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Research Article

Synthesis of ^{2}H- and ^{3}H-labeled *N*-cyclopropylmethyl-7α-[(*R*)-1-hydroxy-1-methyl-3-(2thienyl)-propyl]-6,14-endoethano-6,7,8,14-tetrahydro nororipavine

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Summary

A procedure for deuterium and tritium labeling of the titled compound, an analgesic agent, was developed. A secondary amine intermediate was acylated to an acylamide, then the carbonyl function was reduced by $LiAlD_4$ to yield the tertiary amine. In the tritium-labeled synthesis, the process utilized a bromo-substituted precursor, which was subsequently reduced with $^{3}H_2$ in the presence of a Pd/C catalyst. The labeled compounds were successfully applied in pharmacokinetic and pharmacological studies. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: analgesic agent; deuterium labeling; tritium labeling; synthesis

Introduction

N-cyclopropylmethyl-7α-[(*R*)-1-hydroxy-1-methyl-3-(2-thienyl)propyl]-6,14-endoethano-6,7,8,14-tetrahydro nororipavine (Figure 1, **1**) is a ligand of opioid receptor with partial agonistic activity as a μ-and δ-opioid receptor agonist and a κ-opioid receptor antagonist.[1] It is a promising chemical entity with potent analgesic activity, detoxification and anti-relapse effects. In preclinical study it provides low dosage, good oral absorption, poor dependence and fine therapeutic index. It has a long action time beyond 100 h against morphine addiction and helps to abstain from craving for drugs, decreasing dropout rate of abstainer.[2] However, its dosage is too low ($0.1–1\ mg\,kg^{-1}$) to reach the sensitivity for analytical detection with normal

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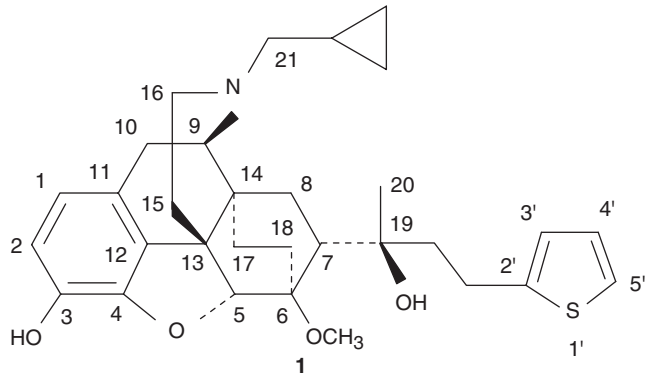

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Figure 1. Structure of *N*-Cyclopropylmethyl-7α-[(*R*)-1-hydroxy-1-methyl-3- (2-thienyl)propyl]-6,14-endoethano-6,7,8,14-tetrahydro nororipavine, 1

methods in the study of pharmacokinetics and pharmacology. To support those *in vitro* and *in vivo* studies, a deuterium/tritium isotopomer was required. In this report, the synthesis of this material is described.

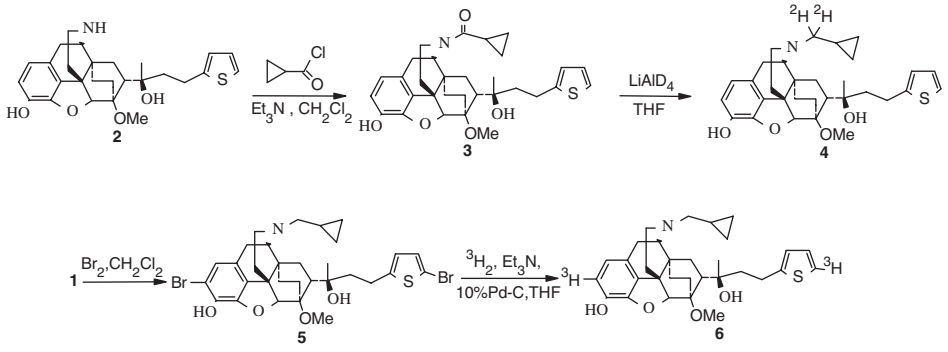
Discussion and results

The semi-synthesis of the thebaine derivatives was carried out according to literature procedures.[3–6] Bearing in mind the available synthetic routes and the likely metabolic pathways of the titled molecule **1**, we set about designing and synthesizing its deuterium/tritium-labeled isotopomer. We had succeeded in the direct reduction of the endoetheno bridge with tritium gas in the presence of catalyst to yield the 17,18-ditritide intermediate, but obtained a certain amount of compound **6** with very low specific activity because subsequent tritium hydrogen exchange may have caused the majority of the specific activity to be lost. Changing the order of hydrogenation in this synthetic route, i.e. synthesizing 6,14-endoetheno **1** first and followed by reduction of the 17,18-C = C double bond with tritium gas lastly, did not work either.

