylethyl 2-carbon were beneficial to inhibitory effects in GPI and MVD.

The data in Tables 1−3 show a good linear correlation between analgesic potencies (ED_{50}) and binding affinities for opioid μ receptors (K_i) (correlation coefficient $r = -0.969$) or inhibitory effects (IC_{50}) in GPI ($r = -0.995$) and MVD ($r = -0.996$), which indicated that the analgesic effect of ohmefentanyl is mediated by interaction between these compounds and opioid μ receptors in the central nervous system.

A theoretical conformational analysis of the two most active and selective isomers, **1a,b**, was carried out in order to examine its conformational behavior in comparison with these two compounds and to determine their possible bioactive conformation.[26] MMP2 program was used in conformation optimization and energy calculation. The results show that the lowest and second lowest energy conformations of these two compounds are very similar (Figure 4). The energy of the lowest conformation of **1b** was only 0.17 kcal/mol lower than that of **1a**, and this might explain why the activity and μ selectivity of these two compounds are so close. Interestingly, both of the lowest conformations (a and c) of **1a,b** are different from their crystal conformations, while their second lowest conformations (b and d) are similar to them. The difference between the two lowest energy conformations in energy was *ca.* 1.2 kcal/mol in the case of **1a** and *ca.* 1.15 kcal/mol in the case of **1b**. These results demonstrate that the lowest energy conformations (a and c) might be the bioactive conformations of ohmefentanyl, while their crystal conformations were not available.

In view of the above results of the study on stereostructure−activity relationships, we can see that the 3-methyl group of the piperidine ring is an important factor in the interaction between the agents and opioid receptors. Portoghese[27] pointed out that introduction of a 3-methyl group into the 4-phenyl-4-propionoxy-1-methylpiperidine molecule enhanced its analgesic activity, since the axial 3-methyl group might bind in a hydrophobic pocket of limited size on the receptor and induce the phenyl group to adopt a favorable configuration. Maybe the 3-methyl group in the ohmefentanyl molecule produces similar effects. In *cis* isomer molecules, when in the 3*R*,4*S* configuration (**1a,b**), the axial 3-methyl group may bind in a hydrophobic pocket of limited size on the receptor; at the same time, it can also influence the position and orientation of the 4-phenyl ring owing to intramolecular steric hindrance and induce the phenyl ring to adopt a favorable conformation. Therefore, the phenyl ring may bind more comfortably to a flat portion on the receptor through van der Waals or hydrophobic interactions. However, when it is in the 3*S*,4*R* configuration (**1c,d**), the 3*S*-methyl group of agents cannot inlay to the hydrophobic pocket of the opioid receptor because it is on another enantiomeric edge of the piperidine ring; in fact, the axial 3*S*-methyl may prevent the agent from binding with the receptors due to steric hindrance, which leads to a sharp decline in analgesic potency and binding affinity. In the *trans* isomer (**1e−h**) molecules, the equatorial 3-methyl groups bind loosely with the receptors and the configuration of the 3-methyl only has a weak influence, so their analgesic potencies and binding affinities were significantly lower than those of *cis*-3*R* isomers (**1a,b**). According to the analysis of relationships between configuration of the isomers' 2′-hydroxyl groups and their individual biological activities, we suggest that the 2′-hydroxyl group may stabilize an interaction with a hydrogen bond binding site or a proton acceptor of the receptor,[1b,6c] and the site has steric structural limits for agents. This may explain why 2′*S*-hydroxyl groups were beneficial to analgesic activities and opioid effects. In *trans* isomers, since the configuration of the piperidine ring only has a weak influence, the influence of the configuration of the 2′-hydroxyl group plays the main role, and therefore, **1e,h** (2′*S* configuration) are more active than **1f,g** (2′*R* configuration) both *in vitro* and *in vivo*.

Conclusion

The stereoisomers of ohmefentanyl mainly act on opioid μ receptors, but the potency and selectivity are different. There is a good parallel relationship between analgesic potencies and binding affinities at opioid μ receptors or the inhibitory effects in GPI and MVD bioassays, which indicated that the analgesic effect of ohmefentanyl is mediated by interaction between these compounds and opioid μ receptors in the central nervous system.

The isomers **1a** (*cis*-(3*R*,4*S*,2′*R*)) and **1b** (*cis*-(3*R*,4*S*,2′*S*)) were the two highest active and μ-selective

compounds among the eight enantiomers. The ratio K_i(DPDPE)/K_i(DAMGO) is 22 800 for **1a** and 22 500 for **1b**. These results indicate that the 3*R*,4*S* configuration at the piperidine 3- and 4-carbon atoms and both the *S* or *R* configuration at the phenylethyl 2-carbon atom are beneficial to μ opioid receptor affinity and selectivity. These two compounds, **1a,b**, were also the most active analgesics among the eight isomers. Analgesic potency ED_{50} values of these two compounds are 0.001 06 and 0.0047 mg/kg, while the corresponding antipodes **1d,c** were the least potent compounds among the eight isomers. The same trend of inhibitory effects in GPI and MVD bioassays was also observed. These results show that not only the 3*R*,4*S* configuration but also the 2′*S* configuration are beneficial to analgesic potency and opioid effects in GPI and MVD. All evidence indicates that **1a,b** are likely to be used widely as pharmacological tools in opioid research and might also have potential as therapeutic agents.

