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## Nicotinic $\alpha 4\beta 2$ Receptor Imaging Agents. Part III. Synthesis and Biological Evaluation of 3-(2-(S)-Azetidylmethoxy)-5-(3'- $^{18}\text{F}$ -Fluoropropyl)Pyridine ( $^{18}\text{F}$ -Nifzetidine)

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### Abstract

Thalamic and extrathalamic nicotinic  $\alpha 4\beta 2$  receptors found in the brain have been implicated in Alzheimer's disease, Parkinson's disease, substance abuse and other disorders. We report here the development of 3-(2-(S)-azetidylmethoxy)-5-(3'-fluoropropyl)pyridine (nifzetidine) as a new putative high affinity antagonist for nicotinic  $\alpha 4\beta 2$  receptors. Nifzetidine in rat brain homogenate assays containing  $\alpha 4\beta 2$  sites labeled with  $^3\text{H}$ -cytisine exhibited a binding affinity,  $K_i = 0.67$  nM. The fluorine-18 analog, 3-(2-(S)-azetidylmethoxy)-5-(3'- $^{18}\text{F}$ -fluoropropyl)pyridine ( $^{18}\text{F}$ -nifzetidine) was synthesized in 20–40% yield and apparent specific activity was estimated to be above 2 Ci/ $\mu\text{mol}$ . Rat brain slices indicated selective binding of  $^{18}\text{F}$ -nifzetidine to thalamus, subiculum, striata, cortex and other regions consistent with  $\alpha 4\beta 2$  receptor distribution. This selective binding was displaced >85% by 150  $\mu\text{M}$  nicotine. PET imaging studies of  $^{18}\text{F}$ -nifzetidine in anesthetized rhesus monkey showed slow uptake in the various brain regions. Retention of  $^{18}\text{F}$ -nifzetidine was maximal in the thalamus and lateral geniculate followed by regions of the temporal and frontal cortex. Cerebellum showed the least uptake. Thalamus to cerebellum ratio was about 2.3 at 180 min post-injection and continued to rise.  $^{18}\text{F}$ -Nifzetidine shows promise as a new PET imaging agent for  $\alpha 4\beta 2$  nAChR. However, the slow kinetics suggests a need for >3 hr PET studies for quantitative studies of the  $\alpha 4\beta 2$  nAChRs.

### Keywords

Nicotine; Autoradiography; Monkey PET; Fluorine-18

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## 1. Introduction

Development of imaging agents for the  $\alpha 4\beta 2$  neuronal nicotinic acetylcholine receptor (nAChR) has attracted much attention (1). These receptors consist of five subunits, each of which contains four transmembrane domains. The  $\alpha 4\beta 2$  nAChR subtype has high affinity for acetylcholine and nicotine and has been implicated in a number of disorders such as Alzheimer's disease, Parkinson's disease, schizophrenia and tobacco dependence (2–5).

A number of imaging agents with 3-pyridyl ethers have been investigated in an effort to optimize *in vivo* imaging properties (6–8). Human studies have been carried out with 2-<sup>18</sup>F-A85380, 6-<sup>18</sup>F-A85380 and 5-<sup>123</sup>I-A85380 for imaging  $\alpha 4\beta 2$  nAChRs (9–12). However, due to the slow binding kinetics of the three pyridylethers currently in human use, several efforts have been made to improve and hasten the binding kinetics (1).

Our goal has been to develop antagonists and agonists for imaging  $\alpha 4\beta 2$  nAChRs. The moderate affinity helps in accelerating the *in vivo* binding kinetics as was observed with the agonist <sup>18</sup>F-nifene, **2** (13). Replacing halogen or hydrogen at the meta-position in 3-pyridyl ethers with an alkyl group such as propyl or butyl group, reduces agonist character and increases the antagonist character (14,15). On the basis of this finding we developed nifrolidine, **3** as an antagonist based PET imaging agent (16). Initially, we had chosen to develop a moderate affinity compound by using the pyrrolidine ring in <sup>18</sup>F-nifrolidine, rather than the azetidine ring (16).

In an effort to increase target to nontarget ratios of nifrolidine, we considered replacing the pyrrolidine ring in nifrolidine with an azetidine ring. Azetidine rings have higher affinity at the  $\alpha 4\beta 2$  receptor site as evidenced by 2-<sup>18</sup>F-A85380, 6-<sup>18</sup>F-A85380 and 5-<sup>123</sup>I-A85380. We anticipated that inclusion of the azetidine ring would result in a more potent compound than nifrolidine.

We have now introduced a 3'-fluoropropyl group at the meta-position in 3-pyridyl ether linked to the azetidine ring. We report here the synthesis of 3-(2-(*S*)-azetidinylmethoxy)-5-(3'-fluoropropyl)pyridine (abbreviated name: nifzetidine, **4**), *in vitro* binding affinity at the nAChRs, radiolabeling with fluorine-18 to provide 3-(2-(*S*)-azetidinylmethoxy)-5-(3'-<sup>18</sup>F-fluoropropyl)pyridine (<sup>18</sup>F-nifzetidine), *in vitro* autoradiographic studies in rat brain slices and PET studies in rhesus monkeys with <sup>18</sup>F-nifzetidine.

## 2. Materials and Methods

All chemicals (except 1-azetidinecarboxylic acid, 2-(hydroxymethyl)-1,1-dimethylethyl ester (*S*), **5**, ChemPacific Corporation, Baltimore, MD) and solvents were obtained from Aldrich Chemical Co. and Fisher Scientific. Electrospray mass spectra were obtained on a Model 7250 mass spectrometer (Micromass LCT). Proton NMR spectra were recorded on Bruker Instruments OMEGA 500- MHz. High specific activity <sup>18</sup>F-fluoride was produced in the MC-17 cyclotron or the CTI RDS-112 cyclotron using oxygen-18 enriched water in a <sup>18</sup>O(p, n)<sup>18</sup>F nuclear reaction. All chemical reactions used high specific activity <sup>18</sup>F-fluoride. Chromatographic separations were carried out on semi-preparative reverse-phase columns using the Gilson high performance liquid chromatography (HPLC) systems. Fluorine-18 radioactivity was counted in a Capintec dose calibrator while low level counting was carried out in a well-counter (Cobra quantum, Packard Instruments Co., Boston, MA). Radioactive thin layer chromatograms were obtained by scanning in a Bioscan system 200 Imaging scanner (Bioscan, Inc., Washington, DC). Rat brain slices were obtained on a Leica 1850 cryotome. Fluorine-18 autoradiographic studies were carried out by exposing tissue samples on storage phosphor screens. The apposed phosphor screens were read by Cyclone Storage Phosphor System (Packard Instruments Co., Boston, MA). Monkey PET were

carried out using a high-resolution ECAT HR+ scanner. All animals were approved by the Institutional Animal Care and Use Committee of University of California-Irvine and Wright State University, Dayton, Ohio.

