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Research Article

Synthesis of multi-labeled [^{14}C]Aripiprazole

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Summary

Development of Aripiprazole as an oral treatment of schizophrenia required the synthesis of a suitably labeled drug product for use in human metabolism and pharmacokinetic studies. Due to the potential metabolic degradation of the molecule, a multi-labeled approach utilizing ^{14}C was adopted. The synthesis of [^{14}C]Aripiprazole was accomplished in separate syntheses from 2,3-dichloro[U-^{14}C]aniline and [3-^{14}C]-cinnamic acid, respectively. Labeled versions were combined on the basis of molar radioactivity giving a final product with a radiochemical purity of 99.9% and a specific activity of 15.5 μCi/mg. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: quinolinone; Aripiprazole; carbon-14 synthesis

Introduction

The novel 2(1H)-quinolinone compound, 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydro-2(1H)-quinolinone (Aripiprazole, **1**) (Figure 1), is currently marketed as a unique antipsychotic agent known commercially as Abilify™. The compound not only possesses postsynaptic dopamine receptor antagonist activity, but also behaves as a dopamine autoreceptor agonist.[1] This dual activity affords Aripiprazole therapeutic efficacy in the treatment of schizophrenia without the serious side-effects often associated with neuroleptic drugs.[2]

Development of Aripiprazole involved a detailed study of its pharmacology. A human clinical study was designed to measure absorption, distribution,

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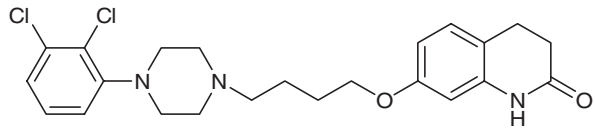


Figure 1. Aripiprazole (1)

metabolism and elimination (ADME) of Aripiprazole. To measure these parameters, a radiotracer study utilizing ^{14}C-labeled Aripiprazole was devised. Conceivably, in addition to oxidative metabolism, the molecule could undergo both *N*- and *O*-dealkylation to give separate dichlorophenylpiperazine and quinolinone fragments. The complex nature of this metabolic situation limited the usefulness of mono-labeled Aripiprazole for human ADME studies. A multi-labeled version of [^{14}C]Aripiprazole was adopted to address this issue, thus assuring that metabolites from *N*- or *O*-dealkylation processes contained a ^{14}C label.

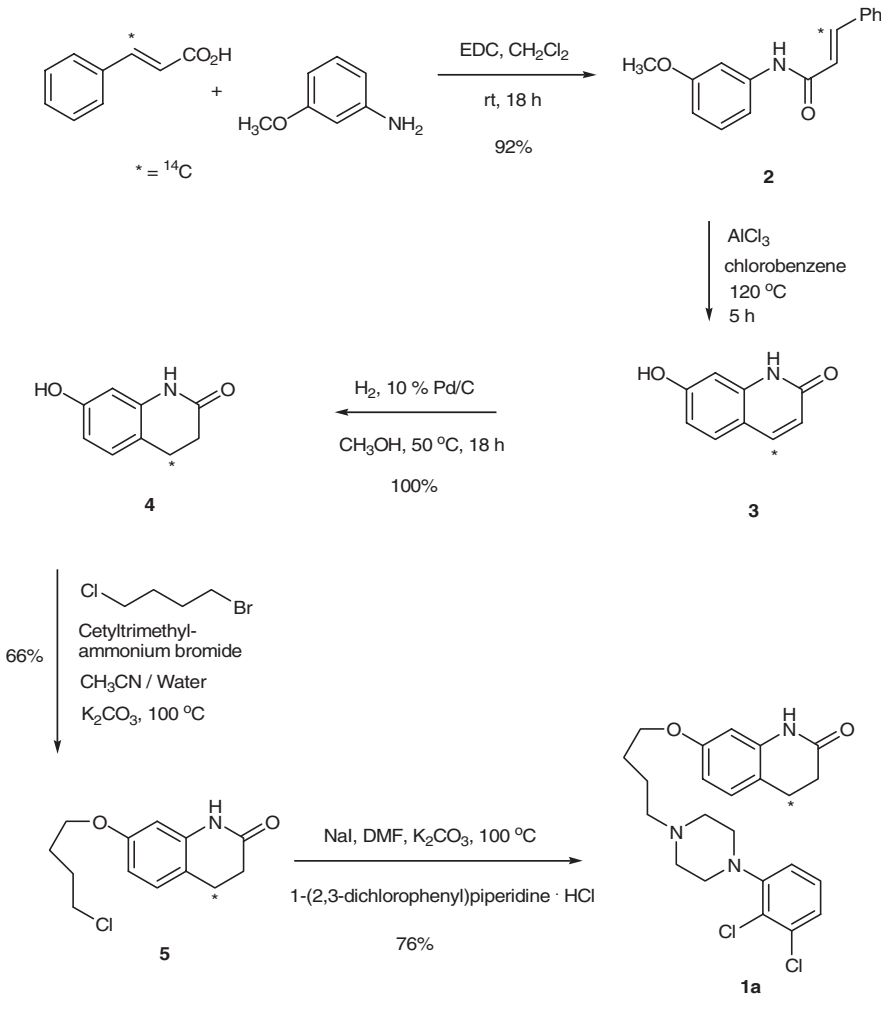
This article describes the synthesis of multi-labeled [^{14}C]Aripiprazole for use in human clinical studies. The synthesis of Aripiprazole has been previously reported.[1,3,4] Multi-step syntheses of separately labeled homologues of Aripiprazole were completed and clinical supplies prepared by combining labeled versions **1a** and **1b** based on equivalent amounts of radioactivity. The final product, [^{14}C]Aripiprazole, was prepared with a radiochemical purity of 99.9% and a specific activity of 15.5 μCi/mg.

