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Synthesis of the olanzapine labeled by carbon-14

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Olanzapine is one of the most widely used antipsychotic drugs, which acts as an antagonist for multiple neurotransmitter receptor sites. 2-Methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2,3-b][1,5] benzodiazepine (Olanzapine) labeled with carbon-14 in the four positions has been synthesized as part of a three-step sequence from 2-amino-5-methylthiophene-3-carbonitrile-[carbonitrile-^{14}C].

Keywords: olanzapine; carbon-14; antipsychotic

Introduction

Olanzapine, an atypical anti-psychotic drug[1,2] with a thienobenzodiazepinyl structure, is indicated for the treatment of schizophrenia. It displays a broad pharmacological profile and is a selective monoaminergic antagonist with high affinity binding to serotonin 5HT2A/2C, dopamine D14, muscrinic M1-5, and adrenergic α1 receptors.[3–5] Atypical antipsychotic drugs are a mainstay of psychiatric pharmacotherapy and are used to treat a variety of psychiatric illnesses, most notably schizophrenia. These second-generation drugs lack the extrapyramidal and other debilitating movement side effects that complicated treatment with first-generation compounds. The popularity of the atypical antipsychotics, especially olanzapine, led to the observation that these drugs have distinct metabolic side effects that were not detected during preclinical testing, including insulin resistance, diabetes, and obesity.[6,7] The mechanisms underlying these metabolic side effects in human beings are not known. However, case reports and other observational studies have suggested that effects of atypical antipsychotics on glucose homeostasis and insulin sensitivity precede weight gain in human beings.[8,9] Therefore to further elucidate the mechanism of action and to support ongoing metabolism studies, there arose a need for analogs of these compounds carbon-14 labeled in a biologically stable site. In our previous papers,[10,11] we reported a convenient method for carbon-14 labeling of Clozapine and Loxapine. [^{11}C]Olanzapine was prepared from desmethyl-Olanzapine with [^{11}C]CH_3OTf through N-[^{11}C]methylation previously.[12] In this paper, synthesis of 2-Methyl-4-(4-methyl-1-piperazinyl)-10H-[4-^{14}C]thieno [2,3-b] [1,5] benzodiazepine (Olanzapine) is described (Figure 1).

Discussion

A synthetic pathway has been designed to get Olanzapine [^{14}C], according to Scheme 1, with appreciable radiochemical yield. In this procedure, reaction of K^{14}CN with 3-Bromo-5-methyl-2-nitrothiophene followed by a selective reduction of the NO_2 group to give 2-amino-5-methylthiophene- 3-[^{14}C]carbonitrile has been described. Then palladium catalyzed coupling of 2-amino-5-methylthiophene- 3-[^{14}C]carbonitrile, [**^{14}C**]-**3** with 1-bromo-2-iodobenzene in the presence of a palladium mediated catalyst, xantphos ligand, and cesium carbonate base to give an intermediate compound 2-(2-bromophenylamino)-5-methylthiophene-3-[^{14}C]carbonitrile, [**^{14}C**]-**4**. The compound 4-^{14}C was further reacted with *N*-methyl piperazine in presence of trimethyl aluminum to afford imine derivative [**^{14}C**]-**5**, which upon subsequent cyclization gave Olanzapine[^{14}C] (Scheme 1).

Experimental

Barium [^{14}C]carbonate was converted to potassium [^{14}C]cyanide according to the standard procedure.[13] Infrared (IR) spectra were recorded on a Bruker FT-IR, Vector 22 instrument, and the ^{1}H- NMR spectra were recorded on a Varian unity plus 400 spectrometer (400 MHz). A Waters HPLC system (Waters Corporation, Milford, MA, USA) was used for chromatographic determination (chemical purity) of olanzapine. It consisted of a 1525 Binary HPLC pump and 2487 Dual λ absorbance detector set at 230 nm. Samples were injected with a Rheodyne 7725i (USA) injection valve equipped with a 20-µl sample loop. WATERS BREEZE chromatography software was used to acquire and process data. A Welcrom C18 column (4.6 × 250 mm, 5 µm) was used for separation. The isocratic mobile phase consisted of 10-mM Phosphate Buffer (pH−2.5): acetonitrile (50:50 v/v) with a flow rate of 1.0 mL/min at

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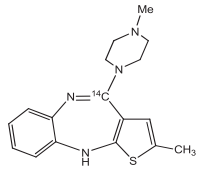
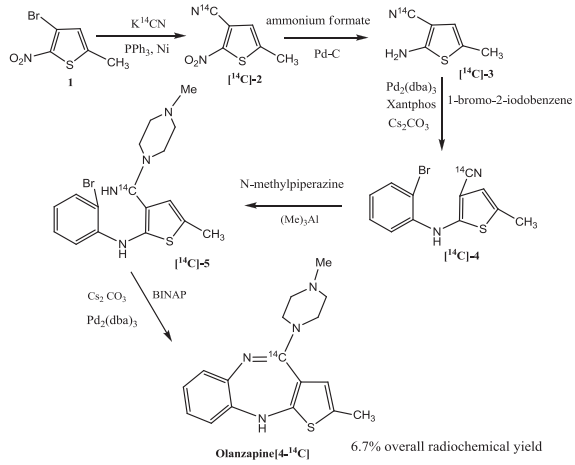


Figure 1. The chemical structures of Olanzapine[**4-^{14}C**].