### 2.1. 3-Bromo-5-(1-*tert*-butoxycarbonyl-2-(*S*)-azetidylmethoxy)pyridine (7)

In THF (12 mL) solution of **5** (0.98 g, 5.28 mmol), 3-bromo-5-hydroxypyridine **6** (0.92 g, 5.36 mmol) and triphenylphosphine (1.71 g, 6.5 mmol) under nitrogen was cooled to 0°C and treated drop wise with diisopropyl azodicarboxylate (1.27 mL, 6.5 mmol) in THF (2 mL). The reaction mixture was stirred for 1 h at 0°C, and then allowed to warm to room temperature and continued stirring overnight. The mixture was concentrated *in vacuo*; the residue was treated with pentane/EtOAc (200 mL, 9:1) and stirred at 0°C until crystalline solid was obtained. The reaction mixture was filtered and the white solid was washed with pentane/EtOAc (9:1), (2 × 10 mL). The combined filtrate was concentrated *in vacuo* and the residue was purified by flash chromatography on silica gel (20×300 mm), eluting with 40% EtOAc in hexane. Column fractions were combined and concentrated *in vacuo* to give 3-bromo-5-(1-*tert*-butoxycarbonyl-2-(*S*)-azetidylmethoxy)pyridine **7** (1.04 g, 58%) as a pale yellow gummy oil. NMR (500 MHz; CDCl<sub>3</sub>) δ 8.28 (m, 2H), 7.43 (dd, J = 1.8, 2.6 Hz, 1H), 4.33 (m, 1H), 4.13 (m, 2H), 3.89 (m, 2H), 2.33 (m, 2H), 1.42 (s, 9H). MS, m/z 343, 345 (35%, [M + H]<sup>+</sup>), 365, 367 (80%, [M + Na]<sup>+</sup>).

### 2.2. 3-Allyl-5-(1-*tert*-butoxycarbonyl-2-(*S*)-azetidylmethoxy)pyridine (8)

3-Bromo-5-(1-*tert*-butoxycarbonyl-2-(*S*)-azetidylmethoxy)pyridine **7** (0.80 g, 2.33 mmol) in anhydrous toluene (25 mL) was treated with tetrakis(triphenylphosphine)palladium (0) (100 mg, 0.08 mmol) followed by addition of allyltri-*n*-butyltin (0.92 g, 2.77 mmol). The reaction mixture was refluxed under nitrogen atmosphere for 20 hours. The reaction mixture was then cooled to room temperature and filtered. The filtrate was evaporated to dryness. The residue was purified by silica column chromatography (25 × 300 mm) eluting with 40% EtOAc in hexane to furnish 3-allyl-5-(1-*tert*-butoxycarbonyl-2-(*S*)-azetidylmethoxy)pyridine **8** (0.49 g, 70%) as a colorless oil. NMR (500 MHz; CDCl<sub>3</sub>) δ 8.19 (d, J = 2.6 Hz, 1H), 8.08 (s, 1H), 7.07 (s, 1H), 5.94 (m, 1H, olefinic H), 5.13 (m, 2H, olefinic H), 4.31 (m, 1H), 4.12 (dd, J = 9.9, 2.8 Hz, 2H), 3.90 (m, 2H), 3.37 (d, J = 6.5 Hz, 2H), 2.32 (m, 2H), 1.42 (s, 9H). MS, m/z 305 (100%, [M + H]<sup>+</sup>), 327 (80%, [M + Na]<sup>+</sup>).

### 2.3. 3-(1-*tert*-Butoxycarbonyl-2-(*S*)-azetidylmethoxy)-5-(3'-hydroxypropyl)pyridine (9)

The olefin **8** (0.47 g, 1.54 mmol) in THF (5 mL) was treated with borane-tetrahydrofuran complex (1.5 M, 3.5 mL) by drop wise addition at 0°C and stirring under nitrogen atmosphere. The reaction mixture was then stirred at room temperature overnight and was then treated with NaOH (3 N, 3 mL) followed by addition of 30% H<sub>2</sub>O<sub>2</sub> (200 μL) at 0°C. Subsequently, this mixture was stirred at room temperature for 12 hours and then concentrated to dryness. The residue was poured into saturated ammonium chloride solution and extracted with ethyl acetate. The organic layer was dried over MgSO<sub>4</sub> and the solvent evaporated *in vacuo*. The residue was purified by preparative TLC (15% MeOH in DCM) to furnish alcohol **9** (0.33 g, 67% yield). The alcohol was obtained in 70% yield. NMR (500 MHz; CDCl<sub>3</sub>) δ 8.29 (s, 1H), 8.15 (s, 1H), 7.45 (s, 1H), 4.42 (bm, 1H), 4.21 (m, 2H), 3.93 (m, 2H), 3.73 (t, J = 6.0 Hz, 2H), 2.82 (t, J = 7.0 Hz, 2H), 2.42 (m, 2H), 1.95-1.92 (m, 2H), 1.47 (s, 9H). MS, m/z 323 (100%, [M + H]<sup>+</sup>), 345 (75%, [M + Na]<sup>+</sup>).

### 2.4. 3-(1-*tert*-Butoxycarbonyl-2-(*S*)-azetidylmethoxy)-5-(3'(4-methylbenzenesulfonyloxy)propyl)pyridine (10)

To a solution of alcohol **9** (0.30 g, 0.93 mmol) and triethylamine (0.40 mL, 2.80 mmol) in anhydrous dichloromethane (3.0 mL), *p*-toluenesulfonyl chloride (0.186 g, 0.97 mmol) was

added and the reaction mixture was stirred at room temperature for 18 hrs. The reaction mixture was then diluted with water and extracted with dichloromethane. The dichloromethane layer was washed with water, dried and concentrated. The residue was chromatographed over silica gel and eluted with ethyl acetate and hexane (1:1) gave tosylate precursor **10** (88 mg, 20% yield). (500 MHz; CDCl<sub>3</sub>) δ 8.18 (s, 1H), 7.99 (s, 1H), 7.78 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 7.34 (m, 1H), 4.31 (m, 1H), 4.13 (m, 2H), 4.04 (t, J = 6 Hz, 2H), 3.89 (m, 2H), 2.66 (t, J = 7.7 Hz, 2H), 2.45 (s, 3H), 2.34 (m, 2H), 1.98–1.89 (m, 2H), 1.41 (s, 9H). MS, m/z 477 (25%, [M + H]<sup>+</sup>), 499 (65%, [M + Na]<sup>+</sup>).

