Shanghai Institute of Materia Medica[1], Chinese Academy of Sciences, Shanghai, and Guanghzou Institute of Chemistry[2], Chinese Academy of Sciences, Guangzhou, China

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

Zou Yong[2], Wu Hao[1], Yuan Weifang[1], Dai Qiyuan[1], Chen Xinjian[1], Jin Wenqiao[1], Zhu Youcheng[1]

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Dr. Zou Yong, Guangzhou Institute of Chemistry, Chinese Academy of Sciences, 1122 Mailbox, Guangzhou/Guangdong, P.R. China
zouyong@gic.ac.cn

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Four stereoisomers **1a–d** of *cis*-fluoro-ohmefentanyl have been synthesized. Their absolute configurations were determined by X-ray analysis of (3*S*,4R,2′*S*)-(−)-*cis*-**l d**. The analgesic activity (mice, sc, hot plate) revealed extreme stereodifferences. The ED_{50} value of (3*R*,4*S*,2′*S*)-(+)-*cis*-**l** (**1a**) was 0.000774 mg/kg (17958 times more potent than that of morphine), while the corresponding antipode **1c** was almost inactive.

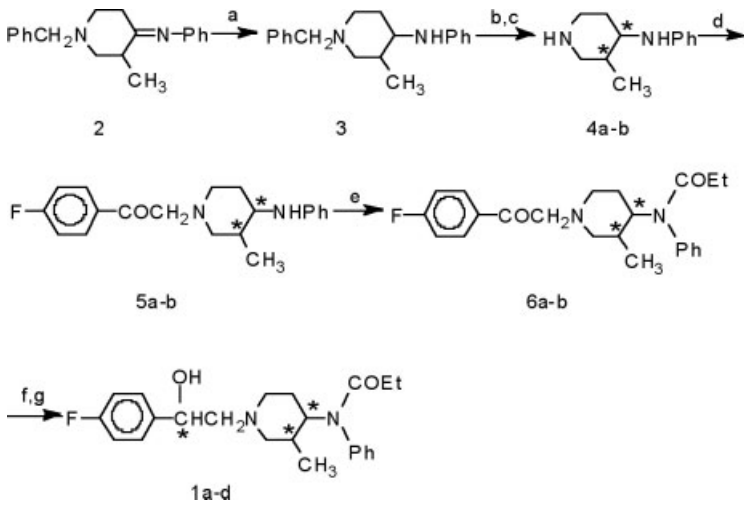
1. Introduction

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 ED_{50} 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-(3S,4R,2′R)-OMF was almost inactive under the same conditions ($ED_{50} > 10$ 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 ^{18}F substituted ohmefentanyl (^{18}F-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) $NaBH_4$/MeOH; (b) 10% Pd−C/H_2; (c) tartaric acid resolution; (d) 2-bromo-4′-fluoroacetophenone; (e) EtCOCl/PhMe; (f) $NaBH_4$/MeOH; (g) fractional crystallization.

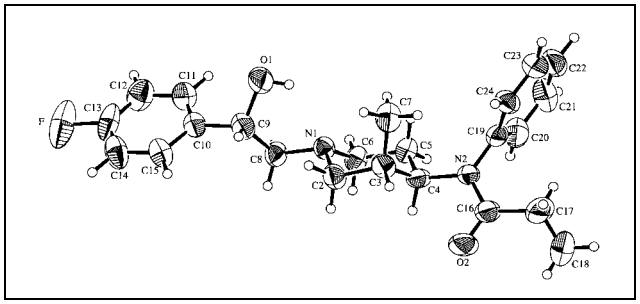


Fig.: X-ray crystal structure of (3S,4R,2′S)-(+)-cis-**1** (**1d**)

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 $NaBH_4$ 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*-**4**(**4a**) as 3*R*,4*S*; (−)-*cis*-**4**(**4b**) as 3*S*,4*R* [3].

Alkylation of **4a** with 2-bromo-4′-fluoroacetophenone at room temprature yielded an unstable intermediate **5a**, which was immediately acylated with propionyl chloride to give **6a**. **6a** was reduced by $NaBH_4$ 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 crystallgraphic 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 bone 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 ED_{50} 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 $ED_{50} = 0.000774$ mg/kg while the ED_{50} 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 $ED_{50} = 0.0676$ 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 ED_{50} 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)	$[\alpha]_D^{25}$ (MeOH)	Analgesic[b] ED_{50} (mg/kg)	Rel potency[c], morphine = 1
(+)-*cis*-(3*R*,4*S*,2′*S*)-**1** (**1a**)	124–125	+19.81 (c 0.31)	0.000774 (0.000624–0.00142)	17958
(−)-*cis*-(3*S*,4*R*,2′*R*)-**1** (**1c**)	124–125	−19.53 (c 0.83)	>10	–
(−)-*cis*-(3*R*,4*S*,2′*R*)-**1** (**1b**)	133–135	−29.78 (c 0.45)	0.00362 (0.00243–0.00539)	3840
(+)-*cis*-(3*S*,4*R*,2′*S*)-**1** (**1d**)	133–135	+27.77 (c 0.66)	>10	–
(−)-*cis*-(3*R*,4*S*)-**6** (**6a**) HCl	236–238	−6.96 (c 0.39)	0.0676 (0.0571–0.0800)	205
(+)-*cis*-(3*S*,4*R*)-**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: $ED_{50} = 13.9$ 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 ^{1}H 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. (3R,4S)-3-Methyl-N-4-phenyl-4-piperidinamine (4a) *and (3S,4R)-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. (3R,4S)-cis-(−)-N-[3-Methyl-1-[2-oxo-2-(4′-fluorophenylethyl)]-4-piperidyl]-N-phenylpropanamide (6a) *and (3S,4R)-cis-(+)-N-[3-methyl-1-[2-oxo-2-(4′-fluorophenylethyl)]-4-piperidyl]-N-phenylpropan-amide* (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, $[\alpha]_D^{25}$ − 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, $[\alpha]_D^{25}$ +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. (3R,4S,2′S)-cis-(+)-N-[1-[2-Hydroxy-2-(4′-fluorophenylethyl)]-3-methyl-4-piperidyl]-N-phenyl-propanamide (1a) *and (3R,4S,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, $[\alpha]_D^{25}$ + 19.81 (c 0.31, MeOH), ^{1}H 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, 4.2 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, $[\alpha]_D^{25}$ − 29.78 (c 0.45, MeOH), ^{1}H 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. (3S,4R,2′R)-cis-(−)-N-[1-[2-Hydroxy-2-(4′-fluorophenylethyl)]-3-methyl-4-piperidyl]-N-phenyl-propanamide (1c) *and (3S,4R,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, $[\alpha]_D^{25}$ − 19.53 (c 0.83, MeOH), ^{1}H 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, $[\alpha]_D^{25}$ + 27.77 (c 0.66, MeOH), ^{1}H 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).

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