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Synthesis of ^{14}C-Labelled CGS 16949A (Fadrozole HCl), a Potent Aromatase Inhibitor

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SUMMARY

CGS 16949A (Fadrozole HCl) is an aromatase inhibitor currently used in Japan for the treatment of estrogen-dependent cancer. A ^{14}C-labeled form of this compound was synthesized for metabolism and pharmacokinetic studies. The synthesis pathway includes six steps, which are shown in synthetic Scheme 2. Initially, 4-bromotoluene was reacted with [^{14}C]CuCN to yield 4-tolunitrile-[^{14}C]-cyano. The nitrile was brominated on the α carbon to give an intermediate that was used to N-alkylate a known imidazole analogue. The product was treated with thionyl chloride to convert a 3-hydroxy propyl moiety to its chloro analogue. Treatment with potassium butoxide gave [^{14}C] fadrozole as the free base via ring closure. Hydrogen chloride gas was used to give the desired salt [^{14}C]CGS 16949A. Using this procedure, [^{14}C]CGS 16949A was prepared with 97% radiochemical purity. The overall radiochemical yield for the synthesis was 1% from [^{14}C]KCN.

KEYWORDS: [^{14}C]CGS 16949A, Aromatase Inhibitor, potassium [^{14}C]cyanide

INTRODUCTION

The novel tetrahydroimidazopyridine CGS 16949A (***9***, Scheme 2) is a potent nonsteroidal inhibitor of aromatase or estrogen synthetase, currently approved in Japan for the treatment of estrogen-dependent breast cancer [1]. It effectively blocks the conversion of androgenic precursors to estrogens as demonstrated in both *in vitro* and *in vivo* studies [2]. Since estrogens have been implicated in the progression of several diseases, the most prominent being breast cancer, inhibitors of aromatase have great therapeutic potential [3]. A ^{14}C-labelled form of CGS 16949A was required for metabolism and pharmacokinetic studies. The details of the radiosynthesis and analysis of this compound are described in this paper.

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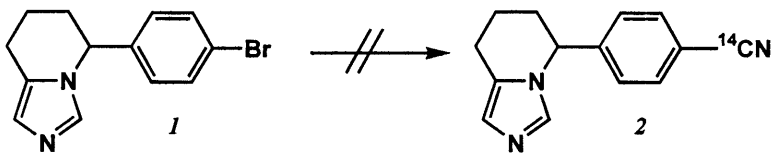
RESULTS AND DISCUSSION

In order to produce appropriately labelled material for metabolic studies, we decided to place the ^{14}C-label in the metabolically non-labile cyano group of the target compound. Initially, we investigated synthesizing [^{14}C]CGS 16949 (*2*), a racemic compound, via direct reaction of [^{14}C]CuCN with the known bromide *1* [4] as displayed in Scheme 1. Attempted cyanations of *1* using [^{14}C]CuCN in DMF at high temperature [5] were unsuccessful, prompting us to prepare the target compound through a pathway based on that previously used for the synthesis of unlabelled CGS 16949A [4].