On the other hand, we successfully employed cyclopropanecarbonyl chloride to react with the secondary amine 7**α**-[(**R**)-1-hydroxy-1-methyl-3-(2-thienyl)-propyl]-6,14-endoethano-6,7,8,14-tetrahydro nororipavine **2**, yielding the acylamide intermediate **3**. The latter was reduced with $LiAlD_4$ to give an amine, 21-di-deuterium-labeled compound **4** (see Scheme 1, top line diagram).

This well-recrystallized product in methanol shows good ESI-MS and ^{1}H-NMR characteristics compared to the original data of **1**, its MW in MS increasing two units by two deuteriums, while its ^{1}H-NMR signals decreasing two hydrogens at 2.1–2.4 ppm (see Figure 2, a and b of top diagram).

Although this deuteride could be used in the *in vitro* pharmacokinetic and pharmacological study, it fell short of meeting the requirements of the *in vivo*



Scheme 1. Synthesis of isotopomers of 1: Top – synthesis of deuteride 4 via the secondary amine intermediate 2; bottom – synthesis of tritide 6 via the dibromo-substituted precursor 5

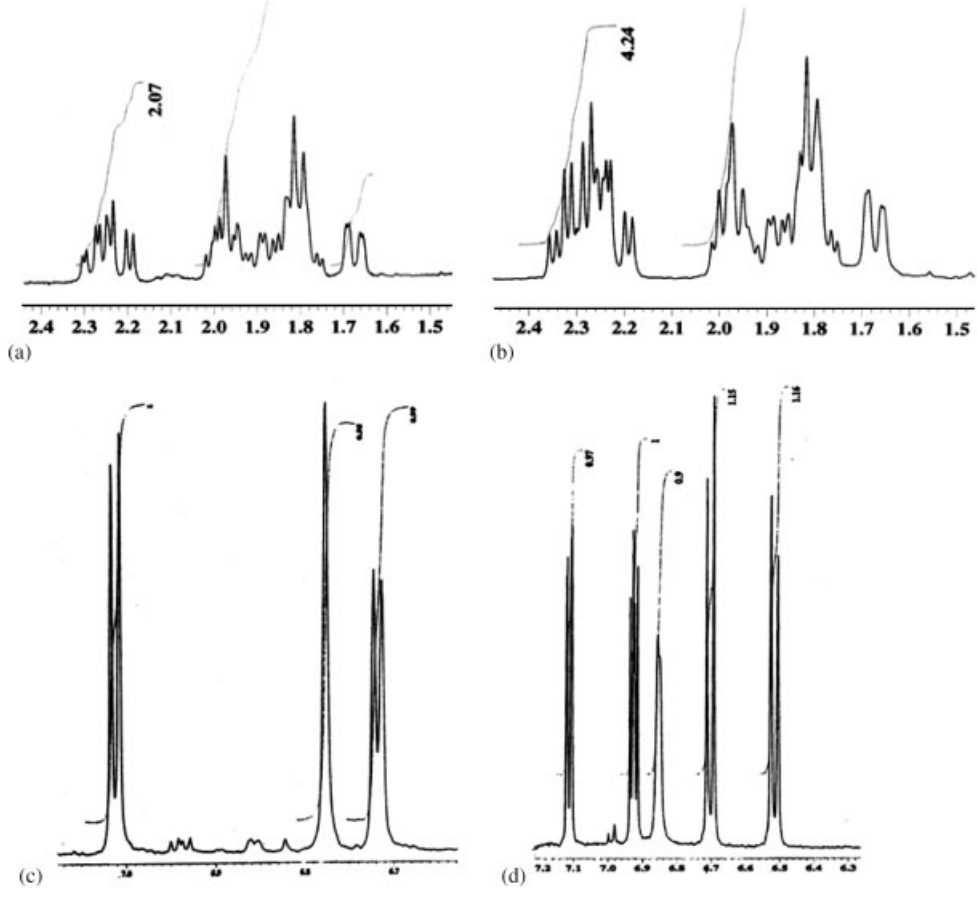


Figure 2. Amplified parts of ^{1}H-NMR spectrum of isotopomers of 1: Top – deuteride 4 (a) in contrast to that of 1 (b); bottom – dibromo-substituted intermediate 5 (c) in contrast to that of 1 (d)

study since the tertiary amine was apt to degrade in ADME, losing the cyclopropylmethyl and reversing back to the secondary amine **2**. Obviously, a new method to introduce isotopes into alternative positions of **1** was required.

