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Synthesis and analgesic activity of stereoisomers of *cis*-fluoro-ohmefentanyl

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Received August 22, 2002, accepted October 28, 2002

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Pharmazie 58: 300–302 (2003)

Four stereoisomers **1a–d** of *cis*-fluoro-ohmefentanyl have been synthesized. Their absolute configurations were determined by X-ray analysis of (3*S*,4*R*,2'*S*')-(–)-*cis*-**1d**. The analgesic activity (mice, sc, hot plate) revealed extreme stereodifferences. The ED₅₀ value of (3*R*,4*S*,2'*S*')-(+)-*cis*-**1a** was 0.000774 mg/kg (17958 times more potent than that of morphine), while the corresponding antipode **1c** was almost inactive.

1. Introduction

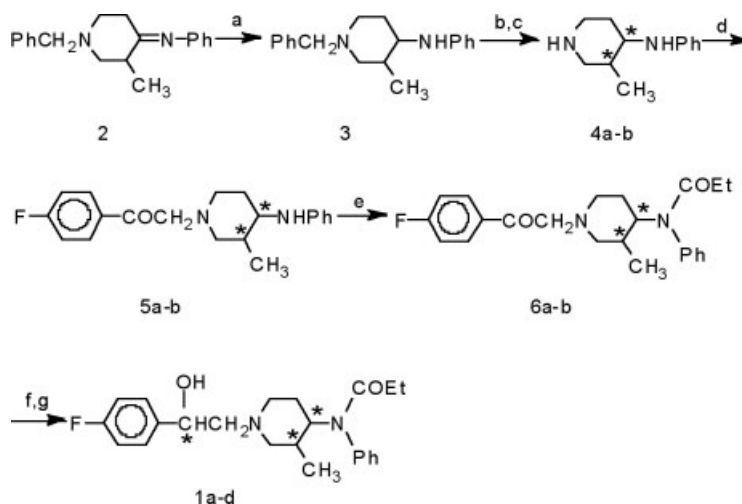
Ohmefentanyl (OMF) is a well-known potent μ -opioid receptor agonist which has firstly been synthesized in our laboratory in the early 1970s [1]. There are 3 chiral carbon atoms in OMF, so eight optically active isomers are possible. In our earlier reports [2, 3], we described the synthesis, stereochemistry, analgesic activity and opioid receptor binding characteristics of OMF. We have also found that the analgesic activity of the four stereoisomers of *cis*-OMF show great differences. For example, the ED₅₀ value (mice, ip, hot plate) of (+)-*cis*-(3*R*,4*S*,2'*S*')-OMF was 0.00106 mg/kg (13100 times more potent than that of morphine); while the enantiomer of which, (–)-*cis*-(3*S*,4*R*,2'*R*')-OMF was almost inactive under the same conditions (ED₅₀ > 10 mg/kg). At the same time, the analgesic activity of the four stereoisomers of *trans*-OMF

reveal little differences. Thus, it is believed that the *cis*-isomers of OMF can provide more information about the stereochemistry of the μ -opioid receptor, and be more useful for further research.

The above-mentioned results stimulated our interest in synthesizing and evaluating the four stereoisomers of *cis*-fluoro-ohmefentanyl (*cis*-FOMF). An F-atom was introduced in the aromatic ring mainly for two reasons, one is that the F-atom is an effective bioisoster known from many potent drugs; the other is that it is part of ¹⁸F substituted ohmefentanyl (¹⁸F-OMF), an extremely useful PET probe for molecular biology studies of the μ -opioid receptor.

Here we describe the synthesis of the four stereoisomers of *cis*-fluoro-ohmefentanyl, the determination of their absolute configurations, and the evaluation of their analgesic activities.

Scheme



Reagents: (a) NaBH₄/MeOH; (b) 10% Pd–C/H₂; (c) tartaric acid resolution; (d) 2-bromo-4'-fluoroacetophenone; (e) EtCOCl/PhMe; (f) NaBH₄/MeOH; (g) fractional crystallization.