Experimental Section

The melting points were determined on a Büchi-510 apparatus and are not corrected. Infrared (IR) spectra were measured with a Perkin-Elmer 983G grating infrared spectrophotometer from KBr pellets (solid) or liquid film (liquid). The ^{1}H NMR spectra were recorded with a Bruker AM-400 MHz spectrometer. Specific rotation measurements were determined with a DIP-180 polarimeter; all determinations were made in MeOH solution in a 10 cm cell at the indicated concentration (g/100 mL). Thin-layer chromatography (TLC) was performed on GF_{254} precoated plates (2.5 × 7.5 cm) in a solvent system consisting of concentrated aqueous NH_3–EtOH–$CHCl_3$ (1:5:94). Elemental compositions for crystalline compounds were performed on a MOD-1106 apparatus.

***cis*- and *trans*-3-Methyl-*N*-phenyl-4-piperidinamine (*cis*- and *trans*-5).** A solution of amine 4[6c] (60 g, 0.214 mol) in absolute ethanol (700 mL) was hydrogenolyzed with 15 psi of H_2 over 10% Pd–C (12 g) at 60 °C for 6 h. The mixture was filtered, concentrated to remove solvent, and then distilled in vacuum to give 36 g (88.5%) of a mixture of *cis*- and *trans*-**5** as a colorless oil: bp 125–130 °C/0.35 mmHg.

A hot solution of a mixture of *cis*- and *trans*-**5** (34.5 g, 0.18 mol) in 150 mL of *i*-PrOH was added to a hot solution of fumaric acid (22 g, 0.18 mol) in 190 mL of MeOH. After a few minutes, fine needles were afforded. The mixture was allowed to cool overnight at 0 °C. The precipitate was filtered and recrystallized with the same solvent three times, giving 17.5 g (29.5%) of pure *cis*-**5** fumaric salt; light yellow needles, mp 197–199 °C. Anal. ($C_{12}H_{18}N_2 \cdot C_4H_4O_4$) C, H, N. The fumaric salt (17.0 g) was treated with dilute aqueous NaOH and extracted with ether; 10 g of free base *cis*-**5** was obtained.

The combined mother solution of fumaric salt was evaporated, and the residue was converted into free base, giving 22.2 g of amine **5**. The amine turned into oxalate with oxalic acid (16 g, 0.12 mol) in 1000 mL of ether. The oxalate was recrystallized with Me_2CO–H_2O–Et_2O to give 15.3 g (25.5%) of pure *trans*-**5** oxalate as fine white needles: mp 167–170 °C (lit.[17] mp 167–169 °C). Anal. ($C_{12}H_{18}N_2 \cdot C_2H_2O_4$) C, H, N. Conversion of the oxalate to free base gave 8.9 g of *trans*-**5**.

Optical Resolution of 5. Optical resolution of *cis*- or *trans*-**5** was performed according to reported procedures by crystallization of L-(+)- and D-(−)-tartaric acid salts from MeOH–Me_2CO to yield after conversion to the free amines **5a–d**.

(3*R*,4*S*)-(+)-*cis*-5a: white solid; mp 97–98 °C (lit.[6b] mp 93.5–94.5 °C); $[\alpha]^{25}_D$ +7.25° (*c* 0.66, MeOH) (lit.[6b,18] $[\alpha]^{25}_D$ +6.1° (MeOH), $[\alpha]^{23}_D$ +6.2° (c 3.7, MeOH)).

(3*S*,4*R*)-(−)-*cis*-5b: white solid; mp 96–98 °C (lit.[6b] mp 91–93 °C); $[\alpha]^{25}_D$ −7.67° (c 0.32, MeOH) (lit.[6b,18] $[\alpha]^{25}_D$ −5.9° (MeOH), $[\alpha]^{23}_D$ −6.8° (*c* 2.6, MeOH)).

(3*S*,4*S*)-(+)-*trans*-5c: light yellow oil; $[\alpha]^{25}_D$ +112.91° (c 0.86, MeOH) (lit.[17] $[\alpha]^{22}_D$ +116.3° (*c* 0.057, MeOH)).

(3*R*,4*R*)-(−)-*trans*-5d: light yellow oil; $[\alpha]^{25}_D$ −110.82° (c 1.0, MeOH) (lit.[17] $[\alpha]^{22}_D$ −116.7° (c 0.163, MeOH)).

(3*R*,4*S*,2′*R*)-*cis*-3-Methyl-1-(2-hydroxy-2-phenylethyl)-*N*-phenyl-4-piperidinamine (6a). (*R*)-(+)-Styrene oxide (0.7 mL, 5.5 mmol) was added to a solution of (3*R*,4*S*)-(+)-*cis*-**5** (**5a**) (1.0 g, 5.3 mmol) in ethanol (4 mL); the reaction mixture was stirred at 40 °C for 5 h. The solvent was evaporated, and the residue was recrystallized with petroleum ether and several drops of ethanol, giving 1.50 g (90%) of **6a** as white needles: mp 104–106 °C; $[\alpha]^{25}_D$ −0.62° (*c* 0.68, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.02 (3H, d, *J* = 7.0 Hz, 3-C*H*$_3$), 1.80 (1H, dd, *J* = 4.9, 10.1 Hz, H-5e), 1.85 (1H, m, H-5a), 2.28 (1H, m, H-3), 2.40–2.60 (3H, m, H-1′, H-2e), 2.64 (2H, m, H-6a, H-2a), 2.73 (1H, m, H-6e), 3.54 (1H, dd, *J* = 4.1, 3.8 Hz, H-4), 3.61 (1H, m, N-*H*), 4.73 (1H, dd, *J* = 9.6, 4.3 Hz, H-2′), 6.6, 6.68, 7.16, 7.27, 7.35 (10H, Ph-*H*). Anal. ($C_{20}H_{26}N_2O$) C, H, N.