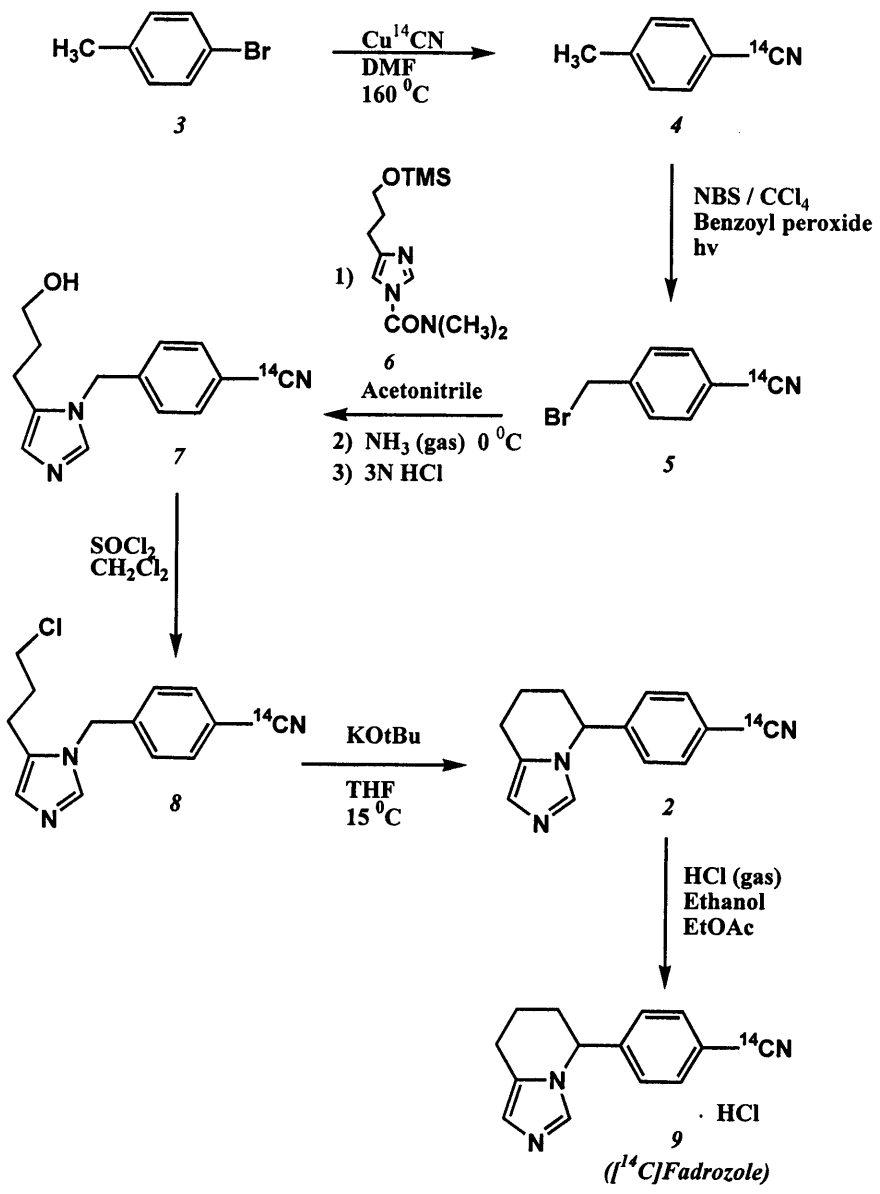
Scheme 1



Using the route outlined in Scheme 2, the radiosynthesis was accomplished in six steps starting with potassium-[^{14}C]-cyanide and 4-bromotoluene (*3*). Potassium-[^{14}C]-cyanide was converted to cuprous-[^{14}C]-cyanide, which was then allowed to react with 4-bromotoluene (*3*) to yield 4-tolunitrile-[^{14}C]-cyano *4* [5]. Bromination of *4* with N-bromosuccinimide and benzoyl peroxide under light [6] gave [^{14}C]bromomethylbenzonitrile *5*, which was then used to alkylate the known imidazole *6*, prepared from 4-imidazoleacrylic acid [4]. This alkylation product was treated successively with anhydrous ammonia and 3N hydrochloric acid to remove the protecting groups yielding [^{14}C]hydroxy compound *7*, which was then directly converted to [^{14}C]chloro derivative *8* with thionyl chloride in methylene chloride. Compound *8* was treated directly with potassium tertiary butoxide in THF giving the desired ring-closed product *2*, [^{14}C]CGS 16949, which was successfully purified by column chromatography. Compound *2* was then converted to its hydrochloride salt and recrystallized twice from acetone to obtain the final product, [^{14}C]CGS 16949A ([^{14}C]fadrozole, *9*).

In conclusion, we have developed a facile method for the preparation of ^{14}C-labelled CGS 16949 (fadrozole). The synthesis was completed in six steps with an overall radiochemical yield of 1 % from potassium-[^{14}C]-cyanide.