### 2.5. 3-(1-*tert*-Butoxycarbonyl-2-(*S*)-azetidylmethoxy)-5-(3'-fluoropropyl)pyridine (**11**; Method 1)

The tosylate **10** (0.23 g, 0.48 mmol) was dissolved in THF (2 mL). The solution was treated with tetrabutylammonium fluoride (TBAF), 1.0M solution in THF (1 mL, 1 mmol) and heated at 60 °C for 2 hr and cooled. The reaction mixture was concentrated to dryness *in vacuo* and the residue was then purified by flash column chromatography (silica gel DCM/MeOH, 95:5) providing **11** (95 mg, 60% yield) as a colorless oil. (500 MHz; CDCl<sub>3</sub>) δ 8.20 (d, J = 2.4 Hz, 1H), 8.10 (s, 1H), 7.11 (s, 1H), 4.50 (t of d, J = 47 Hz, 5.7 Hz, CH<sub>2</sub>-F, 2H), 4.32 (brm, 1H), 4.14 (dd, J = 9 Hz, 2.8 Hz, 2H), 3.89 (m, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.35 (m, 2H), 2.01 (m, 2H), 1.41 (s, 9H). MS, m/z 325 (38%, [M + H]<sup>+</sup>), 347 (100%, [M + Na]<sup>+</sup>).

### 2.6. 3-(1-*tert*-Butoxycarbonyl-2-(*S*)-azetidylmethoxy)-5-(3'-fluoropropyl)pyridine (**11**; Method 2)

To a solution of *N*-BOC alcohol **9** (0.19 g, 0.60 mmol) and triethylamine (250 μL) in dichloromethane (1 mL) was added drop wise a solution of DAST (75 μL) in dichloromethane (0.5 mL) at 0°C under nitrogen atmosphere. The reaction mixture was warmed to ambient temperature and kept stirring at room temperature for 16 hrs. The reaction mixture was neutralized with saturated sodium bicarbonate solution. The aqueous phase was extracted with dichloromethane, and the extracts were washed with water, dried (anhydrous magnesium sulfate), and evaporated to dryness. Column chromatography of the residue over silica gel and elution with dichloromethane:methanol (95:5) gave *N*-BOC fluoro compound **11** (52 mg, 26%). (500 MHz; CDCl<sub>3</sub>) δ 8.22 (d, J = 2.4 Hz, 1H), 8.14 (s, 1H), 7.11 (s, 1H), 4.56 (t of d, J = 47 Hz, 5.7 Hz, CH<sub>2</sub>-F, 2H), 4.35 (brm, 1H), 4.17 (dd, J = 9 Hz, 2.8 Hz, 2H), 3.88 (m, 2H), 2.75 (t, J = 7.5 Hz, 2H), 2.34 (m, 2H), 2.04 (m, 2H), 1.42 (s, 9H). MS, m/z 325 (38%, [M + H]<sup>+</sup>), 347 (100%, [M + Na]<sup>+</sup>).

### 2.7. 3-(2-(*S*)-Azetidylmethoxy)-5-(3'-fluoropropyl)pyridine (**4**)

Deprotection of *N*-BOC fluorinated derivative **11** was carried out by treatment with trifluoroacetic acid (TFA). The *N*-BOC derivative **11** (84 mg, 0.25 mmol) was dissolved in dichloromethane (4 mL) and the solution was cooled at 0 °C. The reaction solution was treated with TFA (0.6 mL) with stirring. The reaction solution was stirred at 0 °C for one hour followed by room temperature for 30 min. The reaction mixture was concentrated to dryness. The residue neutralized with 1N NaOH to pH 11 and the aqueous layer was extracted with dichloromethane, dried (MgSO<sub>4</sub>), filtered, and concentrated to give crude nifzetidine **4**. The product **4** was dissolved in diethyl ether (2 mL) and cooled to 0 °C. The solution was treated with 1M HCl in ether (2 mL) and the temperature was brought into room temperature. The solution was concentrated to dryness. The residue was triturated with diethyl ether to give light brown solid compound **4**. 2HCl was obtained (28 mg, 50%). NMR (CDCl<sub>3</sub>, 500 MHz) δ ppm: 8.21 (d, J = 2.7 Hz, 1H, ArH), 8.17 (d, J = 2.69 Hz, 1H, ArH), 7.17 (s, 1H ArH), 4.45 (t of d, J = 47 Hz, 5.8 Hz, CH<sub>2</sub>-F, 2H), 4.27 (m, 1H), 4.03 (m, 2H), 3.67 (m, 2H), 2.72 (t, J = 7.5 Hz, 2H), 2.59 (m, 2H), 1.98 (m, 2H). MS, m/z 225 (100%, [M + H]<sup>+</sup>), 247 (5%, [M + Na]<sup>+</sup>).

## 2.8. In Vitro Binding Affinity

Rat brain homogenate assays using  $^3\text{H}$ -cytisine were carried out to measure binding affinity of the compounds to  $\alpha_4\beta_2$  receptors using previously described procedures (17). The cerebrum of male Sprague-Dawley rats was isolated and homogenized using Tekmar tissumizer (15 secs, half maxima speed) in the incubation buffer 1:100 (wt:vol). This was then centrifuged at 40,000g for 10 mins and the supernatant discarded. The pellet was then resuspended in the same volume of buffer, homogenized and centrifuged again at 40,000g for 10 mins. The supernatant was then removed and the final pellet was taken up in the incubation buffer described below to a concentration of 120 – 150 mg/mL of tissue (the protein concentration in these homogenate equals 60  $\mu\text{g}/\text{mg}$  tissue). Binding assays were carried out in 50 mM Tris buffer at pH 7.0 containing 120 mM NaCl, 5 mM KCl, 1mM  $\text{MgCl}_2$  and 2.5 mM  $\text{CaCl}_2$  in the presence of 1 nM  $^3\text{H}$ -cytisine at 2 °C for 75 min incubation. The total assay volume was 0.25 mL. Different concentrations of the assay compound, nifzetidine in 0.025 mL (final concentration range  $10^{-10}$  to  $10^{-3}\text{M}$ ) was taken in 0.1 mL of buffer along with 0.025 mL of 1 nM  $^3\text{H}$ -cytisine. Nonspecific binding was measured using 300  $\mu\text{M}$  nicotine. To start the incubation, 0.1 mL of the rat brain homogenate was added into each assay tube. The incubation was terminated by rapid vacuum filtration through Whatman GF/C filter paper (presoaked in 0.1% polyethyleneimine for 60 mins) using Brandel tissue harvester. The filters were washed three times with cold buffer and the filters were suspended in 10 mL of Bio-Safe II scintillation fluid and counted for 10 mins in the scintillation counter. Data was analyzed using following procedure: (a) the non-specific binding of  $^3\text{H}$ -cytisine was subtracted for all samples; (b) the specific binding was normalized to 100% (no competitive ligand) and (c) the binding isotherms were fit to the Hill equation (KELL BioSoft software (v 6), Cambridge, U.K.). The dissociation constant,  $K_d$  for the  $^3\text{H}$ -cytisine to nicotine  $\alpha_4\beta_2$  receptor of 0.59 nM was used (18).