The other isomers (**6b–d**) of *cis*-**6** were prepared from the corresponding **5a,b** by a similar procedure in high yield.

(3*R*,4*S*,2′*S*)-*cis*-6b: white needles; yield 81%; mp 102–103 °C; $[\alpha]^{25}_D$ +56.91° (c 0.55, MeOH).

(3*S*,4*R*,2′*R*)-*cis*-6c: white needles; yield 86%; mp 101–103 °C; $[\alpha]^{25}_D$ −57.66° (c 0.97, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.03 (3H, d, *J* = 6.9 Hz, 3-C*H*$_3$), 1.80 (2H, m, H-5), 2.28 (1H, m, H-3), 2.31–2.60 (2H, m, H-2), 2.47 (1H, dd, *J* = 10.3, 12.4 Hz, H-1′), 2.53 (1H, dd, *J* = 3.7, 12.5 Hz, H-1′), 2.74 (1H, d, *J* = 9.8 Hz, H-6a), 2.93 (1H, m, H-6e), 3.54 (1H, dd, *J* = 4.3, 4.1 Hz, H-4), 3.61 (1H, m, N-*H*), 4.77 (1H, dd, *J* = 10.1, 3.6 Hz, H-2′), 6.6, 6.69, 7.16, 7.27, 7.35 (10H, Ph-*H*).

(3*S*,4*R*,2′*S*)-*cis*-6d: white needles; yield 84%; mp 106–108 °C; $[\alpha]^{25}_D$ +0.56° (c 0.67, MeOH).

(3*S*,4*S*,2′*S*)-*trans*-3-Methyl-1-(2-hydroxy-2-phenylethyl)-*N*-phenyl-4-piperidinamine (6e). Method A. (*S*)-(−)-Styrene oxide (0.51 g, 4.24 mmol) was added to a solution of (3*S*,4*S*)-*trans*-**5** (**5c**) (0.74 g, 3.94 mmol) in 3 mL of ethanol, the reaction mixture was stirred at 50 °C for 5 h, TLC analysis showed **5c** was converted completely, and two products were afforded. The solvent was removed, and the resulting syrup was separated by column chromatography (silica gel, 200–300 mesh, EtOH–$CHCl_3$ (1:30)) to give 0.6 g of **6e** and 0.35 g of **8**.

6e: white needles; mp 133–134 °C; $[\alpha]^{25}_D$ +93.21° (c 0.57, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, d, *J* = 6.6 Hz, 3-C*H*$_3$), 1.38 (1H, ddd, *J* = 12.6, 3.1, 2.5 Hz, H-5), 1.71 (1H, m, H-3), 2.1–2.2 (3H, m, H-5, H-6), 2.49 (2H, m, H-1′), 2.82 (1H, dt, *J* = 10.2, 1.9 Hz, H-2), 2.96 (1H, m, H-4), 3.16 (1H, d, *J* = 11.7 Hz, H-2), 3.38 (1H, d, *J* = 8.0 Hz, N-*H*), 4.05 (1H, m, −O*H*), 4.70 (1H, dd, *J* = 4.5, 9.4 Hz, H-2′), 6.58, 6.66, 7.14, 7.28, 7.33 (10H, Ph-*H*). Anal. ($C_{20}H_{26}N_2O$) C, H, N.

8: light yellow syrup; ^{1}H NMR ($CDCl_3$) δ 0.91 (3H, d, *J* = 6.0 Hz, 3-C*H*$_3$), 1.42 (1H, ddd, *J* = 12.3, 3.8, 2.9 Hz, H-5), 1.57 (2H, m, H-5, H-3), 2.15 (1H, m), 2.39 (1H, dt, *J* = 2.3, 11.7 Hz), 2.78 (1H, m, H-4), 2.89 (2H, m), 3.64 (1H, dd, *J* = 5.1, 10.2 Hz, H-1′), 3.74 (1H, dd, *J* = 5.1, 10.2 Hz, H-1′), 4.01 (1H, t, *J* = 10.2 Hz, H-2′), 6.5, 6.62, 7.10, 7.16, 7.34 (10H, Ph-*H*).

Method B. (*S*)-(−)-Styrene oxide (0.75 g, 6.25 mmol) was added dropwise to melted **5c** (1.1 g, 5.8 mmol) at 100 °C under stirring in a period of 3 h; then the reaction mixture was stirred at this temperature for an additional 1 h. The mixture was recrystallized with petroleum ether with several drops of ethanol, giving 1.35 g (92%) of **6e**: white needles; mp 133–135 °C.

