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SYNTHESIS OF TRITIUM LABELLED 4-FLUORO-1-[1-(2-THIENYL)]CYCLOHEXYLPIPERIDINE ([^{3}H]-FTCP); A TOOL FOR AUTORADIOGRAPHIC STUDY OF THE PHENCYCLIDINE BINDING SITE.

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SUMMARY

4-Fluoro-1-[1-(2-thienyl)-3-cyclohexenyl)]piperidine was efficiently synthesized in four steps starting from cyclohexane-1,4-dione monoethylene ketal. Catalytic tritiation of this key intermediate in the final step afforded the title compound.

Key Words: Phencyclidine, TCP, Autoradiography, Tritiation, Fluoro TCP, NMDA Receptor

INTRODUCTION

Phencyclidine (1-(1-phenylcyclohexyl)piperidine, PCP) was originally developed as an anesthetic agent, but was removed from clinical trials after the observation of psychotic side effects in a significant number of patients. During the last ten years phencyclidine has become a major drug of abuse in the United States, contributing significantly to the number of emergency room admissions in many urban hospitals. Although a large body of research has been directed toward understanding this drug, the exact mode of action of phencyclidine is still uncertain. Binding sites for PCP in the central nervous system have been identified and their regional distribution in rat brain have been recorded autoradiographically using tritiated 1-(2-thienyl)cyclohexyl)piperidine ([^{3}H]-TCP), a potent PCP agonist

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(1). The regional distribution of phencyclidine binding sites has been shown to have excellent correlation with the regional distribution of N-methyl-D-aspartate (NMDA) receptors (2). Present theory indicates that at least some of the pharmacological actions of phencyclidine are mediated through the relationship of the phencyclidine binding site to an NMDA receptor/ion channel complex (3).

In connection with our ongoing study of the phencyclidine binding site in the central nervous system, we required a derivative of PCP or TCP which could be used alternately as a tool for either autoradiographic or positron emission tomography (PET) studies of the mammalian brain. The compound 1-[1-(2-thienyl)cyclohexyl]-4-fluoropiperidine (4-fluoro TCP, FTCP) was chosen because of its high affinity for the phencyclidine binding site and because it was amenable for radiolabelling with either ^{18}F and ^{3}H. The preparation of the [^{18}F] derivative has previously been described (4). We herein report the synthesis of tritiated 4-fluoro TCP ([^{3}H] FTCP, **6**).

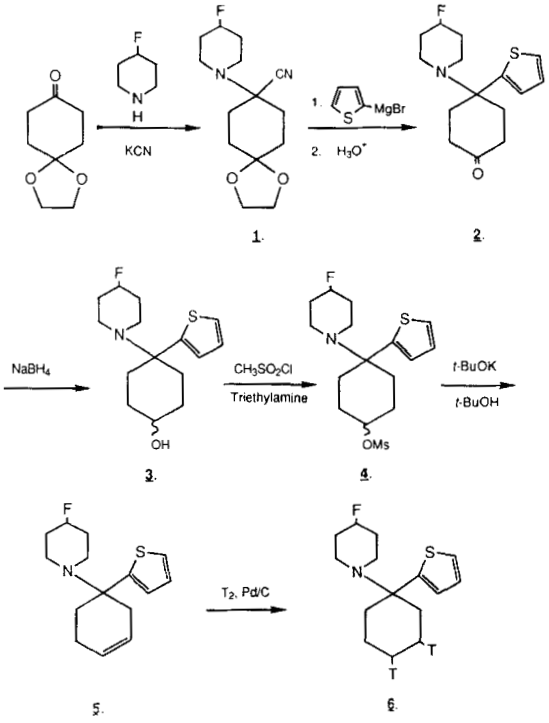
EXPERIMENTAL

All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Gas chromatographic analysis was performed on a Hewlett-Packard 5880 instrument using a flame ionization detector. The ^{1}H NMR spectra were recorded on a Varian XL-300 instrument. Mass spectra were obtained using a Finnigan 1015D instrument. Infrared spectra were obtained using a Beckman 300 instrument. Where elemental analyses are indicated only by symbols of the elements, results obtained were within 0.4% of the theoretical values. Radioactivity determinations were carried out using a Packard Model 2200CA liquid scintillation counter using Hydrofluor scintillation solvent. Thin layer chromatography (TLC) plates were analyzed with a Berthold Model LB 2760 TLC