## 2.9. Radiosynthesis

The radiosynthesis of  $^{18}\text{F}$ -nifzetidine was conducted in the chemistry-processing computer unit (CPCU). Hydrogen  $^{18}\text{F}$ -fluoride in  $\text{H}_2^{18}\text{O}$  from the MC-17 cyclotron was passed through light QMA sep-pak (Waters Corp.), preconditioned with 3 mL of potassium carbonate, 140 mg/mL, followed by 3 mL of anhydrous acetonitrile. The trapped  $^{18}\text{F}$  in QMA was eluted with 1 mL of Kryptofix 2.2.2 (Aldrich)/potassium carbonate solution (stock solution of 360 mg Kryptofix and 75 mg potassium carbonate in 24 mL acetonitrile and 1 mL water) and transferred to the reaction vessel in the CPCU. The SYNTH1 program was used for the synthesis that involved an initial drying step of the  $^{18}\text{F}$ -fluoride, Kryptofix 2.2.2., and  $\text{K}_2\text{CO}_3$  mixture at 120°C for 10 min. The  $^{18}\text{F}$  solution was further dried with acetonitrile (2 mL) at 120 °C for 7 min. Dried  $^{18}\text{F}$  was treated with the precursor **10** (2 mg) in acetonitrile (0.5 mL). This solution was heated at 96 °C for 30 min and cooled.

Radiosynthesis of  $^{18}\text{F}$ -nifzetidine took two steps. As described above, the first step was the nucleophilic displacement of tosylate group by  $^{18}\text{F}$  ion. The crude product *N*-BOC- $^{18}\text{F}$ -nifzetidine was transferred out of CPCU using dichloromethane (5 mL). The dichloromethane containing *N*-BOC- $^{18}\text{F}$ -nifzetidine was evaporated *in vacuo* for the deprotection step. Purity of an aliquot of this intermediate *N*-BOC- $^{18}\text{F}$ -nifzetidine was performed by HPLC using an Alltech  $\text{C}_{18}$  column (10  $\mu\text{m}$ , 250 $\times$ 10 mm) and UV detector (254 nm), mobile phase: 60% acetonitrile-40% 0.1 M ammonium formate, flow rate 2.5 mL/min, r.t. = 25 min (Fig-3).

The above *N*-BOC- $^{18}\text{F}$ -nifzetidine intermediate was taken in dichloromethane (1 mL) and trifluoroacetic acid (0.2 mL). The solution was heated at 80°C for 30 min and evaporated to dryness. The residue was neutralized with 10%  $\text{NaHCO}_3$  solution to pH 6–7.

Semipreparative HPLC purification was performed using an Alltech C<sub>18</sub> column (10  $\mu$ m, 250 $\times$ 10 mm) and UV detector (254 nm), mobile phase: 60% acetonitrile-40% 0.1 M ammonium formate, flow rate 2.5 mL/min, r.t. = 17 min. The collected fraction was taken to near dryness *in vacuo*. The final formulation was carried out using approx. 5 mL of saline (0.9% NaCl INJ) followed by filtration through a membrane filter (0.22  $\mu$ m) into a sterile dose vial.

## 2.10. In Vitro Autoradiographic Studies

Horizontal brain slices (20  $\mu$ m thick) were obtained from male Sprague-Dawley rats. Sets of brain slices were preincubated in buffer (50 mmol/L Tris HCl containing 120 mmol/L NaCl, 5mmol/L KCl, 2.5 mmol/L CaCl<sub>2</sub>, 1 mmol/L MgCl<sub>2</sub>, pH 7.4) for 10 min. Subsequently, the slices were incubated with <sup>18</sup>F-nifzetidine (4  $\mu$ Ci/cc) at 37°C for 60 min. Nonspecific binding was measured in the presence of 15  $\mu$ mol and 150  $\mu$ mol of nicotine. After incubation, slides were washed twice (1 min each) with ice-cold Tris HCl buffer, pH 7.4, followed by a quick rinse in cold deionized water. The slides were then air dried and apposed to phosphor screens overnight and read by the Cyclone Phosphor Imaging System (Packard Instruments Co). The amount of bound <sup>18</sup>F-nifzetidine in the autoradiograms was evaluated in various brain regions (as digital lights units (DLU)/mm<sup>2</sup>) using the OptiQuant acquisition and analysis program (Packard Instruments Co).

## 2.11. PET Imaging

Rhesus monkeys (10 kg) were anesthetized using ketamine (10 mg/kg) and xylazine (0.5 mg/kg) and was subsequently maintained on 1–1.5% isoflurane. Two intravenous catheters were placed, one on each arm, for the purpose of administration of <sup>18</sup>F-nifzetidine and for obtaining blood samples during the study. The head of the animal was placed in the gantry of an ECAT EXACT HR+ PET scanner and positioned in place with adhesive tape as described previously (16). A transmission scan using a <sup>68</sup>Ge/<sup>68</sup>Ga rod source was acquired before administration of <sup>18</sup>F-nifzetidine to correct for tissue attenuation of the coincident radiation. A dynamic sequence of scans for a total of approximately 180 min was acquired in the 3- dimensional mode immediately after administering approximately 3.5–4 mCi (mass injected, <0.2  $\mu$ g of nifzetidine) of <sup>18</sup>F-nifzetidine intravenously. Data in the final form were expressed in units of the percentage injected dose per milliliter (%ID/ml) or kilobecquerels per milliliter (kBq/mL). Areas showing maximal <sup>18</sup>F-nifzetidine binding in the mediodorsal thalamus, ventrolateral thalamus, temporal cortex, occipital cortex, frontal cortex, and cerebellum were delineated in the images. Time-activity curves were obtained for all these regions.

## 3. Results

### 3.1. Chemistry

We followed similar chemistry reported previously for nifrolidine starting from 3,5-dibromopyridine (16). The synthesis route to prepare nifzetidine **4** is shown in Fig-2. Starting with 3-bromo-5-methoxypyridine (available from Aldrich Chemical Co.), 3-bromo-5-pyridinol **6** was prepared in 80% yield by refluxing with 48% hydrogen bromide (16). The 3-bromo-5-pyridyl ether compound **7** was synthesized in 58% yield by etherification of **6** with 1-azetidincarboxylic acid, 2-(hydroxymethyl)-1,1-dimethylethyl ester (*S*) **5** under Mitsunobu conditions (19). The coupling between **5** and **6** took place in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD). The DIAD is more stable and lower in cost compared to diethyl azodicarboxylate (DEAD). The hydrated DIAD adduct can be easily eliminated as a crystalline salt, simplifying isolation of the product from the reaction mixture. The next step was how to introduce 3'-fluoropropyl group at 5 positions of 3-pyridyl ethers. The bromo compound **7** was heated under reflux