The other *trans* isomers, **6f–h**, were prepared from the corresponding **5c,d** by method B in high yield.

(3*S*,4*S*,2′*R*)-*trans*-6f: white needles; yield 81%; mp 103–105 °C; $[\alpha]^{25}_D$ +32.81° (c 0.57, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.04 (3H, d, *J* = 6.7 Hz, 3-C*H*$_3$), 1.55 (1H, ddd, *J* = 3.1, 13.4, 2.8 Hz, H-5), 1.80 (1H, m, H-3), 2.02 (1H, t, *J* = 11.1 Hz, H-2a), 2.18 (1H, dd, *J* = 13.4, 3.0 Hz, H-5e), 2.49 (1H, td, *J* = 11.8, 3.0 Hz, H-6a), 2.59 (2H, d, *J* = 6.7 Hz, H-1′), 2.98 (1H, td, *J* = 3.9, 10.5 Hz, H-4), 3.15 (1H, m, H-6e), 3.26 (1H, d, *J* = 10.7 Hz, H-2e), 4.85 (1H, t, *J* = 6.7 Hz, H-2′), 6.59, 6.67, 7.14, 7.28, 7.33 (10H, Ph-*H*).

(3*R*,4*R*,2′*S*)-*trans*-6g: white needles; yield 79%; mp 103–104 °C; $[\alpha]^{25}_D$ −31.99° (c 0.40, MeOH).

(3*R*,4*R*,2′*R*)-*trans*-6h: white needles; yield 83%; mp 132–135 °C; $[\alpha]^{25}_D$ −89.94° (c 0.26, MeOH).

(3*R*,4*S*,2′*R*)-*cis*-*N*-[1-[2-(Propionyloxy)-2-phenylethyl]-3-methyl-4-piperidyl]-*N*-phenylpropanamide (7a). Freshly distilled propionyl chloride (1.05 mL, 11.9 mmol) was added to a solution of amino alcohol **6a** (1.4 g, 4.5 mmol) in dry toluene (8 mL), and the mixture was stirred at reflux for 5 h, allowed to cool, and then treated with aqueous K_2CO_3 and extracted with ether. The extracts were washed with saturated brine and dried with K_2CO_3, the solvent was evaporated, and the crude product **7a** was used for the next step without further purification.

***cis*-(3*R*,4*S*,2′*R*)-*N*-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-*N*-phenylpropanamide (1a).** K_2CO_3 powder (2.0 g) was added to a solution of crude ester **7a** in 90% aqueous methanol (20 mL), and the mixture was stirred at room temperature overnight. The mixture was filtered to remove solid, and the filtrates were concentrated. The residue was dissolved in ether and washed with water and saturated brine; the ether layers were dried (K_2CO_3) and evaporated to remove solvent. The residual material was recrystallized with petroleum ether (60–90 °C) to give 1.25 g (77% from **6a**) of (3*R*,4*S*,2′*R*)-*cis*-ohmefentanyl (**1a**) as white needles: mp 135–137 °C; $[\alpha]^{25}_D$ −31.91° (c 0.47); ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, J = 7.8 Hz, H-10), 0.93 (3H, d, J = 7.0 Hz, 3-CH_3), 1.24 (1H, qd, J = 11.7, 11.7, 11.7, 4.7 Hz, H-5a), 1.27 (1H, dbr, J = 11.7, 4.7 Hz, H-5e), 1.77, 1.85 (each 1H, dq, J = 16.4, 7.8 Hz, H-9), 2.23 (1H, dd, J = 11.0, 2.4 Hz, H-2a), 2.65 (1H, dt, J = 11.0, 2.4, 2.4 Hz, H-2e), 2.51 (1H, dddq, J = 2.4, 2.4, 4.7, 7.0 Hz, H-3e), 2.07 (1H, td, J = 3.1, 11.7, 11.7 Hz, H-6a), 2.81 (1H, dbr, J = 11.7, 4.7 Hz, H-6e), 2.28, 2.38, 4.57 (3H, ABX, J = 13.3, 6.3, 6.3 Hz, H-1′, 2′), 4.22 (1H, dt, J = 4.7, 4.7, 11.7 Hz, H-4a), 4.77 (1H, s, −O*H*), 7.14–7.48 (10H, m, Ph-*H*). Anal. ($C_{23}H_{30}N_2O_3$) C, H, N.

Similarly, the other isomers, **1b**–**h**, were prepared from the corresponding compounds **6b**–**h**. The absolute configuration and physicochemical constants are listed in Table 1.

(3*R*,4*S*,2′*S*)-*cis*-1b: ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, J = 7.8 Hz, H-10), 0.98 (3H, d, J = 7.0 Hz, 3-CH_3), 1.15 (1H, qd, J = 12.5, 12.5, 12.5, 3.9 Hz, H-5a), 1.22 (1H, dbr, J = 12.5, 4.7, 3.1 Hz, H-5e), 1.76, 1.84 (each 1H, dq, J = 16.4, 7.8 Hz, H-9), 2.25 (1H, dd, J = 11.0, 2.4 Hz, H-2a), 2.73 (1H, dt, J = 11.0, 2.4 Hz, H-2e), 2.52 (1H, dddq, J = 2.4, 4.7, 7.0 Hz, H-3e), 1.97 (1H, td, J = 3.1, 12.5, 12.5 Hz, H-6a), 2.69 (1H, dbr, J = 11.7, 3.9 Hz, H-6e), 2.29, 2.37, 4.59 (3H, ABX, J = 13.3, 6.3, 6.3 Hz, H-1′, H-2′), 4.22 (1H, dt, J = 4.7, 4.7, 12.5 Hz, H-4a), 4.85 (1H, s, −O*H*), 7.12–7.46 (10H, m, Ph-*H*).

