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Research Article

Synthesis of ^{2}H- and ^{3}H-labelled iptakalim hydrochloride

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Summary

Iptakalim hydrochloride (*N*-(1-methylethyl)-1,1,2-trimethyl-propylamine hydrochloride) is a promising antihypertensive drug discovered and developed by Beijing Institute of Pharmacology and Toxicology. Deuterium- and tritium-labelled compounds were designed and synthesized for pharmacokinetic and pharmacological mechanism studies. A derivatization method has been employed in the synthesis procedure. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: iptakalim hydrochloride; tritium labelled; deuterium labelled; synthesis

Introduction

Iptakalim hydrochloride (*N*- (1-methylethyl) -1,1,2-trimethyl- propylamine hydrochloride) (Figure 1) is a new chemical entity with potent anti-hypertension activity. It cannot only decrease blood pressure in a dose-dependent manner, but also reverse the hypertensive cardiovascular remodelling. The molecular mechanisms underlying its antihypertensive action include ATP-sensitive potassium channel activation and endothelin antagonism.[1] The compound is now moving from preclinical study into clinical investigation.

Iptakalim hydrochloride is a small molecular compound. The free base is volatile and lacks a chromophore. Moreover, the steric effect of the multiple methyl groups hinders derivatisation. These features make it very difficult to carry out pharmacokinetic and pharmacological mechanism studies by HPLC or GC. To overcome these problems we designed and synthesized deuterium- and tritium-labelled isotopomers of iptakalim hydrochloride, bearing in mind the available synthetic routes and also the likely metabolite pathways of the compound (Scheme 1). These compounds provided convenient and useful

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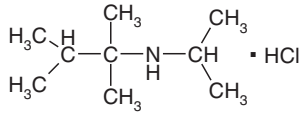
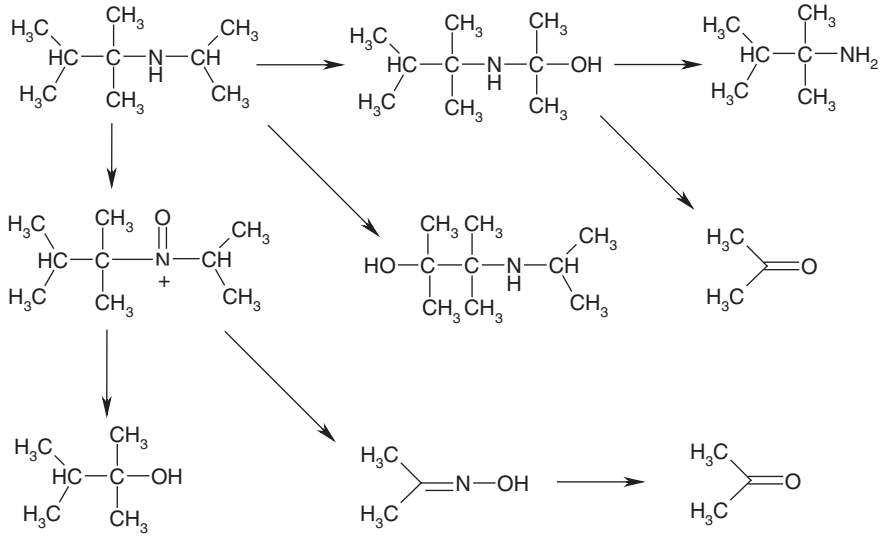


Figure 1. Structure of iptakalim hydrochloride



Scheme 1. Possible metabolic pathways of iptakalim hydrochloride

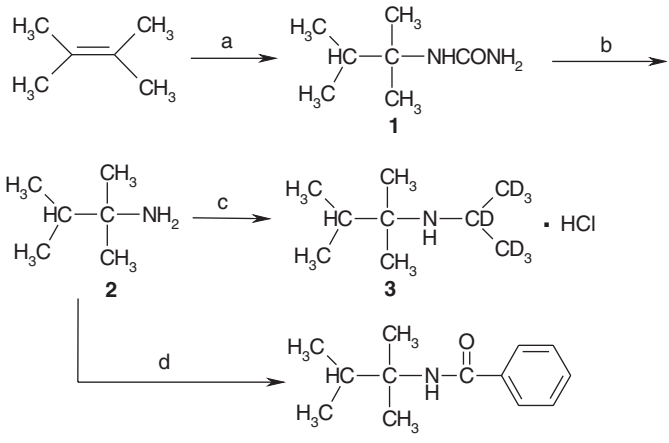
method for the pharmacokinetics and pharmacological mechanism studies of iptakalim hydrochloride.[2]

Results and discussion

The deuterium-labelled iptakalim hydrochloride was obtained by the condensation of 2,3-dimethyl-2-butamine with [$^2H_7$] 2-bromopropane in an autoclave. The synthetic route is shown in Scheme 2.