Results and discussion

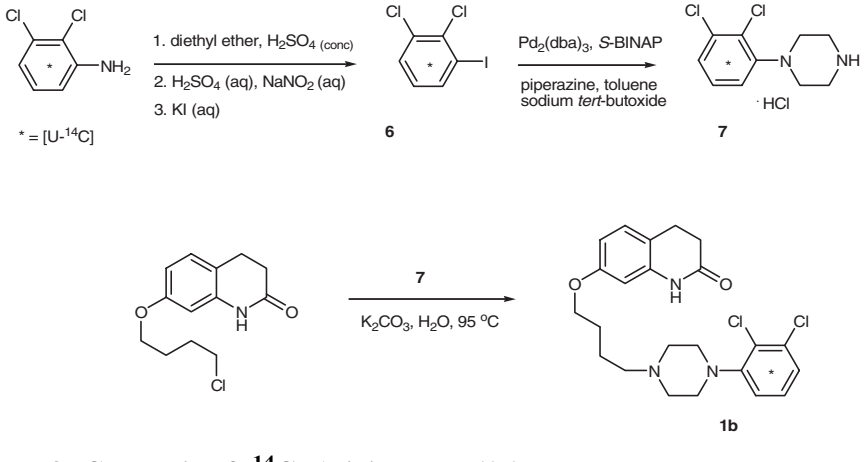
The synthesis of quinolinone-ring-labeled [4-^{14}C] Aripiprazole **1a** was accomplished in 5 steps from [3-^{14}C] *trans*-cinnamic acid (Scheme 1). An EDC mediated coupling of radiolabeled *trans*-cinnamic acid with *m*-anisidine furnished the cinnamanilide **2** in a 92% yield. The amide was reacted with aluminum chloride in chlorobenzene at 150°C to give the cyclized quinolinone **3** via an intramolecular Friedel-Crafts reaction.[5] The quinolinone was sufficiently pure, and without purification it was reduced with H_2 and 10% Pd/C at 50°C for 18 h to give labeled 3,4-dihydroquinolinone **4** in high yield.

Dihydroquinolinone **4** was coupled under basic phase transfer conditions with 1-bromo-4-chlorobutane giving the chloride **5** in 66% yield following purification. Chloride **5** was reacted further with 1-(2,3-dichlorophenyl)piperazine hydrochloride to give **1a** in 76% yield after purification. The overall radiochemical yield of **1a** from [3-^{14}C] *trans*-cinnamic acid was 42%, and the radiochemical purity was 99.5%.