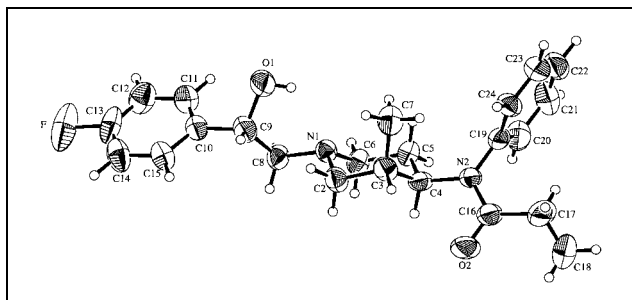


Fig.: X-ray crystal structure of (3*S*,4*R*,2'*S*)-(+)-*cis*-**1** (**1d**)

2. Investigations, results, and discussion

2.1. Chemistry

The Scheme outlines the synthetic route to the stereoisomers of *cis*-fluoro-ohmefentanyl. 1-Benzyl-3-methyl-4-phenyliminopiperidine (**2**) was reduced by NaBH₄ to yield 1-benzyl-3-methyl-*N*-phenyl-4-piperidinamine (**3**) which consisted of an approximately 7:3 mixture of *cis*- and *trans*-diastereoisomers. The *cis*-**3** was obtained by crystallization of the oxalate salt [4]. Debenzylation of *cis*-**3** gave the racemic *cis*-**4**, which was successfully resolved to **4a**, **4b** via fractional crystallization of their tartrates by Janssen's methods [5]. The absolute configurations of **4a–4b** are (+)-*cis*-**4a** as 3*R*,4*S*; (–)-*cis*-**4b** as 3*S*,4*R* [3].

Alkylation of **4a** with 2-bromo-4'-fluoroacetophenone at room temperature yielded an unstable intermediate **5a**, which was immediately acylated with propionyl chloride to give **6a**. **6a** was reduced by NaBH₄ to afford a mixture of diastereoisomers of **1a**, **1b**, which were separated by fractional crystallization to obtain optically pure isomers **1a** (the first which crystallized) and **1b** (the second one crystallized). **1c**, **1d** can be obtained by the same procedure from **4b**. The overall yield was ca. 29%, calculated from optically active compounds **4a–b**.

A second crystallized isomer, **1d** was selected for X-ray crystallographic study (Fig.), since the absolute configuration of the intermediates **4a–b** have been previously established and the transformation from intermediates **4a–b** to final product had no effect on the configurations of the piperidine 3- and 4-carbons [3], the absolute configuration of **1d** was confirmed as (3*S*,4*R*,2'*S*), reasonably the first crystallized isomer **1c** as (3*S*,4*R*,2'*R*). This result agreed with a similar method designed for the synthesis of the four *cis*-stereoisomers of ohmefentanyl by Brine et al. [6].

In addition, X-ray studies showed that there is an intramolecular hydrogen bond at O(1)–H–N(1) (2.167 Å) in the **1d** molecule.

2.2. Pharmacology

The analgesic activity was assessed in mice by the hot plate method after sc administration of the compounds to be tested. All compounds showed a typical morphine-like analgesic action and the ED₅₀ values are given in the Table. Among the six compounds (including two ketone compounds **6a**, **6b**), (3*R*,4*S*,2'*S*)-(+)-*cis*-**1** (**1a**) was found to be 17958 times more potent than morphine with ED₅₀ = 0.000774 mg/kg while the ED₅₀ value of its antipode (3*S*,4*R*,2'*R*)-*cis*-**1** (**1c**) was higher than 10 mg/kg. (3*R*,4*S*)-(–)-*cis*-**6** (**6a**) was found to be 205 times more potent than morphine with ED₅₀ = 0.0676 mg/kg.

3. Discussion

Pharmacology results showed the extreme differences of analgesic activity among stereoisomers of *cis*-FOMF, which were similar to those of OMF. Among the six isomers, (3*R*,4*S*,2'*S*)-(+)-*cis*-**1** (**1a**) was found to be 17958 times more potent than morphine, while its antipode (3*S*,4*R*,2'*R*)-*cis*-(–)-**1** (**1c**) was almost inactive under the same conditions. The order of analgesic potency is **1a** > **1b** > **6a** > **6b** > **1c** ≈ **1d**, which indicates the highly stereo-selective property of the opioid receptor recognition and the great importance of 3-methyl in the piperidine ring, 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. This result is identical with that observed in stereoisomers of 3-methylfentanyl and ohmefentanyl, where all of the most potent isomers were found to have a 3*R*,4*S* configuration [3, 7].