Preparation of [$^2H_7$]-iptakalim hydrochloride was starting from 2,3- dimethyl-2-butene, which was reacted with urea in an acid-catalyzed Ritter-type reaction to give **1**. Compound **1** was hydrolyzed under alkaline condition to give **2**, which was condensed with [$^2H_7$] 2-bromopropane to afford **3**. It is difficult to purify **3** by chromatographic methods and HPLC because of the similar properties of **2** and **3** and of their hydrochlorides. We applied a derivatisation strategy for the purification of **3** in the experiment. Since iptakalim does not react with benzoyl chloride, excessive benzoyl chloride was added to the reaction mixture in order to convert **2** to its amide completely. So [$^2H_7$]-iptakalim was then isolated from the reaction mixture as its hydrochloride with general method.

The most important step for the synthesis of the tritium-labelled iptakalim hydrochloride is to construct a carbon–carbon or carbon–nitrogen double



Scheme 2. Synthesis of [$^{2}H_7$]-iptakalim hydrochloride. Reagents and conditions: (a) Urea, mix of 98% sulfuric acid and glacial acetic acid (1:1,v/v), 9.5 h at 52°C; (b) 50% Sodium hydroxide, glycol, 8 h at 120°C; (c) [$^{2}H_7$] 2-Bromopropane (98% D, Aldrich), glycol, toluene, 170°C, 17 h; (d) 12% Sodium hydroxide, benzoyl chloride, 50°C, 2.5 h

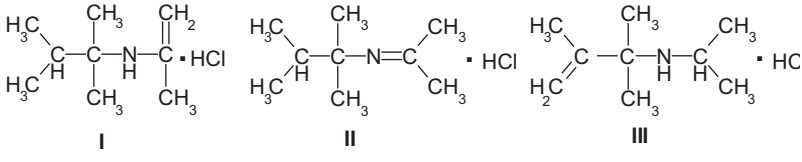
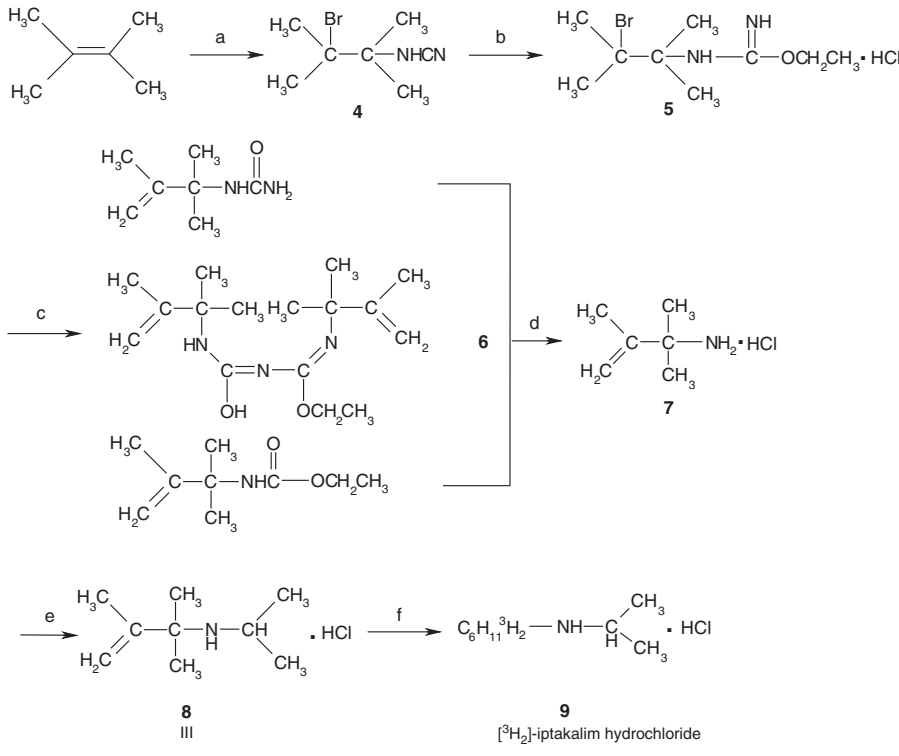


Figure 2. Structures of I, II and III

bond in the core structure of the compound. There are three sites that can be used to construct a double bond (Figure 2 I–III).