Dichlorophenyl-ring-labeled [U-^{14}C]Aripiprazole **1b**, was prepared in 3 steps from 2,3-dichloro[U-^{14}C]aniline (Scheme 2). The hydrogensulfate salt of 2,3-dichloro[U-^{14}C]aniline was reacted with sulfuric acid and sodium nitrite to



Scheme 1. Synthesis of [^{14}C] Aripiprazole (1a)



Scheme 2. Synthesis of [^{14}C] Aripiprazole (1b)

give an intermediate diazonium salt, which was then treated with potassium iodide affording the aryl iodide **6** in 74% yield. Following a modified procedure for the Buchwald reaction,[6,7] **6** was coupled with an excess of anhydrous piperazine. After unsuccessful attempts to purify the crude HCl salt by recrystallization, the salt was purified by semi-preparative HPLC to give the piperazine HCl salt **7** in 59% yield. The reaction of 7-(4-Chlorobutoxy)-3,4-dihydroquinoline-2(1H)-one with **7** in sterile water/K_2CO_3 followed by crystallization provided **1b** in 68% yield and 99.5% radiochemical purity. The overall yield for **1b** from 2,3-dichloro[U-^{14}C]aniline was 30%.

Labeled analogs of Aripiprazole **1a** and **1b** were combined, and dissolved in CH_2Cl_2 to give a final product after solvent removal with a radiochemical purity of 99.9%.

Experimental

Reactions were run under an inert atmosphere of argon and magnetically stirred at a constant rate. Solvent removal under vacuum was accomplished using a Buchi R-124 rotary evaporator. Column chromatography was performed using a Biotage® Flash Chromatography system. Proton NMR spectra were recorded on a Varian Unity/Inova 500 MHz spectrometer. Radiochemical purity was determined by HPLC (Rainin Model SD-200, Varian PDA-2 detector and Beta-Ram detector (IN/US Systems Inc.) and TLC (Merck 60 F_{254} silica-gel-coated plates) using radiochemical detection (QC-Scan, Bioscan Model B-QC). Specific activity was determined by gravimetric analysis using liquid scintillation counting (Wallac Model 1409). Reactions were monitored by HPLC, TLC and NMR, and comparisons were made to authentic materials when available. All reagents were ACS grade or better, and radiolabeled precursors were supplied by GE Healthcare (Formerly Amersham Biosciences).

HPLC. HPLC methods described below were used for in-process and final product analyses as well as purification where described.

Method 1: Column: YMC-ODS-AQ, 3 μm (4.6 × 150 mm). Mobile phase A: 70% 0.02 M $NH_4H_2PO_4$ (aq)/30% CH_3CN. Mobile phase B: 30% 0.02 M $NH_4H_2PO_4$ (aq)/70% CH_3CN. Program: Isocratic (100% A) 0–10 min, gradient (100% B) 10–30 min. Isocratic (100% B) 30–35 min, gradient (100% A) 35–40 min. Isocratic (100% A) 40–45 min; flow rate: 1 ml/min, injection size 20 μl.

Method 2: Column: YMC-Pack Pro 5.0 μm (4.6 × 150 mm). Mobile phase A: 80% Water/20% CH_3CN with 0.1% TFA. Mobile phase B: 20% Water/80% CH_3CN with 0.1% TFA. Program: Isocratic (100% A) 0–10 min, gradient (100% B) 10–30 min. Isocratic (100% B) 30–35 min, gradient (100% A) 35–40 min. Isocratic (100% A) 40–45 min; flow rate: 1 ml/min, injection size: 20 μl.

Method 3: Column: YMC-ODS-AQ, 3 μm (4.6 × 150 mm). Mobile phase A: 95% 0.01 M NH_4OAc (aq)/5% CH_3CN. Mobile phase B: 5% 0.01 M NH_4OAc (aq)/95% CH_3CN. Program: Start (100% A), gradient (100% B) 0–5.5 min. Isocratic (100% B) 5.5–7 min; flow rate: 3 ml/min, injection size: 10 μl.

