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Radiosynthesis of 5-(2-(4-pyridinyl)vinyl)-6-chloro-3-(1-[^{11}C]methyl-2-(*S*)-pyrrolidinylmethoxy)pyridine, a High Affinity Ligand for Studying Nicotinic Acetylcholine Receptors by Positron Emission Tomography

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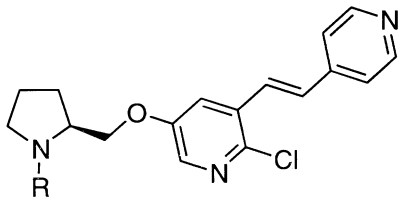
Abstract—5-(2-(4-pyridinyl)vinyl)-6-chloro-3-(1-methyl-2-(*S*)-pyrrolidinylmethoxy)pyridine (**1b**) exhibited high affinity for nicotinic acetylcholine receptors in the in vitro competition binding assays, with a K_d value in the low picomolar range, performed at room temperature and at physiological temperature. An efficient radiochemical synthesis of 5-(2-(4-pyridinyl)vinyl)-6-chloro-3-(1-[^{11}C]methyl-2-(*S*)-pyrrolidinylmethoxy)pyridine (**1c**), a potential tracer for the study of nAChR by positron emission tomography, has been developed.

Introduction

Considerable evidence links neuronal nicotinic acetylcholine receptors (nAChRs) to a variety of brain functions. These receptors have been implicated in the pathophysiology of many human CNS disorders and tobacco dependence. Much of the evidence for the role of nAChRs in these conditions comes from in vitro binding and autoradiographic studies of postmortem human brain tissue.[1–3] Positron emission tomography (PET) offers a non-invasive approach for characterizing changes in nAChR densities that are associated with CNS disorders, nicotine dependence, and the effects of pharmacotherapies. Although advances have been made towards the development of PET tracers for the study of nAChRs, to date, only [^{11}C]nicotine has been used to image nAChRs in human subjects by PET.[4] The use of [^{11}C]nicotine for PET studies, however, is limited due to high non-specific binding and rapid clearance from the brain.[5] Several epibatidine-based PET radiotracers have been used successfully in animal studies for imaging nAChRs, but these derivatives are too toxic for human studies.[6–10] In a search for improved radioligands suitable for non-invasive in vivo imaging of nAChRs in humans, we investigated a series of 3-pyridyl ethers developed by Abbott Laboratories.[11] Included in this series were a number of ligands with high affinity for nAChRs. Previously, halogenated derivatives of one of these compounds, A-85380, were synthesized and evaluated in our laboratory.[12] The compounds with highest affinities were radiolabeled with [^{18}F][13–15] and [^{123}I][16] and used successfully in our and other labs for in vivo imaging of monkey brain nAChRs by PET and SPECT respectively.[16–21] A-85380 based radiotracers have shown substantially less toxicity than epibatidine analogues and may be suitable for use in humans.[14–18]

In this study we report a [^{11}C] radiolabeling of 5-(2-(4-pyridinyl)vinyl)-6-chloro-3-(1-methyl-2-(*S*)-pyrrolidinylmethoxy)pyridine (**1b**) (Fig. 1). This compound, in



1a R=H
1b R=CH_3
1c R=$^{11}CH_3$

Figure 1.

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competition assays with [^{3}H]cytisine at 4 °C, exhibited the highest affinity for nAChRs in subsequent pyridyl ether series developed by Abbott Laboratories.[22] Due to the relevance of the in vitro binding data for the prediction of in vivo binding characteristics, we also evaluated the affinity of **1b** at 23 and 37 °C using the in vitro competition binding assay with the $\alpha_4\beta_2$ nAChRs subtype selective radioligand, 5-[^{125}I]iodo-A-85380.[23]

Results and Discussion

In vitro binding data

In our determination of binding affinity for **1b,** we utilized a competition receptor-binding assay with 5-[^{125}]iodo-A-85380. It has been shown that 5-[^{125}]iodo-A-85380 has high affinity and selectivity for the $\alpha_4\beta_2$ type nAChRs. For example, the nonspecific binding of 5-[^{125}]iodo-A-85380 was 4±1% and 6±1% of the total binding ($n=11$), measured at 23 and 37 °C, respectively. Hence, 5-[^{125}]iodo-A-85380 was established as a suitable ligand for use in competition assays at physiological temperature.[23] The results of these assays (Table 1) demonstrated that receptor-binding of **1b** is temperature dependent. Nevertheless, in all conditions used, **1b** exhibited high affinity for nAChRs; thereby suggesting that **1c** is a strong candidate for use as a PET radioligand.