The introduction of a fluoro atom improved the analgesic activity, the ED₅₀ values of (+)-*cis*-(3*R*,4*S*,2'*S*)-FOMF (**1a**) and (–)-*cis*-(3*R*,4*S*,2'*R*)-FOMF (**1b**) (0.000774 mg/kg and 0.00362 mg/kg respectively) were both lower than that of (+)-*cis*-(3*R*,4*S*,2'*S*)-OMF and (–)-*cis*-(3*R*,4*S*,2'*R*)-OMF (0.00106 mg/kg and 0.00465 mg/kg, respectively), which may indicate that there is possibly a site of hydrogen bond contacting with the fluoro atom in the opioid receptor which increased the binding affinity of the molecules. The introduction of a fluoro atom did not change the mode of action and analgesic character in the hot plate model, indicating that it is reasonable and possible to

Table: Physicochemical constant and analgesic activity of compounds

Compd ^a	M.p. (°C)	[α] _D ²⁵ (MeOH)	Analgesic ^b ED ₅₀ (mg/kg)	Rel potency ^c , morphine = 1
(+)- <i>cis</i> -(3 <i>R</i> ,4 <i>S</i> ,2' <i>S</i>)- 1 (1a)	124–125	+19.81 (c 0.31)	0.000774 (0.000624–0.00142)	17958
(–)- <i>cis</i> -(3 <i>S</i> ,4 <i>R</i> ,2' <i>R</i>)- 1 (1c)	124–125	–19.53 (c 0.83)	>10	–
(–)- <i>cis</i> -(3 <i>R</i> ,4 <i>S</i> ,2' <i>R</i>)- 1 (1b)	133–135	–29.78 (c 0.45)	0.00362 (0.00243–0.00539)	3840
(+)- <i>cis</i> -(3 <i>S</i> ,4 <i>R</i> ,2' <i>S</i>)- 1 (1d)	133–135	+27.77 (c 0.66)	>10	–
(–)- <i>cis</i> -(3 <i>R</i> ,4 <i>S</i>)- 6 (6a) HCl	236–238	–6.96 (c 0.39)	0.0676 (0.0571–0.0800)	205
(+)- <i>cis</i> -(3 <i>S</i> ,4 <i>R</i>)- 6 (6b) HCl	236–238	+6.73 (c 0.52)	10.92 (9.89–12.06)	1.3

^a elemental analysis for C, H, N of all compounds are within ±0.3% of the calculated values; compounds **1a–d** were dissolved by addition of 1.0 equiv. of aqueous HCl.

^b hot plate test (in mice, sc); 95% confidence limits or effective animal number shown in parentheses. ^c morphine: ED₅₀ = 13.9 mg/kg.

choose ^{18}F -OMF as the PET probes in molecular pharmacology research.

(–)-*cis*-(3*R*,4*S*)-**6** (**6a**) was about 205 times more potent than morphine. We have also noticed that the duration of action maintaining above 50% of E_{max} was 300 min, much longer than that of other isomers which no more than 60 min, indicating the existence of a ketone may delay the metabolism of the compound. This result provided useful information for the research of long-acting analgesics.

4. Experimental

M.p.s. were determined with a Büchi 510 apparatus and are uncorrected. IR spectra were measured with a Perkin-Elmer 983G grating infrared spectrophotometer from KBr pellets. The ^1H NMR spectra were recorded on a Varian Genimi-2000 at 200 MHz using TMS as internal reference and CDCl_3 as solvent. MS spectra were recorded on a MAT-95 apparatus. Elemental analysis was performed on a Carlo-Erba 1106 apparatus. Optical rotation were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm, concentrations are given in g/ml.

4.1. *cis*-1-Benzyl-3-methyl-*N*-phenyl-4-piperidinamine (**3**)

Compound **3** was prepared and separated according to the procedure described elsewhere [4].

4.2. (3*R*,4*S*)-3-Methyl-*N*-4-phenyl-4-piperidinamine (**4a**) and (3*S*,4*R*)-3-methyl-4-phenyl-4-piperidinamine (**4b**)

Preparation and optical resolution of **4a**, **4b** was performed according to the general procedure described elsewhere [3, 5].