scanner. All synthetic and analytical operations were initially performed with unlabelled compounds, and the structures of the unlabelled intermediates and products were confirmed spectroscopically.

Scheme 1

Synthesis of Tritium Labelled 4-Fluoro TCP



Synthesis

The synthetic pathway for preparing [^{3}H] labelled **6** is shown in Scheme **1**. 1,4-Cyclohexanedione monoethylene ketal was condensed with potassium cyanide and 4-fluoropiperidine (5) to provide aminonitrile **1**. Treatment of **1** with 2-thienylmagnesium bromide (6) followed by acidic hydrolysis of the ketal gave the thienyl amine **2**. Reduction of the carbonyl with sodium borohydride gave a mixture of the epimeric alcohols **3**. Treatment of **3** with methanesulfonyl chloride and triethylamine provided the

corresponding mesylates **4**, which on dehydration using potassium-tert-butoxide afforded the unsaturated fluoro-TCP derivative **5**. Catalytic tritiation of **5** using palladium on carbon as the hydrogenation catalyst led to the desired **6**. The poor radiochemical yield is possibly attributable to the presence of the thiophene.

8-[1-(4-Fluoropiperidyl)]-8-cyano-1,4-dioxaspiro[4.5]decane (**1**). The pH of a solution of 4-fluoropiperidine hydrochloride (0.95 g, 9.22 mmole) in 3 mL of water was adjusted to 3.5 using 1.0 N HCl solution. 1,4-Cyclohexanedione monoethylene ketal (1.44 g, 6.78 mmol) and potassium cyanide (0.66 g, 10.1 mmol) were added and the resulting solution was stirred for 23 h. The reaction mixture was pipetted into a separatory funnel containing 10 mL of ether and 10 mL of 1.0 N sodium hydroxide solution. The ethereal fraction was separated, dried (Na_2SO_4) and concentrated to give 2.3 g (93%) of the crystalline aminonitrile (**1**), m.p. 106°C. IR (KBr) 2975, 2820, 2225, 1475, 1445 cm^{-1}; ^1H NMR ($CDCl_3$) 4.67 (m, 1H), 3.95 (s, 4H), 2.81 (m, 2H), 2.55 (m, 2H), 1.65-2.2 (m,10H); mass spectrum (CI, NH_3) 269 (M^++1), 242. Anal. C, H, N.

4-[1-(4-Fluoropiperidyl)]-4-(2-thienyl)cyclohexanone (**2**). The aminonitrile **1** (2.2 g, 8.2 mmol) was dissolved in 14 mL of dry tetrahydrofuran (THF) under a nitrogen atmosphere. A solution of 2-thiophenemagnesium bromide (8 mL, 1.07 M) was added dropwise and the resulting mixture was stirred for 1.5 h. The reaction was quenched by pouring into a separatory funnel containing a mixture of 10 mL of ether and 10 mL of 1.0 N HCl solution cooled to 5°C. The aqueous phase was transferred to another separatory funnel and 20 mL of 1.0 N NaOH solution was added. The resulting mixture was extracted twice with 20 mL portions of ether. The combined organic extracts were dried

(Na_2SO_4) and concentrated to give 1.7 g of the crude aminothiophene (mass spectrum (CI, NH_3) 326, M+1). The material was dissolved in 1 N HCl (6 mL) and allowed to stand for 12 h. Sodium hydroxide solution was added and the aqueous mixture extracted with ether (2 X 20 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to give the aminoketone. The free base was recrystallized from isopropanol (820 mg, 36%), mp 81-83 °C. Mass spectrum (CI, NH_3) 282 (M+1). Anal C, H, N.