with allyltributyltin in toluene in the presence of catalytic amount of tetrakis(triphenylphosphine)palladium (20) catalyst; the reaction went smoothly to give allylpyridyl ether derivative **8** in 70% yield. The olefin **8** was treated with  $\text{BH}_3\cdot\text{THF}$  and subsequently oxidized with  $\text{NaOH}/\text{H}_2\text{O}_2$  to furnish the alcohol in 35% yield. We have improved the yield of alcohol **9** from 35% to 67% by stirring the reaction mixture for longer time (12 hrs) before and after adding  $\text{NaOH}/\text{H}_2\text{O}_2$ . The key intermediate alcohol **9** was reacted with *p*-toluenesulfonyl chloride in the presence of pyridine gave a complex mixture. On the other hand, with triethylamine the reaction was cleaner and gave pure tosylate labeling precursor **10** in 20–30% isolated yield. Synthesis of nifzetidine was carried out either from the alcohol or tosylate precursor. Reaction of alcohol **9** with diethylaminosulfur trifluoride (21) (DAST) was investigated, but the *N*-BOC-fluoride **11** was produced only as a minor component of a complex mixture. Alternatively, the compound **11** was prepared in good yield from **10** by the reaction with TBAF.THF (22) suggesting it to be a superior fluorination approach than DAST for these derivatives. Removal of the *N*-BOC group was achieved by treating with TFA at room temperature to provide **4** in 50% yield. Final product **4**, nifzetidine, was used as a hydrochloride salt for in vitro binding assays. The tosylate precursor **10** and salt of Nifzetidine.2HCl **4** were stored at 0 °C to –20 °C.

### 3.2. In Vitro Binding Affinity

In vitro binding affinity of nifzetidine to nicotine  $\alpha 4\beta 2$  receptor was determined using  $^3\text{H}$ -cytisine in rat brain homogenate. The agonist  $^3\text{H}$ -cytisine binds to  $\alpha 4\beta 2$  receptor sub-type and displacement of  $^3\text{H}$ -cytisine binding in the rat brain homogenate suggest that the nifzetidine does indeed have the specificity to the  $\alpha 4\beta 2$  receptor sub-type. Nifzetidine bound to single binding site with a  $K_i$  value of 0.67 nM.

### 3.3. Radiochemistry

Our initial validation studies confirmed *N*-BOC- $^{18}\text{F}$ -nifzetidine to be the major radiochemical product (Fig-3A). The final radiolabeled product  $^{18}\text{F}$ -nifzetidine was obtained in >99% purity, assessed by HPLC. Radiochemical yields of  $^{18}\text{F}$ -nifzetidine were 20–40% range decay corrected, and specific was approx. 2000 Ci/mmol. For routine studies, to reduce the production time only one final step HPLC purification (Fig-3B) was done after completion of *N*-BOC deprotection and the radiosynthesis took approximately 2 hrs.

### 3.4. In Vitro Autoradiographic Studies

As seen in Fig-4A, *in vitro* autoradiography in horizontal rat brain slices indicated selective high binding of  $^{18}\text{F}$ -nifzetidine in the thalamus (TH) as well as extrathalamus regions such as cortex (CO), striata (STR), subiculum (SUB), cerebellum (CB) and other regions consistent with  $\alpha 4\beta 2$  receptor distribution. The anteroventral thalamic nucleus (AVT) exhibited the highest amount of binding. Subiculum also exhibited significant amounts of  $^{18}\text{F}$ -nifzetidine binding, which is known to contain  $\alpha 4\beta 2$  receptors sites. Cerebellum showed some binding and was used as a reference region. Displacement experiments were carried out with different concentrations of nicotine. Nicotine at 15  $\mu\text{M}$  ( Fig-4B) was able to partially displace the selective binding in various brain regions while 150  $\mu\text{M}$  nicotine (Fig-4C) in these brain regions displaced >85% of  $^{18}\text{F}$ -nifzetidine (Fig-4D). The ratios of various brain regions with respect cerebellum were: TH/CER = 12; SUB/CER = 5; STR/CER = 4; COR/CER = 3.7.

### 3.5. Monkey PET Imaging

After intravenous administration of  $^{18}\text{F}$ -nifzetidine, vital signs of the monkeys did not exhibit any unusual derivation from baseline values. PET imaging study revealed a maximal uptake of 0.01%ID/cc in thalamic regions. The results indicated a significant amount of

binding in the thalamic regions in all three sections axial, sagittal and coronal planes (Fig-5a-c). Maximal brain uptake of  $^{18}\text{F}$ -nifzetidine was in thalamus (Fig-5f) and lateral geniculate (Fig-5h,i). Regions outside the thalamus (extrathalamic) were also observed. Frontal cortex (Fig-5c), striatum (Fig-5d), temporal cortex (Fig-5i) showed significant amounts of binding. Cerebellum showed the lowest amount of binding (Fig-5c).

The  $^{18}\text{F}$ -nifzetidine uptake was very slow in various brain regions of rhesus monkey (Fig-6A). Highest uptake of  $^{18}\text{F}$ -nifzetidine was seen in the thalamus and lower uptake in cortical areas such as frontal cortex. Cerebellum had the least amount of uptake as seen in Fig-6A and was used as the reference region. A high level of specific binding was achieved at 2 hours after administration, with a thalamus to cerebellum ratio of 2.4 at 3 hrs postinjection. Ratios of  $^{18}\text{F}$ -nifzetidine continued to rise beyond 3 hrs in the thalamus, indicating slow kinetics. Lateral geniculate to cerebellum ratios was  $>2$  at 3 hrs postinjection. Ratios of striatal and cortical regions with respect to cerebellum were 1.3–1.6 (Fig-6B).