At first, we attempted to synthesize the compounds **I** and **II** by condensing of 2,3-dimethyl-2-butylamine with acetone or 2-brompropene. Unfortunately, none of the desired intermediates were obtained because of the poor reactivity of 2-bromopropene and difficulty of isolating the reactive intermediate **II**. So we chose **III** (2,3-dimethyl-*N*- (1-methylethyl)-3-ene-2-butamine) as the precursor to introduce tritium via a catalytic tritiation of the double bond. The synthetic route of compound **III** and [$^{3}H_2$]-iptakalim hydrochoride are shown in Scheme 3.

In this procedure, 2,3-dimethyl-2-butene was treated with cyanamide and *N*-bromosuccinimide to get **4**, and then stirred in a solution of ethanol containing anhydrous HCl to get **5**. The product was pure enough to be used directly for the next step. After treatment with KI and K_2CO_3 in 1,4-dioxane, **6**, a mixture of three compounds was obtained as reported previously.[3,4] All these three compounds can be converted to **7** by alkaline hydrolysis, so the mixture was subjected to alkaline hydrolysis to provide 2,3-dimethyl-3-butene-2-amine in good yield.



Scheme 3. Synthesis of [$^3H_2$]-iptakalim hydrochloride. Reagents and conditions: (a) NBS, H_2NCN, CH_2Cl_2, room temperature, 72 h; (b) HCl/EtOH, room temperature, 6 h; (c) KI, K_2CO_3, 1,4-dioxane, 90°C, 24 h; (d) KOH, EtOH, H_2O, reflux, 35 h; (e) 2-Bromopropane, K_2CO_3, NMP, 170°C, 17 h; 12% sodium hydroxide, benzoyl chloride, 50°C, 6 h; (f) [$^3H_2$], PtO_2, EtOH, 1,4-dioxane, room temperature, 19 h

The condensation of **7** with 2-bromopropane was carried out smoothly in an autoclave. Because of the similarity of **7** and **8** and their hydrochlorides, the same procedure as used in the purification of **3** was adopted here to scavenge the excess **7** and allow isolation of **8** as its hydrochloride.

The catalytic tritiation of **8** was carried out under the atmospheric pressure. The reaction was modelled several times with hydrogen to ensure the high purity of the desired product and the reliability of the reaction condition before tritium was used. Since our laboratory cannot offer the structural characterisation such as ^3H NMR and ^3H MS for the tritium-labelled compound, we synthesized a deuterium-labelled iptakalim hydrochloride with the same method. The ^1H NMR and MS showed that two deuterium atoms can be introduced to both the original double bond and the adjacent three methyl groups. This result suggested that the tritium labelling would not limit to the original double bond. Since this kind of isotopic scrambling is likewise

not a problem for the pharmacokinetics and pharmacological mechanism studies, we decided to introduce two tritium atoms into the structure of iptakalim hydrochloride with this method. The specific radioactivity of **9** achieved to 53.3 Ci mmol^{-1} and its radiochemical purity was 98%.

Materials and methods

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded as KBr discs on a Nicolet Magna-IR 550 spectrophotometer. Mass spectra were recorded on a Zebspec mass spectrometer. The ^{1}H NMR spectra were recorded at 400 MHz on a JNM-GX400, and the chemical shifts were reported in ppm(δ) relative to trimethylsilane (0.0 ppm). A FJ-2107P liquid scintillation counter was used to determine radioactivity in liquid samples. [$^{2}H_7$] 2-bromopropane (98 at% D) was obtained from Aldrich. Tritium gas was purchased from China Institute of Atomic Energy (CIAE). All other reagents were ACS grade and were obtained from commercial sources.

Experimental

2, 3-Dimethyl-2-buturea, **1**

To a suspension of glacial acetic acid (6.0 ml) and urea (8.0 g, 0.143 mol) in a 50 ml three-necked flask were added 2,3-dimethyl-2-butene (5.6 g, 0.060 mol). A mix of 98% sulfuric acid and glacial acetic acid (1:1, v/v, 17.5 ml) was added dropwise at such a rate that the temperature did not exceed 50°C. The resulting mixture was stirred for 9.5 h at 52°C. The reaction mixture was then poured into an ice-cold solution of 50% sodium hydroxide (180 ml). The mixture was then filtered, washed with cold water (5 × 10 ml). The filtrate was dried under reduced pressure to give 3.06 g (35.4%) **1** as pale yellow solid. m.p. 167–169°C. IR (KBr)cm^{-1}: 3454, 3360, 3216, 2972, 1654, 1612, 1560. ^{1}H NMR($CDCl_3$) δ: 0.88–0.90 (d, 6 H, $J = 6.71$ Hz), 1.26(s, 6 H), 4.20–4.50(2 × br, 3 H). MS(FAB) m/z: 145.2 [$M^+ + H$].

