Published in final edited form as:

Nucl Med Biol. 2013 January ; 40(1): 117–125. doi:10.1016/j.nucmedbio.2012.09.009.

Nicotinic $\alpha 4\beta 2$ receptor imaging agents. Part IV. Synthesis and Biological Evaluation of 3-(2-(S)-3,4-dehydropyrrolinylmethoxy)-5-(3'- ^{18}F -Fluoropropyl)pyridine (^{18}F -Nifrolene) using PET

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Abstract

Imaging agents for nicotinic $\alpha 4\beta 2$ receptors in the brain have been underway for studying various CNS disorders. Previous studies from our laboratories have reported the successful development of agonist, ^{18}F -nifene. In attempts to develop potential antagonists, ^{18}F -nifrolidine and ^{18}F -nifzetidine were previously reported. Further optimization of these fluoropropyl derivatives has now been carried out resulting in 3-(2-(S)-3,4-dehydropyrrolinylmethoxy)-5-(3'- ^{18}F -Fluoropropyl)pyridine (nifrolene) as a new high affinity agent for nicotinic $\alpha 4\beta 2$ receptors. Nifrolene in rat brain homogenate assays—labeled with ^3H -cytisine—exhibited a binding affinity of 0.36 nM. The fluorine-18 analog, ^{18}F -nifrolene, was synthesized in approximately 10–20% yield and specific activity was estimated to be >2000 Ci/mmol. Rat brain slices indicated selective binding to anterior thalamic nuclei, thalamus, subiculum, striata, cortex and other regions consistent with $\alpha 4\beta 2$ receptor distribution. This selective binding was displaced >90% by 300 μM nicotine. Thalamus to cerebellum ratio (>10) was the highest for ^{18}F -nifrolene with several other regions showing selective binding. In vivo rat PET studies exhibited rapid uptake of ^{18}F -nifrolene in the brain with specific retention in the thalamus and other brain regions while clearing out from the cerebellum. Thalamus to cerebellum ratio value in the rat was >4. Administration of nicotine caused a rapid decline in the thalamic ^{18}F -nifrolene suggesting reversible binding to nicotinic receptors. PET imaging studies of ^{18}F -nifrolene in anesthetized rhesus monkey revealed highest binding in the thalamus followed by regions of the lateral cingulate and temporal cortex. Cerebellum showed the least binding. Thalamus to cerebellum ratio in the monkey brain was >3 at 120 min. These ratios of ^{18}F -nifrolene are higher than measured for ^{18}F -nifrolidine and ^{18}F -nifzetidine. ^{18}F -Nifrolene thus shows promise as a new PET imaging agent for $\alpha 4\beta 2$ nAChR.

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Keywords

Rat PET; Monkey PET; Nifene; Fluorine-18

1. Introduction

Nicotinic $\alpha 4\beta 2$ receptors (nAChR) are acetylcholine gated ion channels that are widely distributed in the human and nonhuman brain [1]. Nicotine and acetylcholine have a high affinity for the $\alpha 4\beta 2$ receptor (1.68 nM and 19.2 nM, respectively; [2]). The receptor therefore plays an important role in tobacco dependence [3–6]. Cholinergic mechanisms via the $\alpha 4\beta 2$ receptor have also been implicated in a number of disorders such as Alzheimer's disease, Parkinson's disease and schizophrenia [7,8].

Nicotine analogs, epibatidine analogs and 3-pyridyl ether compounds are nAChR probes for noninvasive in vivo studies. There are several reports in literature describing PET and SPECT radioligands [9–11]. Numerous analogs of epibatidine and 3-pyridyl ethers have been radiolabeled with ^{11}C , ^{18}F , ^{76}Br and ^{123}I . Because of toxicity issues, the epibatidine derivatives were not pursued further for human studies. Pyridyl ether compounds exhibited subnanomolar affinity and high selectivity for the $\alpha 4\beta 2$ receptors [12]. A number of PET tracers with 3-pyridyl ethers have been investigated in an effort to optimize in vivo imaging properties [13, 14]. Human studies have been demonstrated with radioligands such as 2- ^{18}F -A-85380 26- ^{18}F A and 5- ^{123}I -A85380 for the $\alpha 4\beta 2$ receptors [15–19]. Among these agents, 2- ^{18}F -A-85380 is most commonly used in human studies [20,21].

Recently, we have developed Nifene **3** as a potential PET agent for imaging $\alpha 4\beta 2$ receptors [22]. Nifene, which contains a 3,4-dehydroproline ring system, exhibited selective binding to the receptors in the rat brain and was displaced by nicotine in vivo [23]. The selectivity of ^{18}F -nifene binding was further confirmed in $\beta 2$ -knock out mice [24]. In the rhesus monkeys, ^{18}F -Nifene demonstrated rapid uptake, fast kinetics in receptor-rich regions (60 mins) and cleared rapidly from the various brain regions [25]. These properties of ^{18}F -Nifene make it a suitable $\alpha 4\beta 2$ agonist radiotracer for human studies.