4.3. (3*R*,4*S*)-*cis*-(–)-*N*-[1-[2-oxo-2-(4'-fluorophenylethyl)]-4-piperidyl]-*N*-phenylpropanamide (**6a**) and (3*S*,4*R*)-*cis*-(+)-*N*-[3-methyl-1-[2-oxo-2-(4'-fluorophenylethyl)]-4-piperidyl]-*N*-phenylpropanamide (**6b**)

To a solution of **4a** (1.0 g) in dry toluene (25 ml) were added K_2CO_3 (8.0 g) and several crystals of KI. After stirring for 5 min, 2-bromo-4'-fluoroacetophenone (1.14 g) was added, the resultant mixture was stirred at room temperature for 1 h, filtered to remove solid, and obtaining the toluene solution of **5a**. As **5a** is rather unstable, the mixture was immediately acylated with propionyl chloride (2.0 ml) and refluxed for 5 h, allowed to cool, and then treated with aqueous K_2CO_3 and extracted with ether. The organic mixture was extracted with aqueous hydrochloride for several times, the combined acidic extracts were alkalized with K_2CO_3 extracted with ether, dried and concentrated. The residue was treated with EtOH saturated with HCl gas, recrystallization from EtOH/EtOAc/petroleum ether yielded white crystals, **6a**·HCl, m.p. = 236–238 °C, $[\alpha]_{\text{D}}^{25} - 6.96$ (c 0.39, MeOH). MS, m/z (%): 382 (M^+ 10), 335 (25) 259 (79), 160 (40), 122 (76), 105 (100), 77 (56).

6b·HCl was prepared from **4b** in the same procedure as **6a**·HCl, white crystals, m.p. 236–238 °C, $[\alpha]_{\text{D}}^{25} + 6.73$ (c 0.52, MeOH), MS, m/z (%): 382 (M^+ 10), 259 (100), 216 (20), 203 (38), 160 (36), 132 (9), 77 (5).

4.4. (3*R*,4*S*,2'*S*)-*cis*-(+)-*N*-[1-[2-Hydroxy-2-(4'-fluorophenylethyl)]-3-methyl-4-piperidyl]-*N*-phenyl-propanamide (**1a**) and (3*R*,4*S*,2'*R*)-*cis*-(–)-*N*-[1-[2-hydroxy-2-(4'-fluorophenylethyl)]-3-methyl-4-piperidyl]-*N*-phenylpropanamide (**1b**)

Solid NaBH_4 (0.5 g) was added in portions to a solution of **6a**·HCl (1.5 g) in MeOH (50 mL). The resultant mixture was refluxed for 2 h and then cooled, MeOH was evaporated with EtOAc several times, the EtOAc layer was dried and evaporated to obtain a mixture of **1a** and **1b**. Fractional crystallization from petroleum ether afforded the former crystallized **1a** (0.4 g) and the latter crystallized **1b** (0.35 g), respectively.

1a, fine white crystals, m.p. = 124–125 °C, $[\alpha]_{\text{D}}^{25} + 19.81$ (c 0.31, MeOH), ^1H NMR (CDCl_3) δ : 1.01 (3 H, t, $J = 7.4$ Hz, 10- CH_3), 1.17 (3 H, d, $J = 7.1$ Hz, 11- CH_3), 1.36 (1 H, br, 5e-H), 1.43 (1 H, br, 5a-H), 1.94 (2 H, q, $J = 7.6$ Hz, 9- CH_2), 2.11 (1 H, br, 6a-H), 2.38 (2 H, br, CH_2N), 2.66 (2 H, br, 2- CH_2), 2.76 (1 H, br, 3-CH), 3.04 (1 H, br, 6e-H), 3.99 (1 H, br, OH), 4.43 (1 H, dt, $J = 12.6$ Hz, 4a-H), 4.63 (1 H, br, 2'-CH), 6.97–7.9 (9 H, m, PhH). MS, m/z (%): 383 ($M-1$, 3), 366 (64), 323 (18), 259 (100), 216 (37), 203 (63), 160 (92), 149 (59), 132 (18), 77 (30).