Method 4: Column: YMC-ODS, 5 μm, (20 × 100 mm). Mobile phase A: 90% Water/10% CH_3CN. Mobile phase B: 10% Water/90% CH_3CN. Program: Start (100% A), gradient (25% B) 0–4 min, gradient (100% B) 4–6 min. Isocratic (100% B) 6–15 min; flow rate: 6 ml/min, injection size: 1 ml.

3-Methoxy-[3-^{14}C]cinnamanilide (**2**)

A 500-ml round-bottomed flask was charged with [3-^{14}C]-*trans*-cinnamic acid (173 mg, 1.17 mmol, 60 mCi at 51.3 mCi/mmol), *trans*-cinnamic acid (1.480 g, 10 mmol), anhydrous CH_2Cl_2 (25 ml), *m*-anisidine (1.44 g, 11.72 mmol) and *N*-ethyl-*N*′-dimethylaminopropyl carbodiimide hydrochloride (3.21 g, 16.74 mmol). The flask was wrapped in aluminum foil and the reaction gently stirred for 18 h. An ice-cold 20% aqueous solution of H_3PO_4 (25 ml) was added and the resulting bi-phasic mixture stirred rapidly for 15 min. This mixture was transferred to a separatory funnel and partitioned. The aqueous phase was extracted further with CH_2Cl_2 (50 ml). The organic extracts were combined and washed with brine (20 ml), dried over Na_2SO_4, filtered and concentrated to give **2** (2.609 g, 92%). The material was sufficiently pure as judged by TLC, $R_f = 0.24$ (20% ethyl acetate, hexanes), HPLC, (method 1 R_t 26.3 min) and the ^{1}H NMR spectrum was consistent with the published spectrum for **2**.[4]

7-Hydroxy-quinolin[4-^{14}C]-2(1H)-one (**3**)

Crude cinnamanilide **2** (2.61 g, 10.3 mmol) was dissolved in anhydrous chlorobenzene (50 ml). Aluminum chloride (5.49 g, 41.2 mmol) was added, and the reaction heated to 120°C in an oil bath with rapid stirring. After 5 h the reaction was cooled to room temperature (rt), then to 0°C in an ice bath. Chipped ice (3 g) was added to the vigorously stirred mixture followed by water (20 ml). The flask was warmed to rt and stirred further for 20 min. The suspended solids were collected on a medium porosity sintered glass funnel. The highly colored filter cake was rinsed with water (30 ml) followed by $CHCl_3$ (50 ml) and diethyl ether (50 ml). The crude product was dried under high vacuum to give **3** (2.66 g) as a charcoal-gray solid. This material was carried on further without purification; HPLC (method 1 R_t 18.7 min), ^{1}H NMR spectrum was consistent with the published spectrum for **3**.[5]

7-Hydroxy-3-4-dihydroquinolin[4-^{14}C]-2(1H)-one (**4**)

Crude **3** (2.66 g, 16.5 mmol) was placed in a 2-neck round-bottomed flask and dissolved in methanol (150 ml). The resulting solution was sparged with argon

and then charged with 10% Pd/C (1.5 g). The flask was equipped with a gas inlet adapter attached to a balloon containing H_2 and placed under a positive H_2 atmosphere. Slowly, the flask was warmed to 50°C in an oil bath and heated for 18 h with rapid stirring. The reaction was cooled to rt and sparged with N_2 before being filtered through a 5 μm Zylon® filter. The solids were rinsed with additional methanol and the filtrate concentrated to give **4** (1.7 g, 100%) as a light yellow solid. The material was greater than 95% pure based on TLC, $R_f = 0.5$ (10% hexanes, ethyl acetate); ^{1}H-NMR (CD_3OD): $\delta = 2.51$ (m, 2H), 2.82 (m, 2H), 6.33 (d, $J = 2.4$ Hz, 1H), 6.4 (dd, $J = 2.4$, 7 Hz, 1H), 6.95 (d, $J = 7$ Hz, 1H).