#### 4. Discussion

A series of 3-pyridyl ethers with subnanomolar affinity for the major  $\alpha 4\beta 2$  subtype of central nAChRs were synthesized by Abbott laboratories. The 3-pyridyl ethers selectively bind to  $\alpha 4\beta 2$  receptors unlike epibatidine analogs which bind also to  $\alpha 3\beta 2$  and  $\alpha 3\beta 4$  receptors. Several agonist-based radioligands have been explored in the literature for imaging  $\alpha 4\beta 2$  receptors (13, 23). Here we focused our attention towards antagonist-based imaging agents with faster binding kinetics. By placing alkyl groups at 5 positions of 3-pyridyl ethers, agonist character is dramatically decreased (15,16). Based on this approach we previously developed antagonist  $^{18}\text{F}$ -nifrolidine (16), which exhibited reasonable binding kinetics and selectivity for  $\alpha 4\beta 2$  receptors. In an attempt to increase binding affinity and enhance target-to-nontarget ratios, we focused our attention towards nifzetidine which carries azetidene ring. The structure of nifzetidine is similar to that of azetidinylother halogenated analog 2-FA85380 except for an “n-3'-fluoropropyl” at 5-position and lacking the ring fluorine. We accomplished synthesis of nifzetidine by modifying the synthesis of the pyridylethers (16). We used a similar approach for the synthesis of nifzetidine using 1-azetidinecarboxylic acid, 2-(hydroxymethyl)-1,1-dimethylethyl ester (*S*) for the Mitsunobu coupling. We successfully introduced 3'-fluoropropyl group at 5-position via allylation of 5-bromo-3-pyridyl ether compound with an allylating agent allyltri-n-butyltin, hydroboration of the corresponding double bond and conversion of alcohol to fluoride. The corresponding alcohol was the key intermediate for making the precursor for  $^{18}\text{F}$ -nifzetidine. The synthetic approach for preparation of the unlabeled nifzetidine was carried out with diethylaminosulfur trifluoride (DAST) as the fluorinating agent. This was the first approach to make unlabeled nifzetidine directly from the intermediate alcohol **9**. But the DAST reaction did not give satisfactory results. Finally, Nifzetidine was prepared from tosylate **10** by reacting with TBAF followed by deprotection at room temperature. The reaction yields were good to moderate. Nifzetidine was prepared as HCl salt for *in vitro* binding studies.

The binding affinity of nifzetidine is comparable to our previously reported nifrolidine and nifene (Table-1). The four membered ring structure in nifzetidine was expected to significantly influence the binding affinity, since azetidinylother derivatives (e.g., 2- $^{18}\text{F}$ FA85380) have high affinity ( $<100$  pM, 15–19). However, nifzetidine was found only to be marginally better than nifrolidine in brain homogenate binding assays suggesting that other factors may be involved in this class of fluoropropyl derivatives.

Rat brain autoradiographic studies with  $^{18}\text{F}$ -nifzetidine were similar to our previous observations with  $^{18}\text{F}$ -nifrolidine (16). Nicotine displaced binding of  $^{18}\text{F}$ -nifzetidine in a



dose dependent manner in all brain regions (Fig-4). Brain uptake of  $^{18}\text{F}$ -nifzetidine in the monkey brain was slow, although eventual distribution of the radiotracer was consistent with the distribution of  $\alpha 4\beta 2$  receptors. This slow uptake is seen in the time-activity curves for the various brain regions as shown in Fig-6A. The time-activity curves showed the maximum binding in the thalamus. Thalamus to cerebellum ratio was about 1.7 for  $^{18}\text{F}$ -nifrolidine at 2 hours post-injection whereas thalamus to cerebellum ratio was about 2.1 for  $^{18}\text{F}$ -nifzetidine at 2 hours post-injection (Fig-6B). The kinetic profile of  $^{18}\text{F}$ -nifzetidine was slower in all brain regions compared to  $^{18}\text{F}$ -nifrolidine and pseudoequilibrium did not occur during the 180 min period of the PET scan. This behavior of  $^{18}\text{F}$ -nifzetidine seems to be similar to the slow kinetics observed with other azetidone-based radiotracers, such as  $^{18}\text{F}$ -2FA85380 (24).

On the other hand  $^{18}\text{F}$ -nifene showed fast in vivo kinetics, rapid uptake and cleared rapidly from various brain regions (13).  $^{18}\text{F}$ -Nifene has a unique “3,4-dehydropyrrolidine” ring system which provides for its unique imaging properties. In order to take advantage of this unique ring system we have replaced the “pyrrolidine” ring in nifrolidine (16) with “3,4-dehydropyrrolidine” and have developed  $^{18}\text{F}$ -nifrolene (25, 26; manuscript in preparation).

## 5. Conclusions

$^{18}\text{F}$ -Nifzetidine is a high affinity antagonist and has potential as a PET agent for imaging the  $\alpha 4\beta 2$  nAChR. Uptake was seen in thalamus and extrathalamic regions which is consistent with  $\alpha 4\beta 2$  receptor distribution. In vivo imaging studies showed that  $^{18}\text{F}$ -nifzetidine has slow kinetics which may limit its use as PET radioligand for quantitative studies of the  $\alpha 4\beta 2$  nAChR with PET.