1b, compact white crystals, m.p. = 133–135 °C, $[\alpha]_{\text{D}}^{25} - 29.78$ (c 0.45, MeOH), ^1H NMR (CDCl_3) δ : 1.01 (3 H, t, $J = 7.6$ Hz, 10- CH_3), 1.16 (3 H, d, $J = 7.1$ Hz, 11- CH_3), 1.35 (1 H, d, $J = 13.3$ Hz, 5e-H), 1.46 (1 H, qd, $J = 13.3$ Hz, 4.15 Hz, 5a-H), 1.93 (2 H, q, $J = 7.5$, 9- CH_2), 2.36 (4 H, m, 2a-H, 6a-H, 1'- CH_2), 2.69 (1 H, d, $J = 10.7$ Hz, 6e-H), 2.82 (1 H, br, 3e-H), 2.94 (1 H, d, $J = 11.5$ Hz, 2e-H), 4.44 (1 H, dt, $J = 12.9$ Hz, 4.34 Hz, 4a-H), 4.63 (1 H, dd, $J = 7.14$ Hz, 3.2 Hz, 2'-CH), 6.98–7.93 (9 H, m, PhH). MS, m/z (%): 385 ($M+1$, 4), 366 (8), 259 (100), 216 (30), 203 (58), 160 (52), 132 (10), 77 (10).

4.5. (3*S*,4*R*,2'*R*)-*cis*-(–)-*N*-[1-[2-Hydroxy-2-(4'-fluorophenylethyl)]-3-methyl-4-piperidyl]-*N*-phenyl-propanamide (**1c**) and (3*S*,4*R*,2'*S*)-*cis*-(+)-*N*-[1-[2-hydroxy-2-(4'-fluorophenylethyl)]-3-methyl-4-piperidyl]-*N*-phenylpropanamide (**1d**)

1c, **1d** were prepared and separated in the same procedure as **1a**, **1b**.

1c, fine white crystals, m.p. = 124–125 °C, $[\alpha]_{\text{D}}^{25} - 19.53$ (c 0.83, MeOH), ^1H NMR (CDCl_3) δ : 1.01 (3 H, t, $J = 7.4$ Hz, 10- CH_3), 1.16 (3 H, d, $J = 6.9$ Hz, 11- CH_3), 1.36 (1 H, br, 5e-H), 1.44 (1 H, d, $J = 9.1$ Hz, 5a-H), 1.94 (2 H, q, $J = 7.1$ Hz, 9- CH_2), 2.06 (1 H, mbr, 6a-H), 2.36 (2 H, m, 1'- CH_2N), 2.65 (2 H, br, 2- CH_2), 2.78 (1 H, br, 3-CH), 3.05 (1 H, d, $J = 11.0$ Hz, 6e-H), 4.05 (1 H, br, OH), 4.45 (1 H, dt, $J = 12.6$ Hz, 4.3 Hz, 4a-H), 4.61 (1 H, d, $J = 7.97$, 2'-CH), 6.97–7.39 (9 H, m, PhH). MS, m/z (%): 385 ($M+1$, 4), 366 (7), 259 (100), 216 (25), 203 (50), 160 (48), 132 (10), 77 (9).

1d, compact white crystals, m.p. = 133–135 °C, $[\alpha]_{\text{D}}^{25} + 27.77$ (c 0.66, MeOH), ^1H NMR (CDCl_3) δ : 1.01 (3 H, t, $J = 7.56$ Hz, 10- CH_3), 1.16 (3 H, d, $J = 6.87$ Hz, 11- CH_3), 1.34 (1 H, d, $J = 13.4$ Hz, 5e-H), 1.46 (1 H, qd, $J = 13.3$ Hz, 4.15 Hz, 5a-H), 1.92 (2 H, q, $J = 7.6$ Hz, 9- CH_2), 2.35 (4 H, m, 2a-H, 6a-H, 1'- CH_2), 2.68 (1 H, d, $J = 10.2$ Hz, 6e-H), 2.83 (1 H, br, 3e-H), 4.45 (1 H, dt, $J = 12.6$ Hz, 4.3 Hz, 4a-H), 4.61 (1 H, dd, $J = 10.2$ Hz, 2.7 Hz, 2'-CH), 6.98–7.40 (9 H, m, PhH). MS, m/z (%): 385 ($M+1$, 1), 366 (3), 259 (35), 238 (100), 223 (39), 203 (16), 160 (20), 146 (32), 77 (9).

Acknowledgements: This work was supported by The National Natural Science Foundation of China (Grant number: 29790123). The authors are very grateful to the staff of Department of Analysis, Shanghai Institute of Organic Chemistry, Chinese Academy of Science for the X-ray study.

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