*7-(4-Chlorobutoxy)-3,4-dihydroquinolin[4-^{14}**c**]-2(1H)-one* (**5**)

A 1-neck round-bottomed flask was charged with **4** (1.7 g 10.4 mmol), acetonitrile (35 ml), water (15 ml), 1-bromo-4-chlorobutane (3.57 g, 20.8 mmol), potassium carbonate (4.31 g, 31.2 mmol) and cetyltrimethylammonium bromide (20 mg, 0.055 mmol). The reactants were mixed by rapid stirring with heating at 100°C in an oil bath. The progress of the reaction was monitored by TLC and HPLC. After 5 h the reaction was cooled to rt and transferred to a separatory funnel. Additional CH_3CN (50 ml) and brine solution (50 ml) were added and the layers mixed and partitioned. The aqueous phase was extracted further with CH_3CN (50 ml) and combined with the previous extract. The resulting organic solution was dried over a minimum of Na_2SO_4, filtered, then concentrated to give a yellow oil. The crude mixture was purified by chromatography on a Biotage® 40 M column with 40% ethyl acetate/60% hexanes as the mobile phase. The appropriate fractions were pooled and concentrated to give **5** (1.71 g, 66%) as a light yellow solid having an HPLC radiochemical purity of 96.4% (method 2, R_t 24.4 min). The product was purified further by crystallization from hot ethyl acetate (20 ml) and hexanes (40 ml) to give **5** (1.21 g) with a radiochemical purity of 98% and a specific activity of 21.2 μCi/mg; TLC, $R_f = 0.75$ (10% hexanes, ethyl acetate); ^{1}H-NMR spectrum was consistent with the published spectrum for **5**.[4]

7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydroquinolin[4-^{14}C]-2(1H)-one (**1a**)

In a 500-ml round-bottomed flask the following was added; **5** (1.0 g, 3.95 mmol), 1-(2,3-dichlorophenyl)piperazine hydrochloride (1.15 g, 4.34 mmol), 25 ml of DMF, K_2CO_3 (2.02 g, 14.6 mmol) and NaI (888 mg, 5.93 mmol). The mixture was heated at 100°C for 4.5 h. The reaction was monitored by HPLC (method 2, R_t 21.5 min), and at completion, the flask was cooled to rt. Methylene chloride (100 ml) was added along with water (300 ml) and the layers were mixed. The bi-phasic mixture was partitioned, and the aqueous

phase extracted further with CH_2Cl_2 (50 ml). The organic solutions were combined and concentrated to give a yellow semi-solid. The crude product was purified by column chromatography on a Biotage® 40 M column using 95% CH_2Cl_2/5% ethanol, and crystallization from hot ethanol/water to give **1a** (1.35 g, 76%) with a radiochemical purity of 99.5% and a specific activity of 11.4 μCi/mg; ^{1}H NMR spectrum was consistent with the published spectrum for **1a**.[4]

1,2-Dichloro[U-^{14}C]phenyl-3-iodobenzene (**6**)

2,3-Dichloro[U-^{14}C]aniline (50 mCi, 0.76 mmol) was dissolved in Et_2O (30 ml), and added to a flask containing non-labeled 2,3-dichloroaniline (610 mg, 3.76 mmol). Concentrated H_2SO_4 (273 μl, 4.91 mmol) was added in three equal portions via mechanical pipettor. After sonicating the mixture for 10 s, the Et_2O was removed under vacuum affording the hydrogensulfate salt of 2,3-dichloro[U-^{14}C]aniline as a white solid. A magnetic stirring bar, H_2O (6 ml), and concd H_2SO_4 (1.96 ml, 35.3 mmol) were added, and the mixture was cooled to 0°C. A pre-cooled solution of $NaNO_2$ (950 mg, 13.77 mmol) at 0°C and H_2O (17 ml) was added via pipette over 16 min. After 30 min of stirring at 0°C, a solution of KI (2.693 g, 16.22 mmol) in H_2O (17 ml) was added via pipette over 5 min. The resulting purple mixture was stirred at 0°C for 1 h. A condenser was added to the flask and the reaction was heated at 100°C for 2 h. After cooling to rt, hexanes (60 ml) were added through the reflux condenser to rinse off the sublimed material. The resulting bi-phasic mixture was stirred rapidly for 30 min. The organic layer was separated, washed with 5% $NaHSO_3$ (40 ml), dried over $MgSO_4$ and filtered, to give a yellow solution (~5 ml) after concentration. This solution was loaded directly onto a KP-Sil cartridge, and chromatographed with hexanes to give **6** (910 mg, 74% yield) as a white, crystalline solid; TLC, hexanes, R_f 0.49. HPLC radiochemical purity 99.5% (method 3). ^{1}H NMR analysis was consistent with the structure of **6**.[8]