2, 3-Dimethyl-2-butamine, **2**

50% Sodium hydroxide (120 ml) was added to a solution of **1** (196 g, 1.36 mol) in glycol (392 ml). The mixture was stirred for 8 h at 120°C (oil bath temperature). 88.5 g of **2** was obtained by distillation (95–102°C) as colorless liquid. ^{1}H NMR($CDCl_3$) δ: 0.88(d, 6 H), 1.04(s, 6 H), 1.53(m, 3 H). MS(FAB) m/z: 102.1 [$M^+ + H$].

[$^{2}H_7$]-iptakalim hydrochloride, **3**

2 (8.0 g, 0.079 mol), [$^{2}H_7$] 2-bromopropane (5.0 g, 0.038 mol), glycol (5.7 ml) and toluene (40 ml) were added to an autoclave. The mixture was heated at

170°C for 17 h. After cooling to room temperature, a solution of 40% sodium hydroxide (20 ml) was added to dissolve the insoluble solid. The resulting solution was extracted with toluene (3 × 15 ml). The combined organic extracts were washed with water (3 × 10 ml). Then 12% sodium hydroxide (20 ml) and benzoyl chloride (2.0 ml in 4.0 ml toluene) were added to the toluene layer. The mixture was stirred at 50°C for 2.5 h. The toluene layer was then washed with 25% hydrochloride acid (3 × 10 ml) and water (2 × 10 ml). The aqueous layer was combined and washed with ether, and then the pH level was adjusted to 12–13 by addition of 40% sodium hydroxide, and the layer extracted with ether (3 × 15 ml) and dried over anhydrous K_2CO_3. After treatment with a solution of anhydrous HCl in ether (2.5 mol l^{-1}, 10 ml), the precipitation was washed with ether to give a white solid. Recrystallization from **2**-propanol/ether afforded 1.39 g (19.4%) of **3** as white crystals. m.p. 228–230°C. IR (KBr)cm^{-1}: 3436, 2969, 2827, 2755, 2426, 2233, 1588, 1473, 1401, 1155, 1052. ^{1}H NMR(D_2O) δ: 0.96–0.98(d, 6 H), 1.32(s, 6 H), 2.0–2.1(m, 1 H). MS(FAB) *m/z*: 151.2 [M^+-Cl].

(2-Bromo-1,1,2-trimethyl-propyl) cyanamide, **4**

N-bromosuccinimide(25.0 g, 0.140 mol) was added to a stirred, cooled (5–10°C) solution of the 2,3-dimethyl-2-butene (10.7 g, 0.127 mol) and cyanamide (21.3 g, 0.508 mol) in methylene chloride (70 ml). The resulting reaction mixture was stirred for 1 h at 5–10°C and then stirred for 72 h at room temperature. The methylene chloride layer was washed with water (6 × 100 ml), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was treated with cyclohexane and methylene chloride to give 3.5 g (13.4%) of **4** as white powder. m.p. 109–111°C.

Ethyl ((2-bromo-1,1,2-trimethyl-n-propyl) amino) carboximidate, **5**

A solution containing 1.3 g of anhydrous HCl in ethanol (30 ml) was added to a solution of **4** (13.0 g, 0.063 mol) in ethanol (30 ml) at 5–10°C, and another 30 ml of ethanol was added. The resulting solution was stirred for 6 h at room temperature and then concentrated *in vacuo*. The resulting solid was washed several times with ether and dried to yield 17.0 g (93.8%) of **5** as white solid. m.p. 119–121°C. IR (KBr)cm^{-1}: 3242, 3149, 1676, 1558, 1385, 1085. ^{1}H NMR($CDCl_3$) δ: 1.42–1.46 (t, 3 H, J = 6.94 Hz), 1.54 (s, 6 H), 1.85 (s, 6 H), 4.55–4.61 (q, 2 H, J = 6.94 Hz), 8.31 (s, 1 H), 8.71–9.12 (d, 2 H). MS(FAB) *m/z*: 250.9[M^+-HCl].