4-[1-(4-Fluoropiperidyl)]-4-(2-thienyl)cyclohexanol (**3**).
Sodium borohydride (0.31 g, 8.3 mmol) was added in one portion to a stirred solution of the ketone (0.78 g, 2.8 mmol) in 5 mL of 95% ethanol at 20°C. After 20 min the solution was partitioned between 20 mL of water and 20 mL of ether. The ether layer was removed and the aqueous layer was extracted with a second 20 mL portion of ether. The combined ether extracts were dried (Na_2SO_4) and concentrated to give 0.68 g (87%) of the epimeric alcohols (**3**, ratio 1:1.2 by GC). IR (neat) 3400, 2950, 1450, 1430 cm^{-1}; ^1H NMR ($CDCl_3$) 7.18 (m, 1H), 6.99 (m, 1H), 6.83 (m, 1H), 4.5 (br d, J = 52 Hz), 3.85 and 3.75 (b, 1H, CHOH), 2.6 (m, 2H) 2.45 (m, 2H), 2.3 (m, 2H), 1.4-2.0 (m, 10H).

4-[1-(4-Fluoropiperidyl)]-4-(2-thienyl)cyclohexene (**5**).
The epimeric alcohols (0.66 g, 2.33 mmol) were dissolved in 8 mL of dry THF. The mixture was cooled to 0°C and 2 mL of triethylamine and 0.20 mL (0.294 g, 2.56 mmol) of methanesulfonyl chloride was added. After stirring for 1 h at room temperature the reaction was poured into a separatory funnel containing 10 mL of water and 10 mL of ether. The water layer was removed and the ether extract dried (Na_2SO_4) and concentrated to give 0.81 g (96%) of the crude epimeric mesylates. The crude mesylates were dissolved in 8 mL of t-butanol. To this was added 2.50 g (10

equiv) of potassium-t-butoxide and the resulting mixture warmed to 45°C for 11 h. After cooling to 0°C the reaction was poured into a separatory funnel containing 10 mL of water and 10 mL of ether. The aqueous layer was removed and the organic layer reextracted with a second 10 mL portion of water. After drying and concentration of the organic fraction the crude product mixture was fractionated by column chromatography (20% ethyl acetate/hexane) to give 0.33 g (56%) of the desired olefin. The hydrochloride salt was prepared in ethyl acetate, mp. 207-211 °C (dec.). 1H NMR ($CDCl_3$) 7.18 (d, J = 4.9 Hz, 1H), 6.86 (m, 2H), 5.75 (m, 1H), 5.62 (m, 1H), 4.60 (br d, J = 49 Hz, 1H), 2.8 (m, 1H), 2.65 (m, 1H), 2.3-2.55 (m, 3H), 1.5-2.1 (m, 9H); mass spectrum (CI, NH_3) 266 (M+1), 211, 163, 104. Anal C, H, N.

[^{3}H]-4-Fluoro-1-[1-(2-thienyl)]cyclohexylpiperidine (**6**). An atmosphere of carrier free tritium gas (58 Ci/mmol) was applied for 5 h to a stirred solution of 10 mg of **5** hydrochloride in 3 mL of methanol containing 10 mg of palladium on carbon (10%). After removal of the tritium atmosphere, the reaction was filtered and concentrated under a stream of nitrogen. Purification by preparative thin layer chromatography (20% ethyl acetate/hexane) gave **6** (25.6 mCi, 14.0 Ci/mmol, 1.2% radiochemical yield) TLC R_f = 0.61 (5 % methanol/89% chloroform/1% NH_4OH), identical to cold material. The [^{3}H]-FTCP was shown to be >99% radiochemically pure using a Berthold TLC scanner.

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