Radiochemistry

Compounds **1a** and **1b** were prepared as described elsewhere.[22] The radiosynthesis of **1c** was achieved by reacting **1a** with [^{11}C]methyliodide (Fig. 2). The choice of reaction solvents and conditions were crucial since **1a** was susceptible to degradation. Initial attempts to react **1a** with [^{11}C]methyliodide in acetonitrile at 100 °C gave **1c** in low yields (<5%). Improved radiochemical yields (20%) were obtained from the reaction of **1a** with [^{11}C]methyliodide in acetonitrile at 180 °C (under pressure) for 8 min. However, the reaction also produced a substantial quantity of unlabeled byproduct. Due to the short half-life of Carbon-11, purification of the final product (**1c**) required fast and effective HPLC methods. We therefore altered the experimental procedure to reduce the formation of byproducts. Satisfactory radiochemical yields (20–26%) and significantly fewer byproducts were formed when **1a** and [^{11}C]methyliodide were reacted in dimethylformamide at 130 °C for 4–6 min. The final product, **1c**, was separated from [^{11}C]methyliodide and two radioactive peaks near void volume via HPLC using a Hamilton PRP-1 HPLC column (CH_3CN/CH_3OH/0.1M HCO_2NH_4/Et_3N 300:300: 320:4.6; 7 mL/min). Compound **1c** was eluted after 13.5 min and collected in a flask containing 100 μL of glacial acetic acid. Failure to add acetic acid to the collection flask resulted in significant degradation of **1c** during rotary evaporation. After a total synthesis time of 21–26 min, doses of 20–50 mCi were produced and the final products were chemically and radiochemically pure (>99%). The non-decay corrected radiochemical yields were all determined with respect to [^{11}C]methyliodide, and the specific radioactivity of **1c**, prepared from 200–250 mCi of [^{11}C]methyliodide, ranged from 1477 to 3723 mCi/μmol.

Table 1. In vitro binding data

Compound	5-[^{125}I]iodo-A-85380 binding K_i (pM), Mean±SEM		[^{3}H]-cytisine binding
	37 °C	23 °C	K_i (pM), 4 °C[22]
1a	16±1 ($n=6$)	15±1 ($n=4$)	39
1b	56±3 ($n=7$)	28±3 ($n=5$)	4.4
2-Fluoro-A85380	127±8	61±5	

Conclusion

In summary, we developed a facile synthesis for 5-(2-(4-pyridinyl)vinyl)-6-chloro-3-(1-[^{11}C]methyl-2-(*S*)-pyrrolidinylmethoxy)pyridine, **1c**, and we demonstrated that **1b** exhibits a high affinity for nAChRs at physiological temperature. Therefore, **1c** will be further evaluated as a PET tracer for imaging nAChRs.

Experimental

All reagents were ACS or HPLC grade and were purchased from Aldrich. HPLC analyses and purification were performed with two Waters 600/610 HPLC pumps, an in-line UV-detector (Waters, 254 nm), and a flow-count radioactivity detector (Bioscan 3200). HPLC chromatograms were recorded by a Rainin Dynamax dual channel control/interface module connected to a Macintosh computer with Dynamax v.1.4 software. Hamilton PRP-1 columns were used in all HPLC analyses and semi-preparative separations. A dose calibrator (Capintec CRC-35R) was used for all radioactivity measurements. [^{11}C]methyliodide was

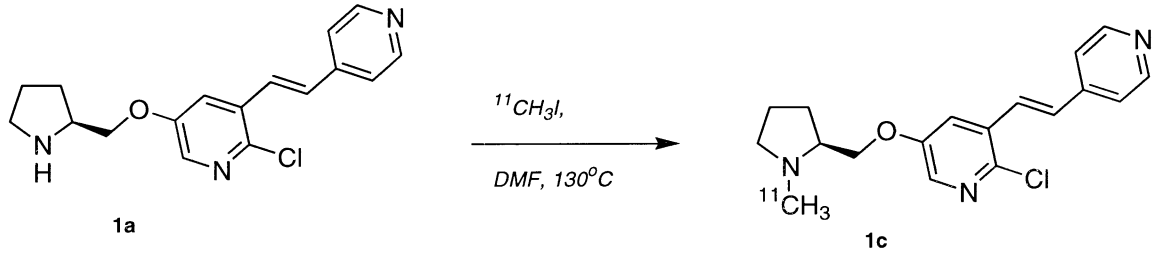

Figure 2.

