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Research Article

Radiosynthesis of the α4β2 nicotinic acetylcholine receptor ligand: 5-((1-[^{11}C]-methyl-2-(*S*)-pyrrolidinyl)methoxy)-2-chloro-3-((*E*)-2-(2-fluoropyridin-4-yl)vinyl)pyridine

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Summary

5-((1-[^{11}C]-methyl-2-(*S*)-pyrrolidinyl)methoxy)-2-chloro-3-((*E*)-2-(2-fluoropyridin-4-yl)-vinyl)pyridine ([^{11}C]-FPVC) was synthesized from [^{11}C]-methyl iodide and the corresponding normethyl precursor. The average time of synthesis, purification, and formulation was 42 min with an average non-decay-corrected radiochemical yield of 19%. The average specific radioactivity was 359 GBq/μmol (9691 mCi/μmole) at end of synthesis (EOS). Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: α4β2 nicotinic acetylcholine receptor; carbon-11; positron emission tomography

Introduction

Central nicotinic acetylcholine receptors (nAChR) are known to play a key role in a number of brain functions and may be involved with many pathological disorders.[1,2] Due to the low density of nAChRs, there is a need for the development of a lipophilic high-affinity radioligand for imaging by positron emission tomography (PET). Numerous radioligands have been synthesized for imaging the α4β2 unit of the nAChR, the most abundant subtype, and recently reviewed.[3] The radioligands include nicotine and closely related analogs of cytosine, epibatidine, and pyridyl ethers of A-84543. Recently, a series of pyridyl ether compounds based on the A-84543 and

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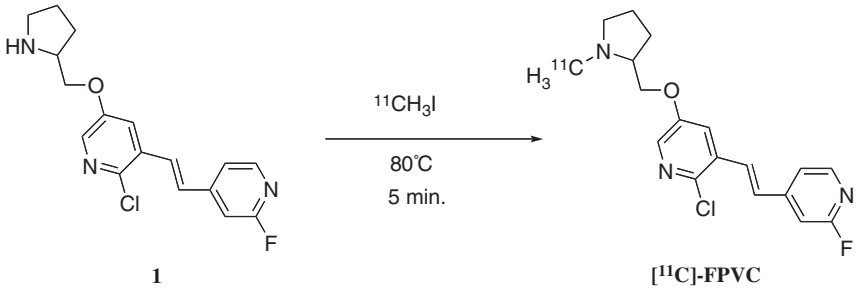

Figure 1. Radiosynthetic scheme for [^{11}C]-FPVC

A-85380 backbones were synthesized.[4] The compounds in this series were found to have higher clog $D_{7.4}$ and K_i values than currently available PET nAChR radioligands. For example, 2-[^{18}F]fluoro A-85380 (2FA) with a clog $D_{7.4}$ of -1.99 and a K_i of 61 pM has slow brain kinetics *in vivo* possibly due to its hydrophilic character. Of the pyridiyl ethers described above, 5-(((*S*)-pyrrolidin-2-yl)methoxy)-2-chloro-3-((*E*)-2-(2-fluoropyridin-4-yl)-vinyl)-pyridine (**1** in Figure 1) was found to have a clog $D_{7.4}$ of 0.6 with a K_i of 9.4 pM. The *N*-methyl analog, 5-((1-methyl-2-(*s*)-pyrrolidinyl)methoxy)-2-chloro-3-((*E*)-2-(2-fluoropyridin-4-yl)vinyl)pyridine (FPVC), has a clog $D_{7.4}$ of 1.6. Both compounds, being more lipophilic than 2FA, should cross the blood brain barrier more easily showing better brain kinetics. In a continuing effort to develop a PET radiotracer with rapid brain kinetics for imaging nAChR, 5-((1-[^{11}C]-methyl-2-(*S*)-pyrrolidinyl)methoxy)-2-chloro-3-((*E*)-2-(2-fluoropyridin-4-yl)-vinyl)pyridine ([^{11}C]-FPVC) (Figure 1) was synthesized from [^{11}C]-methyl iodide ([^{11}C]MeI).

Results and discussion

In order to confirm the identity, purity and specific radioactivity of the final radiolabeled product authentic FPVC was produced. FPVC was synthesized by a typical reductive alkylation of **1** using formaldehyde and sodium cyanoborohydride. The isolation and purification resulted in pure FPVC which when stored as a standard solution in absolute ethanol at -18°C was stable for over 1 month.

The carbon-11 radiosynthesis of [^{11}C]-FPVC was facile. After extraction of the free base from the trifluoroacetic acid (TFA) salt of the normethyl precursor, the anhydrous dimethylformamide (DMF) solution was cooled and [^{11}C]MeI was bubbled into the capped vial. After heating, the solution was diluted with aqueous ammonium formate and injected onto the semi-preparative high-pressure liquid chromatography (HPLC) column. The product was collected into a large volume of water and subsequently eluted onto a C-8 solid phase extraction cartridge (SPE). The SPE was rinsed with

saline and eluted with absolute ethanol. The eluted ethanol was diluted with saline to reduce the ethanol concentration to 10% or less.