(3*S*,4*S*,2′*R*)-*trans*-1f: ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, J = 7.8 Hz, H-10), 0.92 (3H, d, J = 7.0 Hz, 3-CH_3), 1.25 (1H, qd, J = 11.7, 11.7, 11.7, 3.1 Hz, H-5a), 1.61 (1H, dbr, J = 11.7, 1.6 Hz, H-5e), 1.85 (2H, q, J = 7.8 Hz, H-9), 1.79 (1H, t, J = 11.0 Hz, H-2a), 2.92 (1H, dbr, J = 10.9, 3.1 Hz, H-2e), 1.47 (1H, dddq, J = 11.0, 11.0, 3.1, 7.0 Hz, H-3a), 2.09 (1H, td, J = 11.7, 11.7, 1.6 Hz, H-6a), 2.91 (1H, dbr, J = 11.7, 3.1 Hz, H-6e), 2.29, 2.39, 4.60 (3H, ABX, J = 12.5, 8.6, 3.9 Hz, H-1′, H-2′), 4.30 (1H, m, J = 11.0, 11.7 Hz, H-4a), 4.88 (1H, s, −O*H*), 7.14–7.51 (10H, m, Ph-*H*).

(3*S*,4*S*,2′*S*)-*trans*-1e: ^{1}H NMR (DMSO-d_6) δ 0.89 (3H, t, J = 7.8 Hz, H-10), 0.91 (3H, d, J = 7.0 Hz, 3-CH_3), 1.22 (1H, qd, J = 12.5, 12.5, 12.5, 3.1 Hz, H-5a), 1.60 (1H, dbr, J = 12.5, 1.9 Hz, H-5e), 1.84 (2H, q, J = 7.8 Hz, H-9), 1.87 (1H, t, J = 11.0 Hz, H-2a), 2.93 (1H, dbr, J = 10.9, 3.1 Hz, H-2e), 1.50 (1H, dddq, J = 11.0, 11.0, 3.1, 7.0 Hz, H-3a), 2.03 (1H, td, J = 12.5, 12.5, 1.6 Hz, H-6a), 2.91 (1H, dbr, J = 12.5, 3.1 Hz, H-6e), 2.28, 2.39, 4.59 (3H, ABX, J = 12.5, 8.6, 3.9 Hz, H-1′, H-2′), 4.30 (1H, m, J = 11.0, 12.5 Hz, H-4a), 4.89 (1H, s, −O*H*), 7.13–7.51 (10H, m, Ph-*H*).

X-ray Analysis for 1a,g. A *cis* form isomer, **1a**, and a *trans* form one, **1g**, were selected from eight isomers for X-ray crystallographic studies. The single crystal for X-ray analysis recrystallized from petroleum ether (60–90 °C) as rectangular colorless crystals, $C_{23}H_{30}N_2O_2$, M_r = 366.5. A computer-controlled R 3m/E diffractometer with Cu Kα radiation and an incident beam graphite monochromator was used for the X-ray data collection. The scan range was $0° < \theta \leq 57°$ with ω scanning. The space group of **1a** is rhombic, $P2_12_12_1$, with a = 7.583(1) Å, b = 11.732(8) Å, c = 23.214(7) Å, V = 2065.2(16) Å^3, D_x = 1.179 g cm^{-3}, and Z = 4. A total of 1623 unique reflections were measured; 1284 of them were observed reflections ($I \geq 3\sigma(I)$). The space group of **1g** is rhombic, $P2_12_12_1$, with a = 8.398(1) Å, b = 12.712(3) Å, c = 20.064(5) Å, V = 2142.23(96) Å^3, D_x = 1.137 g cm^{-3}, and Z = 4. A total of 1682 unique reflections were measured; 1369 of them were observed reflections ($I \geq 3\sigma(I)$). The structures were solved by direct methods with the aid of the program SHELEX-86. As for **1a**, the coordinates of 26 non-hydrogen atoms were observed in an E map, while the coordinates of all 27 non-hydrogen atoms were observed in an E map of isomer **1g**. The coordinates and anisotropic thermal parameters for all C, N, and O atoms were refined by the full-matrix least-squares method and the difference Fourier combined method. Hydrogen atoms were refined by the difference Fourier combined method and the geometric calculated method. The final R factor for **1a** was 0.072 and for **1g** was 0.0822.

Analgesic Pharmacological Tests. Female mice of 18–22 g of body weight were used. The analgesic activity was assessed by hot plate tests after ip administration of the compounds to be tested. The ED_{50} values and the 95% confidence limits were calculated by the method of Litchfield and Wilcoxon.