Scheme 1. The synthetic pathway for synthesis of Olanzapine-[4-^{14}C] from 2-amino-5-methylthiophene-3-carbonitrile-[carbonitrile-^{14}C]

ambient temperature (25 °C). Radioactivity was determined using a Beckman LS6500 liquid scintillation spectrometer. Mass spectra were obtained on a Finnigan TSQ-70 (San Jose, CA, USA) instrument at 70 eV.

2-nitro-5-methylthiophene-3-[^{14}C]carbonitrile [^{14}C]-2

A three-necked round-bottomed flask, equipped with a reflux condenser (bulbs), a mechanical stirrer and a gas inlet, was charged with 1.5 mmol of 3-Bromo-5-methyl-2-nitrothiophene **1** (333 mg), 2 mL of 100% ethanol, 400 mg of triphenylphosphine, and Potassium [^{14}C]cyanide (297 MBq, 100.5 mg). The air in the flask was completely replaced by inert gas, and the mixture was brought at reflux temperature, and then added Ni powder (10 mg, 0.17 mmol) as catalyst.[14] The mixture was stirred at a vigorous rate and heated under reflux for 60 min, while a slow stream of inert gas was passed through the apparatus. The very dark suspension was then cooled to room temperature, and 10 mL of water was added with vigorous stirring, after which four extractions with diethyl ether (4 × 5 mL) were carried out. The solid material that was formed was filtered off through a sintered-glass funnel and rinsed well with ether. The combined ethereal solutions were washed three times with water (3 × 1 mL) and dried over $MgSO_4$. After concentration under reduced pressure, the residue consisted mainly of triphenylphosphine. Redistillation (under reduced pressure) afforded the 2-nitro-5-methylthiophene-3-[^{14}C]carbonitrile [**^{14}C**]-**2** as a yellow crystalline powder (105.4 mg, 184 MBq, 0.62 mmol) in 62% yield (HPLC Chemical Purity 98.5%). IR (KBr) 3016, 2199, 1626, 1510, 1455, 1276, 1106, 797, 412 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ ppm) δ 6.80 (s, 1H), 2.37 (s, 3H), MS: m/z 170 (M + 1).

2-amino-5-methylthiophene-3-[^{14}C]carbonitrile [^{14}C]-3

To a stirred suspension of 2-nitro-5-methylthiophene-3-[^{14}C]carbonitrile [**^{14}C**] **2** (100 mg, 174.5 MBq, 0.58 mmole) and 10% Pd-C (30 mg) in dry methanol (2 mL), anhydrous ammonium formate was added (2.7 mmol) in a single portion.[15] The resulting reaction mixture was stirred at room temperature for 1.5–2 h under argon, the catalyst was removed by filtration through a celite pad and washed with dry methanol (2 mL). The filtrate was evaporated under reduced pressure. The resulting residue was triturated with water (1–2 mL), product was extracted with ethylacetate (3 × 2 mL) then organic layer isolated and dried over Na_2SO_4. The organic layer on evaporation gave the 2-amino-5-methylthiophene-3-[^{14}C]carbonitrile [**^{14}C**]-**3** 58% (HPLC Chemical Purity 96.8%) as a colorless crystal (50.5 mg, 101.2 MBq, 0.36 mmol). IR (KBr); 3420, 3332, 3227, 2916, 2199, 1626, 1519, 1385, 1276, 1106, 897, 812, 505 cm^{-1}; ^{1}H NMR ($CDCl_3$) d 6.34 (s, 1H), 4.72 (br s, 2H), 2.27 (s, 3H), MS: m/z 140 (M + 1).