2,3-Dimethyl-3-butene-2-aminehydrochloride, **7**

KI (9.1 g, 0.055 mol) and anhydrous K_2CO_3 (12.0 g, 0.087 mol) were added to a solution of **5** (8.0 g, 0.028 mol) in 1,4-dioxane (220 ml). The resulting reaction

mixture was stirred at 90°C for 24 h. The insoluble material in the 1,4-dioxane was separated and washed with 1,4-dioxane, and the filtrate was concentrated *in vacuo*. The residue was treated with cyclohexane to give 3.0 g of **6** as white solid. The mixture of **6** was then mixed with KOH (3.50 g), ethanol (25 ml) and water (10 ml), refluxed for 35 h, and distilled to collect the volatile component as colorless liquid. A solution of anhydrous HCl in ether (2.5 mol l^{-1}, 20 ml) was then added to give the desired product as white solid. Recrystallization from ether/ethanol gave 1.1 g (29.2%) of **7** as white crystals. m.p. > 300°C. IR (KBr)cm^{-1}: 3435, 2986, 2912, 1596, 914. ^{1}H NMR ($CDCl_3$) δ 1.58 (s, 6 H), 1.88 (s, 3 H), 5.00 (s, 1 H), 5.14 (s, 1 H) 8.6–8.8 (br, 3 H). MS (FAB) *m/z*: 100.1[M^+-Cl].

2,3-Dimethyl-N-(1-methylethyl)-3-butene-2-amine hydrochloride, **8**

7 (1.1 g, 0.0081 mol), 2-bromopropane (2.0 g, 0.016 mol), anhydrous K_2CO_3 (2.5 g, 0.018 mol) and *N*-methyl-pyrrolidone (20 ml) were added to an autoclave, the mixture was stirred at 170°C for 17 h. After cooling to room temperature, 50% sodium hydroxide (15 ml) was added and the reaction mixture was extracted with toluene (3 × 25 ml). The toluene layer was washed with water and then 12% sodium hydroxide (4.5 ml) and benzoyl chloride (2.0 ml in 4 ml toluene) were added. The mixture was stirred at 50°C for 6 h. The toluene layer was then washed with 25% hydrochloric acid (3 × 10 ml) and water (2 × 10 ml). The aqueous layers were combined and washed with ether, and then the pH level was adjusted to 12–13 by addition of 50% sodium hydroxide, extracted with ether (3 × 15 ml). The ether layer was washed with water (30 ml), dried over K_2CO_3, treated with a solution of anhydrous HCl in ether (2.5 mol l^{-1}, 10 ml), and the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography with chloroform, petroleum ether (b.p. 60–90°C) and methanol (3:4:1, v/v/v) as eluent to give **8** (Rf 0.42) as a white solid (300 mg, 20.8%). m.p. 199–201°C. IR (KBr)cm^{-1}: 3408, 2974, 2760, 1587, 932. ^{1}H NMR($CDCl_3$) δ: 1.47–1.50 (d, 6 H, J = 6.36 Hz), 1.70 (s, 6 H), 2.01 (s, 3 H), 3.20–3.30 (m, 1 H), 5.14 (s, 1 H), 5.18 (s, 1 H), 9.10–9.30 (br, 2 H). MS(FAB) *m/z*: 142.2[M^+-Cl].

[1,2-$^{3}H_2$]-N- (1-Methylethyl)-1,1,2-trimethyl-propylamine hydrochloride ([$^{3}H_2$]-iptakalim hydrochloride), **9**

5 Drops of ethanol were added to a mixture of **8** (12.4 mg, 0.070 mmol), PtO_2 (7.4 mg, 81%Pt, Fluka) and 1,4-dioxane (4.0 ml). The resulting solution was stirred under a tritium atmosphere (4.7 ml, 12.0 Ci) at room temperature for 19 h when the uptake of gas had stopped. Upon completion of the reaction, the solution was filtered to remove catalyst and the residue was washed with chloroform. The solvent was removed *in vacuo*. The residue was re-dissolved in ethanol and was lyophilized to remove volatile tritium. This procedure was

repeated three times. Then the residue was evaporated to dryness under reduced pressure to yield 10 mg of **9** as white solid which was then re-dissolved in 75% ethanol to 500 ml. The total radioactivity of **9** was determined to be 3.0 Ci. The specific radioactivity of 53.3 Ci $mmol^{-1}$ was calculated from liquid scintillation counting. The radiochemical purity was shown to be 98%, the active material co-eluting with the inactive reference.

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