prepared with MicroLab MeI box (GEMS)[24] by conversion of [^{11}C]CO_2 into [^{11}C]methane followed by a high temperature iodination. [^{11}C]Carbon dioxide was prepared using RDS111 cyclotron (CTI). Compounds **1a** and **1b** were prepared as described elsewhere.[22]

5-(2-(4-pyridinyl)vinyl)-6-chloro-3-(1-[^{11}C]methyl-2-(*S*)-pyrrolidinylmethoxy)pyridine (1c). 1a*TFA (3–5 mg) was dissolved in 5%K_2CO_3 (0.2 mL) and extracted with diethyl ether (1.0 mL) to give **1a**. The diethyl ether was evaporated via an argon flow [^{11}C]Methyl iodide was swept, by helium flow, into the vial containing a solution of **1a** in 0.35 mL of dimethylformamide. The reaction vessel was heated for 4 min at 130 °C. Afterwards, the reaction mixture was cooled, diluted with 0.5 mL of a mixture of 50% acetonitrile and 50% water, and injected onto a Hamilton PRP-1 (250×7 mm) HPLC column (CH_3CN/CH_3OH/0.1M HCO_2NH_4/Et_3N 300:300:320:4.6, 6 mL/min for 1 min, then 7 mL/min). Compound **1c** was eluted, collected in a flask containing acetic acid (100 μL), and rotary evaporated. The product was then redissolved in 5 mL of sterile saline.

An aliquot of **1c** (100–150 μL) was applied to an analytical Hamilton PRP-1 (250×4.6 mm) HPLC column (CH_3CN/CH_3OH/0.1M HCO_2NH_4/Et_3N 300:300:320:4.6; 2 mL/min). **1c** was coeluted with **1b** at 3.6–3.8 min. and the specific radioactivity was calculated using a standard curve relating mass to UV absorbance. The specific radioactivity ranged from 1477 to 3700 mCi/μmol. The product's integrity was confirmed via additional HPLC and MS analyses. A sample of **1b** prepared under the same conditions as **1c** was evaluated against a standard sample of **1b** prepared via 'cold' synthetic methods. Both samples eluted at 3.86 min on an analytical Supelcosil ABZ HPLC column (CH_3CN/CH_3OH/0.1M HCO_2NH_4/Et_3N 300:300:320:4.6; 2 mL/min). Also, the parent ion for **1b** prepared using radiosynthesis conditions (331 amu), along with a fragmentation peak due to the loss of a chlorine ion (296 amu), were the major peaks identified in the mass spectrum.

Binding assays. Competition assays with 5-[^{125}I]iodo-A-85380 were performed as described previously.[23] Briefly, rat brain P2 membrane fractions (5–10 μg of protein) were incubated at 37 °C for 1 h or 23 °C for 3 h in 0.4 mL of HEPES-salt solution (pH 7.4) in the presence of 20 pM (for 37 °C) or 10 pM (for 23 °C) 5-[^{125}I]iodo-A-85380 and 9 to 11 concentrations of the test compound. Incubations were performed in polystyrene titer plates (Beckman Instruments Co., Columbia, MD). Nonspecific binding was determined in the presence of 300 μM (−)-nicotine. The nonspecific binding of 5-[^{125}]iodo-A-85380 was 4±1% and 6±1% of the total binding ($n=11$), measured at 23 and 37° C, respectively. The binding was terminated by a vacuum filtration through GF/B filters pretreated with 1% polyethyleneimine. Experiments were performed in duplicate. The K_i values were calculated from measured IC_{50} and K_D values by the Cheng–Prusoff equation $K_i = IC_{50}/(1 + F/K_D)$, where F is the added radioligand concentration. The K_D values for 5-[^{125}I]Iodo-A-85380 of 10 pM for 23° C and 20 pM for 37° C were used for calculation. These K_D values were determined as described previously[23] in three to six independent experiments performed on the same membrane preparations that were used for the competition assay.

MALDI MS. Spectra were acquired on a DE PRO MALDI mass spectrometer from Applied Biosystem (Framingham, MA) using a nitrogen laser, in both positive and negative ion mode. Each spectrum was the average of 50 laser shots. Samples were prepared by mixing an equal volume of sample and matrix. The matrix was a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% ethanol. 1 μL of the mixture was deposited on the target and left to dry at room temperature.

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