Scheme 2



EXPERIMENTAL

Potassium-[^{14}C]-cyanide was purchased from Amersham Corporation of Arlington Heights, IL or NEN DuPont of Boston, MA. Reagents were purchased from Aldrich Chemical Company of Milwaukee, WI. All thin layer chromatography (TLC) was performed on EM Silica -Gel 60 F254 plates, 0.250 mm thickness, using dichloromethane:ethyl acetate:triethylamine

(9:1:1.5). Radiochemical purity was determined by scanning the TLC plates with a Bioscan System 200 imaging scanner. HPLC analyses were performed on a Waters system that included a Model U6K injector, a Model 510 HPLC pump, a Model 486 tunable absorbance detector adjusted to 254 nm wavelength, and a Baseline 810 Chromatography Workstation. The HPLC system was interfaced with an on–line radioactivity detector (Flo–One/Beta Series A–100 detector of Radiomatic Instrument Co.). The HPLC effluent was mixed with liquid scintillation solution (Flo–Scint III) at a ratio of 1:3. Reverse phase HPLC analysis utilized a 3.9 x 150 mm Waters μ-Bondapak CN analytical column. The column was eluted isocratically at a flow rate of 1 mL/min with a mobile phase consisting of methanol and 0.01 M KH_2PO_4 solution (1:1). All melting points were taken in capillary tubes on a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected.

Cuprous-[^{14}C]-cyanide.

To a solution of cupric sulfate (8.3 g, 33.2 mmol) in 30 mL of water at 60 °C was added a solution of sodium metabisulfite (4.1 g, 21.5 mmol) in 30 mL of water at 60 °C. A solution of [^{14}C]KCN (2.15 g, 32.1 mmol, 210 mCi) in 12 mL of water was immediately added to the above mixture with stirring. The precipitate was filtered, and the solid was washed successively with water, ethanol and ether, then dried under vacuum at 70 °C for 48 h to yield 2.7 g (29.5 mmol, 92.8% yield) of product.

4-Tolunitrile-[^{14}C]-cyano (*4*).

A mixture of 4-bromotoluene (5.8 g, 33.9 mmol), [^{14}C]CuCN (2.7 g, 29.5 mmol) and 50 mL of dimethylformamide was heated at 160 °C for 24 h. The mixture was cooled to ambient temperature and then treated with 10 mL of water followed by 80 mL of ammonium hydroxide. The mixture was then extracted four times with ethyl acetate and the extract washed with water and brine and dried over $MgSO_4$. Removal of the solvent by distillation under reduced pressure followed by chromatography on silica gel (9:1 ethyl acetate:petroleum ether) gave a white solid *4* (1.6 g, 13.4 mmol, 45% yield). The above two steps were repeated to give another batch of product *4* (1.5 g, 12.6 mmol, 43% yield). TLC comparison (9:1 ethyl acetate:petroleum ether) of the combined batches (3.1 g, 26.1 mmol) with the authentic compound, showed that only the product was present (UV visualization).

4-(Bromomethyl)benzonitrile-[^{14}C]-cyano (*5*).

To a solution of nitrile *4* (3.1 g, 26.1 mmol) in 50 mL of carbon tetrachloride was added N-bromosuccinimide (5.1 g, 28.7 mmol) and the resulting solution was heated at reflux. A

solution of benzoyl peroxide (70 mg, 0.29 mmol) in 10 mL of carbon tetrachloride was added dropwise, and the solution at reflux was irradiated with light from a 200 W lamp for 1 hour. The mixture was then cooled, filtered, and the filtrate washed with dilute NaOH solution. After drying over $MgSO_4$, the solution was evaporated to dryness affording the crude product ***5*** (3.1 g, 15.6 mmol, 59.8% yield) as a yellow semisolid material.

4-[5-(3-Hydroxypropyl)-1H-imidazol-1-yl)methyl]benzonitrile-[^{14}C]-cyano (*7*).