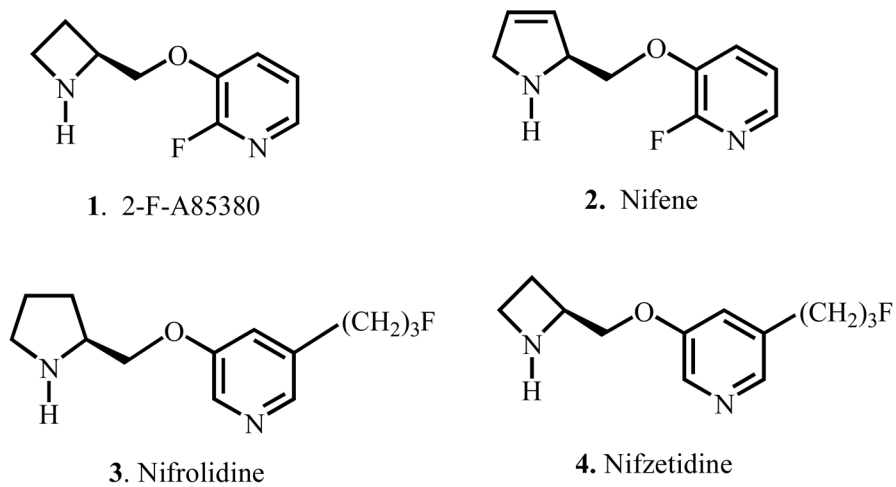
## Acknowledgments

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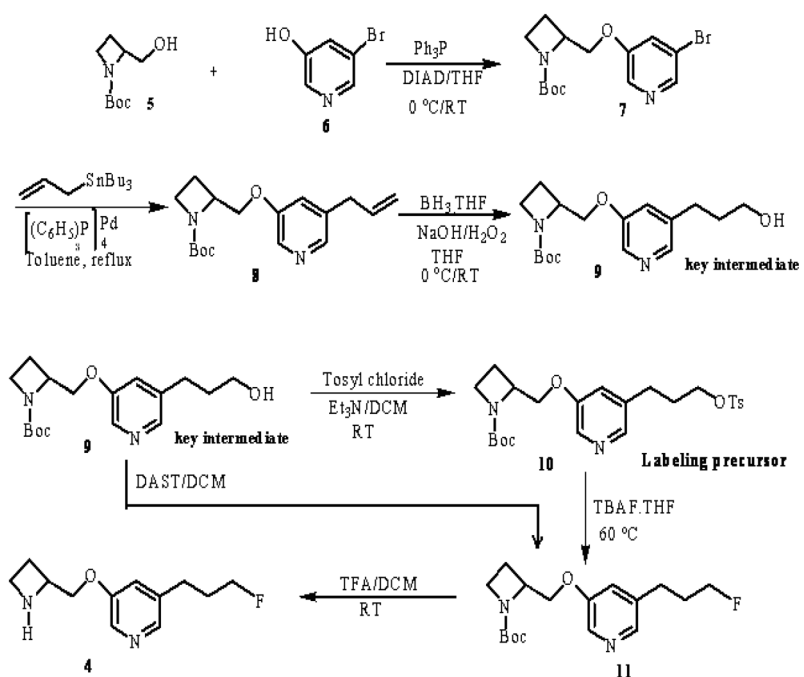
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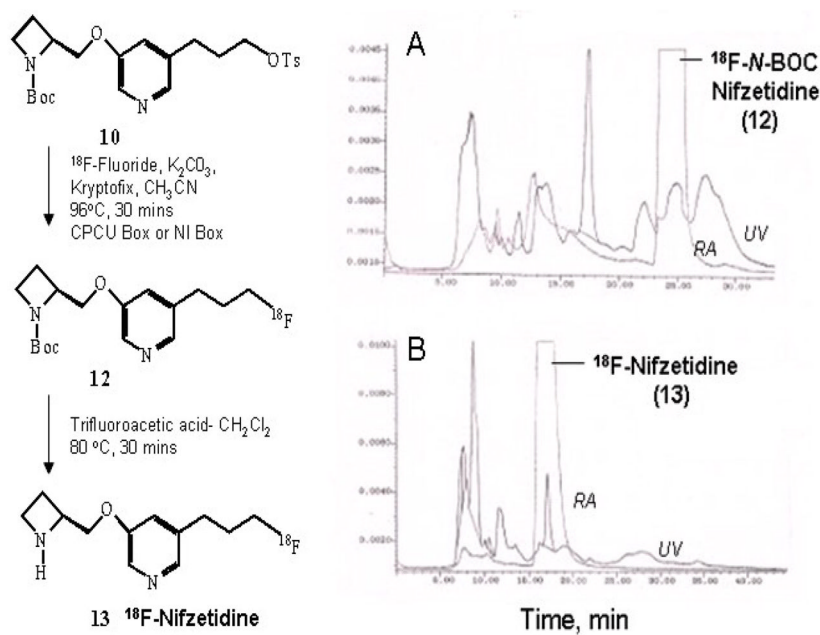
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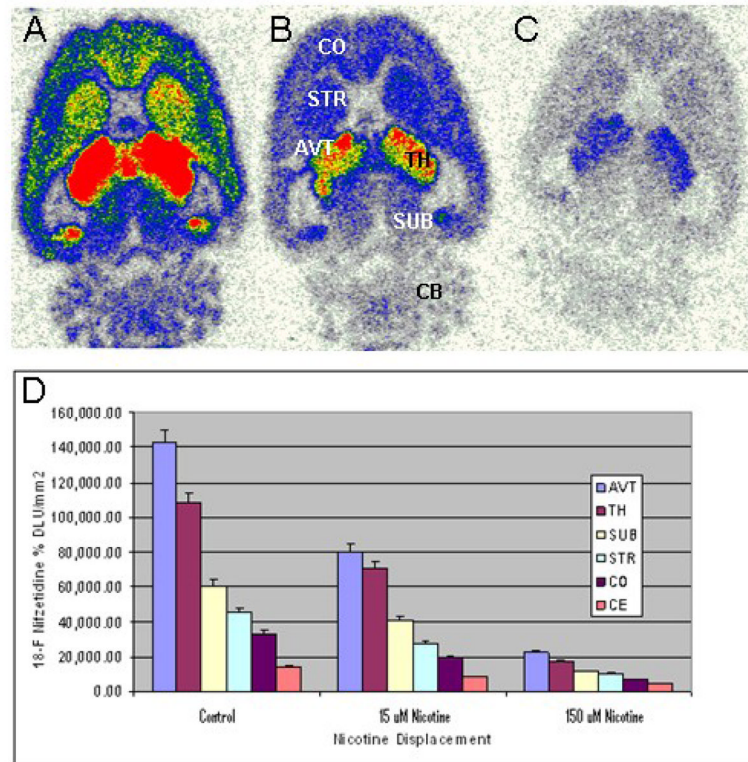
**Figure-1.**  
Chemical Structures of  $\alpha 4\beta 2$  radioligands: (1). 2-F-A85380 ; (2). Nifene; (3). Nifrolidine;  
(4). Nifzetidine (this work).



**Figure-2.** Synthesis scheme for 3-(2-(*S*)-Azetidylmethoxy)-5-(3'-fluoropropyl)pyridine, **4** and labeling tosylate precursor, **10**.