The radiosynthesis, purification and formulation was completed in 42 min ($n = 4$). [^{11}C]-FPVC had an average specific radioactivity of 359 GBq/μmol (9691 ± 4817 mCi/μmol EOS) and was obtained in a non-decay corrected yield of 19%. The introduction of the commercially available gas phase [^{11}C]MeI production unit to our laboratory in 2000 (and the continued use of a tight no carrier added targetry system) has increased the final specific radioactivity dramatically over our wet method (lithium aluminum hydride and hydriodic acid) system that has been used to prepare [^{11}C]MeI in our laboratory since 1982. An average remote radiosynthesis started with 21.7 GBq (584 mCi) of [^{11}C]MeI with isolation of 4.1 GBq (112 mCi) of formulated [^{11}C]-FPVC. The identity, purity and specific radioactivity of the final product was determined by comparison and coinjection with FPVC. Radiochemical purity was greater than 98%.

Experimental

[^{11}C]MeI was synthesized using a GE Methyl Iodide Microlab from [^{11}C]carbon dioxide produced by 18 MeV proton bombardment of a nitrogen target containing 0.5% oxygen using a GE PETtrace biomedical cyclotron. All reagents were ACS or HPLC purity. The normethyl precursor, **1**, was synthesized as the TFA salt as previously described.[4] DMF was distilled under vacuum from barium oxide prior to use. Reverse-phase HPLC analysis and purification were performed with two Waters 590 HPLC pumps, a Waters 440 fixed wavelength (254 nm) UV detector, and a Ortec 1 inch NaI crystal radioactivity detector. All HPLC chromatograms were recorded with Varian Galaxy hardware and software (version 1.8). The analytical and semi-preparative chromatography was performed using a Phenomenex Luna C-18 5 μm column (analytical – 4.6 × 250 mm and semi-preparative – 10 × 250 mm) and a mobile phase of 32:68 acetonitrile:water (0.1 M ammonium formate) for both systems at a flow rate of 3 and 10 ml/min, respectively. A dose calibrator (Capintec 15R) was used for all radioactivity measurements.

Synthesis of FPVC

A mixture of 5-((2-(*S*)-pyrrolidinyl)methoxy)-2-chloro-3-(2-(2-fluoro-pyridin-4-yl)vinyl)pyridine (**1**, 128 mg, 0.330 mmol) and 37% formaldehyde (0.39 ml, 4.8 mmol) in acetonitrile (2.7 ml) was stirred for 20 min at room temperature under argon, then sodium cyanoborohydride (95 mg, 1.5 mmol) was added in small portions. The mixture was stirred for 15 min at room temperature and a complete reaction was confirmed by analytical HPLC as described above. An aliquot of glacial acetic acid was added with stirring over 2 h, while maintaining a pH near neutrality. The solvent was evaporated at reduced pressure and 7 ml of 2% K_2CO_3 was added to the residue. The resulting

mixture was extracted with three 15 ml portions of ether. The combined ether extracts were washed with water (10 ml), brine (15 ml), dried with anhydrous Na_2SO_4 and evaporated *in vacuo*. After purification by column chromatography on silica gel with $CHCl_3$/MeOH (1:1), the product was obtained as a white solid (50% yield). ^{1}H NMR ($CDCl_3$/TMS) δ: 8.24 (d, $J = 5.2$ Hz, 1 H), 8.08 (d, $J = 2.9$ Hz, 1 H), 7.58 (d, $J = 16.4$ Hz, 1 H), 7.53 (d, $J = 2.9$ Hz, 1 H), 7.32 (dt, $J = 1.5$, 5.2 Hz, 1 H), 7.03 (s, 1 H), 7.00 (d, $J = 16.4$ Hz, 1 H), 4.05 (m, 2 H), 3.16 (m, 1 H), 2.74 (m, 1 H), 2.52 (s, 3 H), 2.34 (m, 1 H), 2.04 (m, 2 H), 1.83 (m, 2 H). Elemental analysis: Calculated for $C_{18}H_{19}ClFN_3O \bullet 1.2\ H_2O$: C, 58.53; H, 5.80; N, 11.38; Cl, 9.60; F, 5.14. Found: C, 58.98; H, 5.48; N, 11.06; Cl, 9.94; F, 4.69.

Radiosynthesis and purification of [^{11}C]-FPVC

The TFA salt of normethyl precursor, **1**, (2.5 mg, 7.8 nmol) was added to 1 ml of 2% potassium carbonate and 2 ml ether. After vortexing the mixture, the ether layer was separated and passed through a small potassium carbonate column. After evaporation of the ether, the residue was dissolved in 200 µl DMF, capped in a small v-vial and cooled to −40°C. [^{11}C]MeI was bubbled into the vial. After the radioactivity reached a plateau, the vial was assayed in the dose calibrator then placed in an 80°C water bath for 5 min. Aqueous ammonium formate (200 µl of 0.1 M) was added and the solution injected onto the semi-preparative HPLC column. The product ($T_R = 7.3$ min, $k' = 4.2$; normethyl precursor: $T_R = 4.8$ min, $k' = 2.4$) was collected in 50 ml of HPLC water. The water solution was transferred through an activated Waters C-8 Sep Pak Plus (10 ml ethanol then 10 ml HPLC water) to waste. After washing the SPE with 10 ml of 0.9% saline, the product was eluted with 1 ml of absolute ethanol into a vial. The ethanol was diluted with 9 ml of 0.9% saline. An aliquot was removed to determine chemical and radiochemical purity of the final solution ($T_R = 4.9$ min, $k' = 2.8$; precursor: $T_R = 3.5$, $k' = 1.7$) by analytical HPLC.

Conclusion

[^{11}C]-FPVC was easily synthesized from the nor-methyl precursor in good yield, purity and specific radioactivity. Animal studies are currently under way to determine the utility of [^{11}C]-FPVC for imaging the α4β2 nAChR.

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