To an acetonitrile (50 mL) solution of the protected imidazole ***6***, prepared *in situ* from 1H-imidazole-4-propanol (2.2 g, 17.5 mmol) [4], was added compound ***5*** (3.1 g, 15.6 mmol) and the mixture was heated at reflux for 10 h. It was then cooled to 0 ^{o}C and anhydrous ammonia gas was bubbled through the solution for 4 h. The cooling bath was removed and stirring continued for 2 h at ambient temperature. After removing the solvent *in vacuo*, the resulting residue was stirred overnight at room temperature with 40 mL of 3N HCl. The mixture was then extracted with toluene, the layers separated, and the aqueous layer treated with NaOH solution to pH 9 and then extracted with ethyl acetate. The extract was dried over $MgSO_4$ and evaporated to give crude product ***7*** (3.1 g, 12.8 mmole, 81% yield) as an oil. TLC analysis (ethyl acetate/methanol/NH_4OH 70:30:0.1) showed a major UV-visible product that matched authentic compound and several minor products. This crude material was used directly for the next step.

4-[(5-(3-Chloropropyl)-1H-imidazol-1-yl)methyl]benzonitrile-[^{14}C]-cyano (*8*).

Crude compound ***7*** (3.1 g, 12.8 mmol) was dissolved in 50 mL of dichloromethane, and 2.1 mL (28.7 mmol) of thionyl chloride was added dropwise to the solution with stirring. The resulting solution was heated at reflux for 2 h and then evaporated to give 3.2 g (10.8 mmol) of a dark viscous oil. After dissolving this oil in 30 mL of tetrahydrofuran, 1.5 mL (10.8 mmol) of triethylamine was added. This mixture was stirred for 30 min at ambient temperature and then filtered to remove triethylamine hydrochloride. The resulting solution of crude ***8*** in tetrahydrofuran was used directly for the next step.

4-(5,6,7,8-Tetrahydroimidazo[1,5-a]pyridin-5-yl)benzonitrile-[^{14}C]-cyano (*2*).

To the solution of crude ***8*** in tetrahydrofuran was added dropwise a solution of potassium tertiary butoxide (1.21 g, 10.8 mmol) in 10 mL of tetrahydrofuran. The reaction temperature was kept at 15 ^{o}C during the addition and this temperature was maintained for 2 h after the addition. Glacial acetic acid was then added until the solution was at pH 7. Following the evaporation *in vacuo*, the residue was dissolved in water and the solution extracted several times with toluene. The toluene extract was washed with water and brine and then dried over $MgSO_4$. Removal of

the solvent afforded 900 mg of crude *2* as an oil. Purification by chromatography on silica gel (chloroform:methanol 80:20) gave compound *2* (350 mg, 1.56 mmole, 12.2% yield in two steps from *7*) as a light-yellow oil. TLC analysis of compound *2* in dichloromethane:ethyl acetate:triethylamine (90:10:15) showed one UV visible spot identical to authentic unlabeled compound *2*. Radioscan analysis of the thin layer plate showed that the radiochemical purity of compound *2* was 95%.

4-(5,6,7,8-Tetrahydroimidazo[1,5-a]pyridin-5-yl)benzonitrile-[^{14}C]-cyano monohydrochloride (*9*) ([^{14}C]CGS 16949A).

Nitrile *2* (350 mg) was dissolved in 3 mL of ethyl acetate, and the solution was cooled to 0 °C. A solution of 1 mL of ethanol saturated with hydrogen chloride gas was then added in one part. After 15 min the solvents were removed by evaporation, and the solid residue recrystallized twice from acetone to yield 175 mg (0.67 mmol, 43% yield) of a white solid; m.p., 220 - 230 °C. The specific activity, as determined by liquid scintillation counting was 24 μCi/mg with a total activity of 4.2 mCi (1% yield from 210 mCi x 2 batches of [^{14}C]KCN). TLC analysis of [^{14}C]CGS 16949A with dichloromethane:ethyl acetate:triethylamine (90:10:15) showed a single UV visible spot identical to that of authentic CGS 16949A. TLC analysis of the final product with radioscanning showed three spots: an impurity at the origin (1.5%), a second impurity (<1%) with an RF lower than that of [^{14}C]CGS 16949A, and [^{14}C]CGS 16949A at 97% purity. HPLC of [^{14}C]CGS 16949A using a μ-Bondapak CN column (Waters) with UV detection at 254 nm and a mobile phase of methanol: 0.01 M KH_2PO_4 (1:1) showed only one peak identical to that of authentic CGS 16949A. The same analysis with radiodetection showed a radiochemical purity of 98%.

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