2-(2-bromophenylamino)-5-methylthiophene-3-[^{14}C] carbonitrile [^{14}C]-4

To a stirred solution of three (45 mg, 90 MBq, 0.32 mmol), tris (dibenzylidineacetone)dipalladium (15 mg), cesium carbonate (300 mg), and Xantphos (10 mg) in 1,4-dioxane (1.0 mL) was added a solution of 1-bromo-2-iodobenzene (90.5 mg, 0.32 mmol) in 1.0 mL of xylene at room temperature. After complete addition, the reaction mass was heated to 90 °C and further stirred for the reaction completion. After completion of the reaction, water was added, and the reaction mixture was extracted with ethyl acetate (3 × 3 mL). The organic layer was separated and washed with brine solution and dried over sodium sulfate. The mixture was concentrated under reduced pressure, and the resulting residue was purified by plate chromatography (chromatography was performed on 150 mm × 200 mm glass plates precoated with 2-mm layers of silica gel Si 60 HF_{254}, by using 20: 80 ethyl acetate: n-hexan as eluent) to yield compound [**^{14}C**]-**4** as a yellow oil (62 mg, 60.3 MBq, 0.21 mmol) Yield: 67% and having the chemical purity of 97.3% by HPLC analysis. IR (cm^{-1}): 3320 (NH), 2223 (CN); ^{1}H NMR ($CDCl_3$, δ ppm): 2.40 (s, 3H, Ar-CH_3), 6.73 (s, 1H, Ar-H), 6.76 (m, 1H, Ar-H), 7.45 (dd, 2H, J = 1.6, 8.4, Ar-H), δ 7.69(dd, 1H, J = 1.2, 8.0, Ar-H). MS: m/z 295 (M + 1).

N-(2-bromophenyl)-3-[^{14}C](imino(4-methylpiperazin-1- yl) methyl)-5-methylthiophen-2-amine [^{14}C]-5

To a solution of [**^{14}C**]-**4** (50 mg, 48.6 MBq, 0.170 mmol) and N-methylpiperazine (34 mg, 0.34 mmol] in xylene (1.0 mL) was added trimethyl aluminum (0.5 mL, 0.22 mmol, 2.0 M solution in toluene) at 90–100 °C with stirring. The resulting solution was heated to 110 °C and stirred for the reaction completion. After completion of the reaction, the solution was cooled to 0 °C, and sodium potassium tartrate salt solution (1.5 mL, 30% solution) was added. The resulting mixture was extracted with ethyl acetate (2 × 2 mL) and washed with 3.0 N hydrochloric acid solution (2 × 1.0 mL). The combined aqueous layer was washed with ether and basified with aqueous sodium hydroxide solution (20%). The resulting mixture was extracted with ethyl acetate (2 × 2 mL), and the combined organic layers were washed with water brine and dried over sodium sulfate. The mixture was concentrated under reduced pressure to yield the targeted compound [**^{14}C**]-**5** as a brown colored crystal (47 mg, 34 MBq, 0.12 mmol) Yield: 70.0% (HPLC Chemical Purity 99.1%). IR (cm^{-1}): 3360 (NH), 3265 (NH); ^{1}H NMR (DMSO-d6, δ ppm): 2.20 (s, 6H, $2CH_3$), 3.36 (br, 8H, CH_2), 6.41 (s, 1H, Ar-H), 6.77 (m, 1H, Ar-H), 7.25 (m, 2H, Ar-H), 7.56 (d, 1H, J = 8.0, Ar-H). MS: m/z 395 (M + 1).

2-Methyl-4-(4-methyl-1-piperazinyl)-10H-[4-^{14}C]thieno[2,3-b] [1,5]benzodiazepine (Olanzapine[4-^{14}C])

The compound [**^{14}C**]-**5** (40 mg, 0.10 mmol, 29 MBq), cesium carbonate (42 mg, 0.77 mmol), BINAP (10 mg) in toluene (0.8 mL) was added catalytic amount (15 mg) of tris [dibenzylidineacetone] dipalladium and stirred at room temperature for 1.0 h. The resulting reaction was heated to 60 °C for 4.0 h. After completion of the reaction, the solution was cooled to room temperature, water (1 mL) was added and extracted with ethyl acetate (3 × 1 mL), and the combined organic layers were washed with brine and dried over sodium sulfate. The mixture was concentrated under reduced pressure to afford crude compound. The solid was recrystallized from methylene chloride, to give olanzapine[4-^{14}C] compound (12.6 mg, 11.6 MBq, 0.04 mmol) as a light yellow solid in

40.0% yield (HPLC Chemical Purity: 99.6%, R_t: 3.50 min from HPLC analysis according to aforementioned condition). IR (cm^{-1}): 3236 (NH); ^1H NMR (DMSO-d6, δ ppm): 2.21 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.38 (br, 4H, CH_2), 3.32-3.20 (br, 4H, CH_2), 6.32 (s, 1H, Ar-H), 6.68 (d, 1H, J = 8.0, Ar-H), 6.85-6.78 (m, 3H, Ar-H), 7.57 (s, 1H, NH); MS: m/z 314 (M + 1).

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