Ligand Binding Assays. The mouse brain membranes (P_2 fraction) were prepared as described previously.[28] The μ binding sites were labeled utilizing [^{3}H]DAMGO (Amersham; 2.22 TBq/mmol), and δ binding sites were labeled using [^{3}H]-DPDPE (New England Nuclear, 1.04 TBq/mmol).[7,10] The nonspecific binding was measured by incubation in the presence of 10 μM DAMGO or DPDPE. Briefly, the binding assays were conducted at 30 °C in 0.05 M Tris-HCl buffer (pH 7.4) with radioligand in the presence of different concentrations of test compounds; each assay mixture (1.0 mL) contained 1 mg of crude synaptic plasma membranes of mice brain. After a 45 min incubation, the samples were immediately cooled in an ice bath and filtered through glass fiber filters (Whatman GF/C) on a Millipore Model 1225 sampling manifold instrument, and then the samples were washed with three 4 mL portions of ice-cold Tris-HCl buffer. The fiber filters were dried and transferred to counting vials with scintillation cocktail. The radioactivity was determined by a Beckman LS6500 multipurpose scintillation counter. The IC_{50} values and slope factors were obtained by linear regression from probit-log plots. The corresponding K_i values were calculated utilizing standard equations.

Isolated Tissue Bioassays. The myenteric plexus-longitudinal muscle from guinea pig ileum was prepared as previously described by Kosterlitz *et al.*[29] The vas deferens from mouse or rabbit were prepared by the manner reported by Hughes *et al.*[30] and Oka *et al.*,[31] respectively. All preparations were suspended in an organ bath containing 6 mL of Kreb's solution. The bath fluid was kept at 37 ± 1 °C and gassed continuously with 95% O_2 and 5% CO_2. After equilibration for 30 min, longitudinal contractions were evoked by field stimulation through Pt electrodes at the upper and lower ends of the bath. The parameters of stimulation were as follows. For GPI and RVD, single puses were used (50 V, 1.0 ms duration, 15 s interval). For the MVD, the trains consisted of three pulses at intervals of 200 ms (40 V, 1.0 ms duration, 15 s interval). The contractions were recorded by means of a force displacement transducer and recorder. The IC_{50} values were obtained using curves of concentration–response.

Theoretical Conformational Analysis. All calculations were carried out using the MMP2 program[32] on an AST/486 microcomputer. The standard MMP2(87)/QCPE force field was employed for the energy calculations, and the torsional parameters of the N-propanamide group, which were short-handed in MMP2(87), were chosen from the parameters of amide groups reported by Kontoyianni.[33] Starting with the X-ray crystallographic data of **1a**, its conformational property was analyzed using molecular mechanics by a simplified systematic search. The chair conformation of the piperidine ring part in **1a** was chosen, and the piperidine ring divided the flexible groups into two large sections, the 1β-hydroxyphenylethyl group and the 4-*N*-phenylpropanamide group. The

conformations of the two sections were searched with a simplified strategy to give the corresponding energetic favorable conformations, respectively. Combination of the favorable conformations of the two sections produced 21 basic structures, and for each of the structures a grid search encompassing all rotatable bonds and using 20° increments was performed. Conformations within 2.5 kcal/mol of the lowest energy structure were grouped into a family. Structurally, **1b**,**a** are different in the configuration of the 1β-hydroxyl group, so the conformation of **1b** was analyzed on the basis of results from the systematic conformation analysis of **1a**.

Acknowledgment. This work was supported by The National Science and Technology Commission of China and the National Natural Science Foundation of China (Grants 39470805). We thank Professor Yang Lu and Qi-Tai Zhen (Beijing Institute of Materia Medica, CAMS) for the X-ray crystallographic data on compounds **1a**,**g** and Dr. Hong-Wu Wang for theoretical conformational analysis.

Supporting Information Available: Tables of atomic coordinates, bond lengths, bond angles, torsion angles, isotropic thermal parameters, and H atom coordinates for compounds **1a**,**g** (10 pages). Ordering information is given on any current masthead page.

References

(1) (a) This work was presented in part at the XIIIth International Symposium on Medicinal Chemistry (Paris, France, September 19–23, 1994; Abstr. 00428(02A)) and the 25th International Narcotis Research Conference (*Regul. Pept.* **1994**, *54*, 59–60). (b) For a preliminary account of this work, see: Wang, Z. X.; Zhu, Y. C.; Chen, X. J.; Ji, R. Y. Ohmefentanyl Enantiomers and Its Analgesic Activity. *Chin. Sci. Bull.* **1994**, *39*, 2004–2008.

(2) Reisine, T.; Bell, G. I. Molecular Biology of Opioid Receptor. *Trends Neurosci.* **1993**, *16*, 506–510 and references cited therein.

(3) Zimmerman, D. M.; Leader, J. D. Selective Opioid Receptor Agonists and Antagonists: Research tools and potential therapeutic agents. *J. Med. Chem.* **1990**, *33*, 895–902.

(4) Janssen, P. A. J. A Review of the Chemical Features Associated with Strong Morphine-like Activity. *Br. J. Anaesth.* **1962**, *34*, 260–268.

(5) Casy, A. F.; Parfitt, R. T. *Opioid Analgesics: Chemistry and Receptors*; Plenum Press: New York, 1986; pp 287–301.