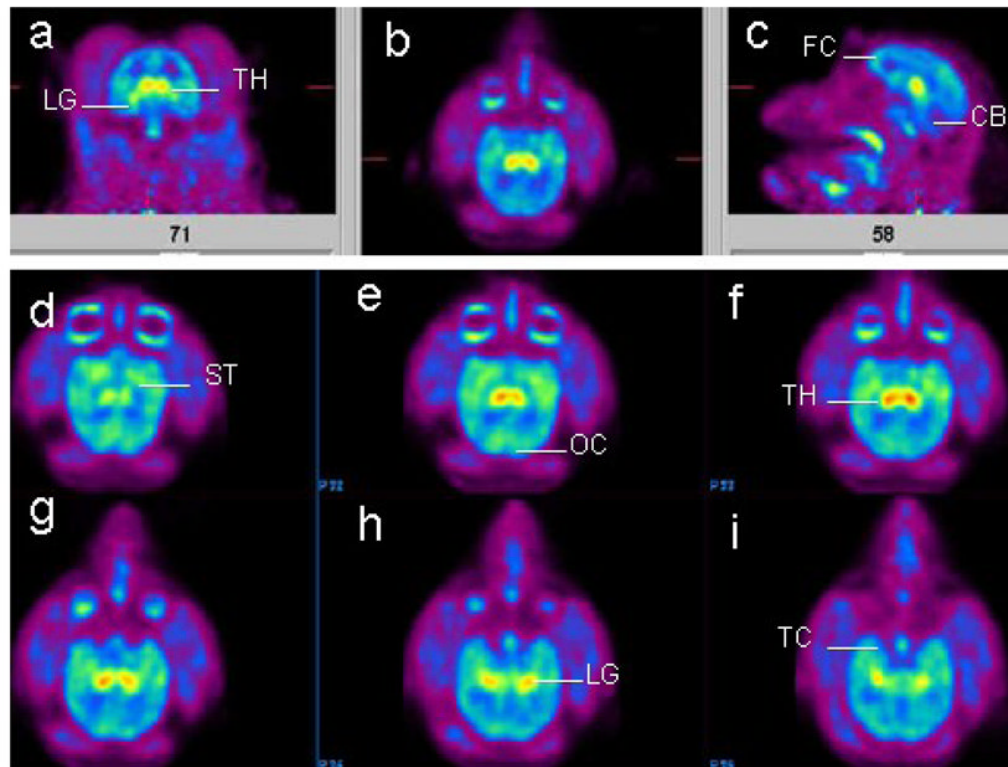


**Figure-3.** Reaction scheme showing the radiosynthesis of  $^{18}\text{F}$ -N-BOC-nifzetidine and  $^{18}\text{F}$ -nifzetidine. (A) Radioactivity and UV trace at 254 nm of HPLC analysis of  $^{18}\text{F}$ -N-BOC-nifzetidine intermediate using  $\text{C}_{18}$  reverse-phase semi-preparative column eluted with 60% acetonitrile-40% 0.1M ammonium formate at a flow rate of 2.5 mL/min. Retention of  $^{18}\text{F}$ -N-BOC-nifzetidine was found to be 24.5 min. (B). Radioactivity and UV trace at 254 nm of HPLC purification of  $^{18}\text{F}$ -nifzetidine after two-step radiosynthesis using  $\text{C}_{18}$  reverse-phase semi-preparative column eluted with 60% acetonitrile-40% 0.1M ammonium formate at a flow rate of 2.5 mL/min. Retention of  $^{18}\text{F}$ -nifzetidine was found to be 17.5 min.

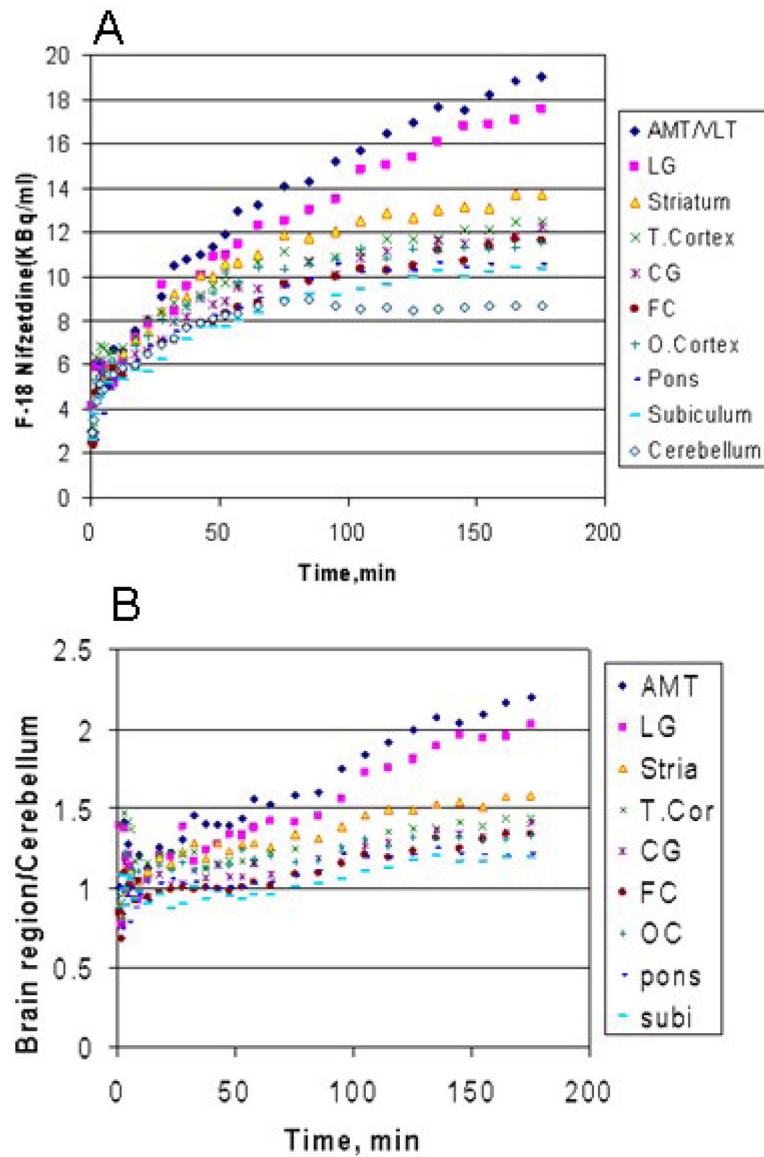


**Figure-4.**

In vitro autoradiographic studies of  $^{18}\text{F}$ -nifzetidine in rat brain slices. Binding of  $^{18}\text{F}$ -nifzetidine in 20  $\mu\text{m}$  horizontal slices (4  $\mu\text{Ci/cc}$  at 37  $^{\circ}\text{C}$ . Horizontal brain slices of rat brain showing binding of  $^{18}\text{F}$ -nifzetidine (red = highest binding and white = lowest binding). (A). total binding (CO: frontal cortex; STR: striatum; AVT: anteroventral thalamus; TH: thalamus; SUB: subiculum; CB: cerebellum). (B). binding in the presence of 15  $\mu\text{M}$  nicotine showing partial displacement of  $^{18}\text{F}$ -nifzetidine. (C). binding in the presence of 150  $\mu\text{M}$  nicotine showing >80 displacement of  $^{18}\text{F}$ -nifzetidine. (D). Plot showing amount of  $^{18}\text{F}$ -nifzetidine binding in brain regions.



**Figure-5.** Distribution of  $^{18}\text{F}$ -nifzetidine in the rhesus monkey brain. Summed PET images (100–200 mins) showing binding of  $^{18}\text{F}$ -nifzetidine in select brain slices (a-c: orthogonal planes; d-i: horizontal planes). Brain regions include thalamus (TH), lateral geniculate (LG), frontal cortex (FC), cerebellum (CB), striatum (ST), temporal cortex (TC), occipital cortex (OC).



**Figure-6.** (A). Time-activity curves of the binding of  $^{18}\text{F}$ -nifzetidine in select areas of the monkey brain corresponding to the regions identified in Fig-5 (AMT/VLT: anteriomedial thalamus/ventrolateral thalamus; CG: cingulate gyrus; subi: subiculum) (B). Ratio plot of brain regions to cerebellum.



**Table I**Binding Affinity at Nicotinic  $\alpha 4\beta 2$  Receptors

<b>Drug</b>	<b>K<sub>i</sub><sup>a</sup>, nM</b>
Nicotine	2.83
Nifene	0.50
Nifrolidine	0.80
Nifzetidine	0.67

<sup>a</sup> Binding assay with <sup>3</sup>H-cytisine in rat brain homogenates using reported methods (Pabreza et al., 1991);

<sup>b</sup> K<sub>i</sub> calculated from IC<sub>50</sub> using Cheng-Prusoff equation (1973) and K<sub>d</sub> = 0.59 nM for <sup>3</sup>H-cytisine.