The mechanism of electrophilic aromatic substitution provides the possibility of direct halogenation of the titled molecule. Once the halide compound was available, its conversion to tritide by catalytic reduction was easily achieved.[7] We succeeded in preparing of the bromo-substituted precursor **5** and its reduction compound **6**. The key to the bromo-substitution of **1** was to control the reaction time and temperature. The experiment gave complicated and viscous products. To meet the requirement of specific activity in pharmacokinetic and pharmacological study, we isolated **5** using silica gel 60 (230–400 mesh) chromatographic column, then reduced the compound to **6** in the presence of 10% Pd/C catalyst (see Scheme 1). The reason we employed Pd/C instead of PtO as catalyst is because the latter would be poisoned by the alkalinity of the amine **5** and the commercially available PtO is expensive.

The structure of the important intermediate, the dibromo-substituted compound **5**, was confirmed by ESI-MS and ^{1}H-NMR spectroscopy. The mass spectrum of **5** showed a $[M+1]^+$ peak at m/z 680.4, while $[M+1]^+$ of **1** at m/z 521 indicating that the double bromo-substitution had occurred. Further comparison of the ^{1}H-NMR data of **5** with that of **1**, hydrogen in low field decreased two resonance signals and the two hydrogens located at $\delta = 7.01$ and $\delta = 6.72$ showing coupling constant $J = 3.7$, confirming the two bromine atoms located at 2-C of oripavine and 5′-C of thiophene (see Figure 2, c and d of bottom diagram). These characteristic data can indirectly authenticate the structure of tritiated compound **6**. As our laboratory cannot offer the isotope analytic instruments, such as ^{3}H-NMR, ^{3}H-MS or ^{3}H-detective HPLC, we based on the pretest synthetic results and these data to confirm the structure of compound **6**.

These methods for deuterium and tritium labeling of **1** were finally developed and carried out in preparation of the resulting compound **4** and **6.** The tritide with high radioactivity (35 mCi), specific activity (18 Ci mmol^{-1}) and radiochemical purity (>95%), together with the deuteride, was successfully applied in the study of pharmacokinetics and pharmacology.

Materials and methods

Elemental analysis was carried out with a CarloErba-1106 Auto Elemental Analyzer. Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded as KBr discs on a Nicolet Magna-IR 550 spectrophotometer. Mass spectra were scanned on a Finnigan MAT-MS90 spectrometer. The ^{1}H-NMR spectra were obtained at 400 MHz on a JNM-ECA400 nuclear magnetic resonance spectrometer, and the chemical shifts are reported in parts per million (ppm, δ) downfield from

tetramethylsilane (0.0 ppm). An FJ-2107P liquid scintillation counter was used to determine the radioactivity in liquid samples. Lithium aluminum deuteride (98 atom% D) was purchased from Aldrich and directly used in synthetic experiments. Tritium gas was purchased from China Institute of Atomic Energy (CIAE). Other commercially available reagents were ACS grade and were purified by standard procedures. All reactions were monitored by a 25×75 mm HS-GF_{254} silica gel TLC provided by China Qingdao Haiyang Chemical Co., Ltd.

Experimental

Synthesis of N-cyclopropanecarbonyl-7α-[(R)-1-hydroxy-1-methyl-3-(2-thienyl)propyl]-6,14-endoethano-6,7,8,14-tetrahydro nororipavine, **3**

To a solution of 7α-[(R)-1-hydroxy-1-methyl-3-(2-thienyl)-propyl]-6,14-endoethano-6,7,8,14-tetrahydro nororipavine (**2**, 4.0 g, 8.5 mmol), triethylamine (2.7 g) in methylene chloride (125 ml), the liquid cyclopropanecarbonyl chloride (2.8 g, 26.8 mmol) in methylene chloride (25 ml) was added slowly with stirring at room temperature, then allowed to stir overnight at the same temperature. Solid in the mixture was filtered. The filtrate was evaporated *in vacuo* to dryness. The residue was dissolved with methylene chloride, dried (with anhydrous Na_2SO_4) and evaporated to dryness and redissolved in absolute ethanol to recrystallize to colorless crystals of **7** (3.3 g, 71%). m.p. 164–-166°C. Element analysis ($C_{31}H_{37}NO_5S$): calculated: C 69.50, H 6.91; found: C 69.87, H 6.93. MS (ESI):m/z 536.1$[M+1]^+$.