1-(2,3-Dichloro-[U-^{14}C]phenyl)piperazine Hydrochloride (**7**)

A mixture of **6** (910 mg, 3.33 mmol), anhydrous piperazine (1.741 g, 20.21 mmol), and anhydrous toluene (20 ml) was sparged with argon for 15 min. Sodium *tert*-butoxide (449 mg, 4.67 mmol), *S*-BINAP (64.3 mg), and *tris*(dibenzylideneacetone)dipalladium(0) (29 mg, 0.03 mmol) were added to the reaction flask followed by additional toluene (20 ml) as a rinse. The reaction mixture was sparged further with Ar for 10 min. The flask was then equipped with a condenser, and heated to reflux in a 120°C bath. The reaction was monitored by HPLC (method 3) and after 18.5 h found to be 18% complete. Additional *S*-BINAP (65.6 mg, 0.11 mmol) and *tris*(dibenzylideneacetone)dipalladium(0) (31.8 mg, 0.035 mmol) were added, and after a total of

24 h of heating the conversion was determined to be 25% complete. Again the flask was charged with *tris*(dibenzylideneacetone)dipalladium(0) (30.1 mg, 0.03 mmol), and after 44.5 h at reflux, the reaction was >98% complete. The mixture was filtered and the filtrate washed with H_2O (2 × 20 ml) and dried over Na_2SO_4. The organic solution was filtered and ethereal 1 M HCl (8.5 ml, 8.5 mmol) added to the filtrate over 5 min with stirring. The resulting mixture was kept cool at 0°C for 2 days and the solids collected by vacuum filtration. The crude product was dissolved in H_2O:CH_3CN (90:10, ~11 ml) at reflux and after cooling to rt, purified by semi-preparative HPLC (method 4). Pure fractions were immediately concentrated on a rotary evaporator to give aqueous solutions which were combined, frozen at −78°C and lyophilized. This gave **7** (530 mg, 59%) as a light yellow solid; ^{1}H NMR spectrum was consistent with the published structure of **7**.[7] HPLC radiochemical purity was 99.7% (method 3).

*7-[4-[4-(2,3-**d**ichloro-[U-^{14}C]phenyl)-1-piperazinyl]butoxy]-3,4-dihydroquinolin-2(1H)-one* (**1b**)

A heterogeneous mixture of **7** (473 mg, 1.77 mmol), 7-(4-Chlorobutoxy)-3, 4-dihydroquinolin-2(1H)-one (450 mg, 1.77 mmol), K_2CO_3 (269 mg, 1.95 mmol), and sterile H_2O (9.0 ml) was stirred at 95°C for 6 h and then rt for 19 h. The solvent was removed via pipette, and the solid washed with sterile H_2O (8 ml). Acetonitrile (46 ml) was added to the solid, and the mixture was stirred at 60°C for 5 h then cooled slowly to rt and stirred overnight. The solid was collected and dried under high vacuum to a constant weight (540 mg, 1.20 mmol, 68% yield) giving **1b** as a light tan solid (24.79 μCi/mg); HPLC (method 2, R_t 21.5 min) radiochemical purity 99.5%.

*7-[4-[4-(2,3-**d**ichloro-[U-^{14}C]phenyl)-1-piperazinyl]butoxy]-3,4-dihydroquinolin[4-^{14}C]-2(1H)-one* (**1**)

Labeled **1a** (496.1 mg 5.62 mCi) and **1b** (226.7 mg, 5.62 mCi) were combined in a flask and dissolved in anhydrous methylene chloride (20 ml). The solution was filtered through a 0.2 μm syringe filter into a clean vial. The vial and syringe were then rinsed with additional CH_2Cl_2. The solvent was removed by rotary evaporation at 30°C under partial vacuum, and the powdery solid dried to constant weight under high vacuum to give multi-labeled [^{14}C]Aripiprazole **1** with a radiochemical purity of 99.9% (method 2, R_t 21.5 min) and a specific activity of 15.5 μCi/mg.

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