(6) (a) Riley, T. N.; Hale, D. B.; Wilson, M. C. 4-Anilinopiperidine Analgesics I. Synthetic and Analgesic Activity of Certain Ring-Methylated 1-Substituted 4-Propanilidopiperidines. *J. Pharm. Sci.* **1973**, *62*, 983–986. (b) Van Bever, W. F. M.; Niemegeers, J. E.; Janssen, P. A. J. Synthetic Analgesics. Synthesis and Pharmacology of the Diastereoisomers or N-[3-Methyl-1-(2-phenylethyl)-4-piperidyl]-N-phenylpropanamide and N-[3-Methyl-1-(1-methyl-2-phenylethyl)-4-piperidyl]-N-phenylpropanamide. *J. Med. Chem.* **1974**, *17*, 1047–1051. (c) Jin, W. Q.; Xu, H.; Zhu, Y. C.; Fang, S. N.; Xia, X. L.; Huang, Z. M.; Ge, B. L.; Chi, Z. Q. Studies on Synthesis and Relationship Between Analgesic Activity and Receptor Affinity for 3-Methylfentanyl Derivatives. *Sci. Sin. (Engl. Ed.)* **1981**, *24*, 710–720.

(7) Xu, H.; Chen, J.; Chi, Z. Q. Ohmefentanyl-A New Agonist for μ-Opiate Receptor. *Sci. Sin., Ser. B* **1985**, *28*, 504–511.

(8) In ref 9, four diastereoisomers of ohmefentanyl were prepared in a nine-step synthesis and a two-step fractional crystallization. After *cis*- and *trans* form separation (the first fractional crystallization), the first crystallized isomer was named *cis*- or *trans*-A-ohmefentanyl and the second one from mother solution was *cis*- or *trans*-B-ohmefentanyl in the second fractional crystallization, respectively. Recently, we have confirmed that *cis*-A-1 is a racemic pair of (3*R*,4*S*,2′*R*)-**1b** and (3*S*,4*R*,2′*R*)-**1c** and *cis*-B-1 is a racemic pair of (3*R*,4*S*,2′*S*)-**1a** and (3*S*,4*R*,2′*S*)-**1d** by HPLC and ^{1}H NMR. See: Wang, Z. X.; Zhu, Y. C.; Jiang, N.; Ji, R. Y. Determination of Compositions and Configurations of *cis*-A- and *cis*-B-Ohmefentanyl by HPLC and ^{1}H NMR. *Yaoxue Xuebao* **1995**, *30* (7), in press.

(9) Zhu, Y. C.; Wu, R. Q.; Chou, D. P.; Huang, Z. M. Studies on Potent Analgesics. VII. Synthesis and Analgesic Activity of Diastereoisomers of 1-β-Hydroxyl-3-methylfentanyl(7302) and Related Compounds. *Yaoxue Xuebao* **1983**, *18*, 900–904.

(10) Xu, H.; Yao, Y. H.; Zhu, Y. C.; Chen, J.; Chi, Z. Q. Potent 3-Methylfentanyl Analogs: Morphine-like Catalepsy and Receptor Binding Characteristics. *Zhonguo Yaoli Xuebo* **1987**, *8*, 289–292.

(11) Jin, W. Q.; Chen, X. J.; Chi, Z. Q. The Choice of Opioid Receptor Subtype in Isolated Preparations by Ohmefentanyl. *Sci. Sin., Ser. B* **1987**, *30*, 176–181.

(12) (a) Ye, S. Z.; Li, G. F.; Chi, Z. Q. Autoradiography of [^{3}H]-Ohmefentanyl Binding with Opiate Receptors in Rat Brain. *Zhongguo Yaoli Xuebao* **1986**, *7*, 193–198. (b) Wang, H.; Pelaprat, D.; Roques, B. P.; Vanhove, A.; Chi, Z. Q.; Rostene, W. [^{3}H]Ohmefentanyl Preferentially Binds to μ-Opioid Receptors But Also Labels σ-Sites in Rat Brain Sections. *Eur. J. Pharmacol.* **1991**, *193*, 341–350. (c) Wang, H.; Sarrieau, A.; Pelaprat, D.; Roques, B. P.; Vanhove, A.; Kopp, N.; Chi, Z. Q.; Rostene, W. Characterization and Distribution of [^{3}H]Ohmefentanyl Binding Sites in the Human Brain. *Synapse* **1991**, *8*, 177–184.

(13) Goldstein, A.; Naidu, A. Multiple opioid receptors: Ligand Selectivity Profiles and Binding Site Signatures. *Mol. Pharmacol.* **1989**, *36*, 265–272.

(14) Wang, H.; Ye, S. Z.; Li, G. F.; Chi, Z. Q. Comparison of Outogenices of Opioid Receptors in Rat, Guinea Pig, and Rabbit CNS by Autoradiography. *Zhongguo Yaoli Xuebao* **1988**, *9*, 205–212.

(15) Yao, Y. H.; Xu, H.; Chi, Z. Q. Cataleptic Effect of Ohmefentanyl in the Rat. *Chin. J. Physiol. Sci.* **1985**, *1*, 151–158.

(16) Wang, Z. X.; Zhu, Y. C.; Chen, X. J.; Ji, R. Y. Stereoisomers of 3-Methylfentanyl: Synthesis, Absolute Configuration and Analgesic Activity. *Yaoxue Xuebao* **1993**, *28*, 950–910.