Synthesis of N-cyclopropylmethyl-21,21-[^{2}H]$_2$-7α-[(R)-1-hydroxy-1-methyl-3-(2-thienyl)propyl]-6,14-endoethano-6,7,8,14-tetrahydro nororipavine, **4**

To a mixture of $LiAlD_4$ (0.2 g, 4.8 mmol) and absolute THF (6 ml), a solution of **3** (0.5 g, 0.9 mmol) in THF (5 ml) was slowly added with stirring, and the whole heated gently to reflux. After reaction for about 6 h, the contents cooled to 5°C in an ice-water bath, and 20 ml of saturated aqueous salt solution was added to the mixture with stirring. The product was extracted from the aqueous layer with ether (3×25 ml), the combined organic phase dried with anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by recrystallization from methanol to give colorless crystals **4** (0.35 g, 73%). m.p. 174–176°C. ($C_{31}H_{37}D_2NO_4S$).

^{1}H-NMR spectrum ($CHCl_3$-*d*): δ = 8.98 (s, 1H, 3-Ar-OH), 7.10(d, 1H, J = 5.0 Hz, 5′-thien-H), 6.93(m, 1H, 4′-thien-H), 6.85(m, 1H, 3′-thien-H), 6.68(d, 1H, J = 7.9 Hz, 2-Ar-H), 6.51(d, 1H, J = 7.9 Hz, 1-Ar-H), 4.66(s, 1H, 19-OH), 4.44(s, 1H, 5β-H), 3.55(s, 3H, 6-OCH_3), 2.95–3.0(m, 5H), 2.62–2.63(m, 1H), 2.23–2.26(m, 2H), 1.79–1.97(m, 8H), 1.40(s, 3H, 20-CH_3), 1.08–1.25(m, 3H), 0.78(m, 2H, CH), 0.49(m, 2H).

MS (ESI):m/z 524.3$[M+1]^+$.

*Synthesis of N-cyclopropylmethyl-2-bromo-7α-[(**R**)-1-hydroxy-1-methyl-3-(5-bromo-thien-2-yl)-propyl]-6,14-endoethano-6,7,8,14-tetrahydro nororipavine,* **5**

To a solution of **1** (2.6 g, 5 mmol) in methylene dichloride (20 ml) in an ice-water bath, liquid bromine (0.6 ml, 11 mmol) in methylene dichloride (5 ml) was added with magnetic stirring and reacted for 15 min. The resulting mixture was washed with H_2O, saturated aqueous $NaHCO_3$ and brine. The organic phase was dried with anhydrous Na_2SO_4, filtered and evaporated to yield a crude yellow solid 1.0 g. This product was further purified by column chromatography on silica gel 60 (230–400 mesh) eluting with hexane/methylene dichloride/methanol (16:4:1, v/v/v) to yield pure **5** (0.2 g, 6%).

1H-NMR spectrum ($CHCl_3$-*d*): δ = 9.51(s, 1H, 3-Ar-OH), 7.00–7.01(d, 1H, *J*= 3.7 Hz, 4′-thien-H), 6.78(s, 1H, 1-Ar-H), 6.71–6.72(d, 1H, *J*= 3.7 Hz, 3′-thien-H), 4.64(s, 1H, 19-OH), 4.36(s, 1H, 5β-H), 3.45(s, 3H, 6-OCH_3), 3.00–1.61(m, 14H), 1.29(s, 3H, 20-CH_3), 1.20–0.08(m,11H).

MS (ESI) *m/z*: 680.4$[M+1]^+$.

Synthesis of N-cyclopropylmethyl-2-[^{3}H]-7α-[(R)-1-hydroxy-1-methyl-3-(5-[^{3}H]-thien-2-yl)-propyl]-6,14-endoethano-6,7,8,14-tetrahydro nororipavine, **6**

A solution of **5** (30.0 mg, 0.044 mmol) in absolute THF (2 ml), 10%Pd/C (12.0 mg) and anhydrous triethylamine (20 μl) was placed on a tritium manifold system (RC. TRITEC AG, Switzerland) and stirred for 24 h at room temperature until the uptake of the gas had stopped. After recovery of tritium gas, the mixture was filtered to remove solids (catalyst and triethylamine hydrobromide) and the filtrate was evaporated to dryness. The residue was dissolved in absolute ethanol (5 ml) and the solution evaporated to dryness three times for removal of labile tritium. The final compound **6** was purified by preparative silica gel TLC, developing with methylene dichloride/methanol (20:1, v/v, $R_f = 0.55$), and yielding a pale white solid (35 mCi). The specific radioactivity of 18 Ci mmol^{-1} was calculated from liquid scintillation data. The radiochemical purity was shown to be >95%.

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