(17) Kim, C.-H.; Rothman, R. B.; Jacobson, A. E.; Mattson, M. V.; Bykov, V.; Streaty, R. A.; Klee, W. A.; George, C.; Long, J. B.; Rice, K. C. Probes for Narcotic Receptor Mediated Phenomena. 15. (3*S*,4*S*)-(+)-*trans*-3-Methylfentanyl Isothiocyanate, A Potent Site-directed Acylating Agent for the δ Opioid Receptors in vitro. *J. Med. Chem.* **1989**, *32*, 1392–1398.

(18) Burke, T. R., Jr.; Jacobson, A. E.; Rice, K. C.; Silverton, J. V.; Simonds, W. F.; Streaty, R. A.; Klee, W. A. Probes for Narcotic Receptor Mediated Phenomena. 12. *cis*-(+)-3-Methylfentanyl Isothiocyanate, A Potent Site-directed Acylating Agent for δ Opioid Receptors. Synthesis, Absolute Configuration and Receptor Enantioselectivity. *J. Med. Chem.* **1986**, *29*, 1087–1093.

(19) Brown, H. C.; Pai, G. G. Asymmetric Reduction of Prochiral α-Halo Ketones with *B*-3-Pinanyl-9-brorabicyclo[3,3,1] nonane. *J. Org. Chem.* **1983**, *48*, 1784–1786.

(20) Brine, G. A.; Streak, P. A.; Carroll, F. I.; Xu, H.; Rothman, R. B. Enantiomers of (±)-*cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-N-phenylpropanamide: Influence of the Hydroxyl Group. *Med. Chem. Res.* **1992**, *2*, 34–40.

(21) Gao, J. H.; Wang, Z. X.; Song, G. Q.; Zhu, Y. C.; Ji, R. Y. ^{1}H-NMR and Stereochemistry of Ohmefentanyl Enantiomers. *Acta Chim. Sin.* **1995**, *53* (9), in press.

(22) Handa, B. K.; Lane, A. C.; Lord, J. A. H.; Morgan, B. A.; Rance, M. J.; Smith, C. F. C. Analogues of β-LPH$_{61-64}$ Possessing Selective Agonist Activity at μ-Opiate Receptors. *Eur. J. Pharmacol.* **1981**, *70*, 531–540.

(23) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. Bis-penicillamine Enkephalins Possess Highly Improved Specificity toward δ Opioid Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5871–5874.

(24) Rothman, R. B.; Xu, H.; Seggel, M.; Jacobson, A. E.; Rice, K. C.; Brine, G. A.; Carroll, F. I. FTI-4614-4: An Analog of (+)-*cis*-3-Methylfentanyl with a 27,000-fold Binding Selectivity for μ versus δ Opioid Binding Sites. *Life Sci.* **1991**, *48*, PL-111-116.

(25) Brine, G. A.; Stark, P. A.; Liu, Y.; Carroll, F. I.; Singh, P.; Xu, H.; Rothman, R. B. Enantiomers of Diastereomeric *cis*-N-[1-(2-Hydroxy-2-phenylethyl)-3-methyl-4-piperidyl]-N-phenylpropanamides: Synthesis, X-ray Analysis, and Biological Activities. *J. Med. Chem.* **1995**, *38*, 1547–1557. The authors thank Dr. Brine for sending a reprint of their recent publication.

(26) Wang, H. W.; Chen, K. X.; Ji, R. Y. A Systematic Conformation Search for High Analgesic Agent Ohmefentanyl. *Chin. J. Med. Chem.* **1994**, *4*, 121–127.

(27) Protoghese, P. S. Stereoisomeric Ligands as Opioid Receptor Probes. *Acc. Chem. Res.* **1978**, *11*, 21–28.

(28) Jin, W. Q.; Fan, L. Q.; Chen, X. J.; Chi, Z. Q. P-7521 – A New Irreversible Opioid Ligand. *Zhongguo Yaoli Xuebao* **1989**, *10*, 205–210.

(29) Kosterlitz, H. W.; Lydon, R. J.; Watt, A. J. The Effects of Adrenaline, Noradrenaline and Isoprenaline on Inhibitory α- and β-Adrenoceptors in the Longitudinal Muscle of the Guineapig Ileum. *Br. J. Pharmacol.* **1970**, *39*, 398–413.

(30) Hughes, J.; Kosterlitz, H. W.; Leslie, F. M. Effect of Morphine on Adrenergic Transmission in the Mouse Vas Deferens. Assessment of Agonist and Antagonist Potencies of Narcotic Analgesics. *Br. J. Pharmacol.* **1975**, *53*, 371–381.

(31) Oka, T.; Negishi, K.; Suda, M.; Matsumiya, T.; Inazu, T.; Ueki, M. Rabbit Vas Deferens: A Specific Bioassay for Opioid κ-Receptor Agonists. *Eur. J. Pharmacol.* **1980**, *73*, 235–236.

(32) Burkert, U.; Allinger, N. L. *Molecular Mechanics, ACS Monograph Series 177*, 1982.

(33) Kontoyianni, M.; Bown, J. P. An *Ab Initio* and Molecular Mechanics Investigations of Ureas and Amide Derivatives. *J. Comput. Chem.* **1992**, *13*, 657–665.

JM9501487