Our goal now has been to further optimize putative antagonists for imaging $\alpha 4\beta 2$ nicotinic receptors. Replacing halogen or hydrogen at the 5 position in 3-pyridyl ethers with an alkyl group such as propyl or butyl group, causes the agonist character to dramatically reduce and increases the antagonist character [26,27]. Initially, we chose the moderate affinity compound containing the pyrrolidine ring (^{18}F -nifrolidine, **4**). Pseudoequilibrium in the monkeys was achieved in the thalamus between 120–150 mins postinjection of ^{18}F -nifrolidine [28]. Subsequently, to evaluate the effect of azetidine ring (found in 2-F-A85380, **2**) on both affinity and in vivo kinetics, ^{18}F -nifzetidine, **5** was developed [29]. The in vivo kinetics of ^{18}F -nifzetidine were found to be significantly slower than ^{18}F -nifrolidine, suggesting its similarity to 2-F-A85380 [10] binding kinetics and required >3 hours of imaging time.

Since both ^{18}F -nifrolidine and ^{18}F -nifzetidine exhibited slower in vivo kinetics in the brain compared to ^{18}F -nifene it was envisaged that the relatively faster kinetics of ^{18}F -nifene is likely due to the presence of the 3,4-dehydroproline unique ring system. Therefore our design for the new putative antagonist included the replacement of the pyrrolidine ring (in nifrolidine) or azetidine ring (in nifzetidine) with 3,4-dehydroproline. The new radiotracer resulting from this change, 3-(2-(*S*)-3,4-dehydropyrrolinylmethoxy)-5-(3'-fluoropropyl)pyridine, Fig-1, **6** (nifrolene) was developed. We anticipated that this new

tracer might have faster in vivo binding kinetics compared to ^{18}F -nifrolidine and ^{18}F -nifzetidine.

Reported here is the synthesis of nifrolene, radiolabeling of ^{18}F -nifrolene, in vitro binding affinity, autoradiographic studies in rat brain slices, PET studies in rats and PET imaging studies in rhesus monkey.

2. Materials and Methods

All chemicals and solvents were obtained from Sigma-Aldrich Chemical Co. and Fisher Scientific. *N*-Boc-L-3,4-dehydroproline was purchased from CNH Technologies, Inc., Woburn, MA and was used to prepare (*S*)-*N*-*tert*-butoxycarbonyl-2-hydroxymethyl-3-pyrroline **7** as previously reported [22]. 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 ^{18}F -fluoride was produced in the MC-17 cyclotron or the CTI RDS-112 cyclotron using oxygen-18 enriched water target (^{18}O to ^{18}F using p, n reaction). The high specific activity ^{18}F -fluoride was used in subsequent reactions. Chromatographic separations were conducted 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 chromatographs 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 done by exposing tissue samples on storage phosphor screens. The apposed phosphor screens were read by Cyclone Storage Phosphor System (Packard Instruments Co., Boston, MA). Inveon preclinical Dedicated PET (Siemen's Inc) was used for the MicroPET studies which has a resolution of 1.45 mm [30]. *In vivo* and *ex vivo* images of rat brains were obtained using the Inveon MicroPET scanner and analyzed using Acquisition Sinogram Image Processing (ASIPRO) and Pixelwise Modeling Software (PMOD) softwares. 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 Chemistry

2.1.1. 3-Bromo-5-(1-*tert*-butoxycarbonyl)-2-(*S*)-3,4-dehydropyrrolinylmethoxy)pyridine (9**)**—To a mixture of alcohol **7** (1.49 g, 7.48 mmol), phenol **7** (1.52 g, 8.73 mmol) and triphenylphosphine 2.71 g, 10.33 mmol) in tetrahydrofuran (THF, 30 mL) was treated with a solution of diisopropyl azodicarboxylate (DIAD, 2.2 mL, 11.34 mmol) in THF (3 mL) at 0°C under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 16 hrs. The solvent was removed by rotary evaporation and the crude product was treated with dichloromethane (DCM, 3 × 10 mL), stirred well, and then the solution was filtered and the filtrate was concentrated to dryness. The residue was purified by column chromatography using 1:1 mixture of ethyl acetate and hexane. The compound **9** was obtained as yellow oil (2.28 g, 86%). NMR (500 MHz; CDCl_3) δ 8.27 (m, 1H), 8.23 (d, $J = 2.15$ Hz, 1H), 7.38 (m, 1H), 5.88 (m, 2H, olefinic H), 4.80 (m, 1H), 4.35-3.97 (m, 4H), 1.50 and 1.47 ($2 \times s$, 9H, ^tBu). MS, m/z 377 (65%, $[\text{M} + \text{Na}]^+$), 379 (63%, $[\text{M} + \text{Na}]^+$).

2.1.2. 3-Allyl-5-(1-*tert*-butoxycarbonyl)-2-(*S*)-3,4-dehydropyrrolinylmethoxy)pyridine (10**)**—The bromo compound **9** (2.2 g, 6.21 mmol) was treated with allyltri-*n*-butyltin (5.5 mL, 17 mmol) in anhydrous toluene (70 mL)

under nitrogen atmosphere. The solution was stirred at room temperature for 5 min followed by the addition of tetrakis(triphenylphosphine)palladium (0) (80 mg) under the nitrogen atmosphere. The reaction mixture was heated under reflux for 17 hrs and cooled to room temperature. The TLC showed the reaction did not complete. So the additional amount of allyl-n-butyltin (1 mL) and palladium catalyst (33 mg) was added to the reaction mixture. The reaction mixture was heated under reflux for 12 hrs and cooled to room temperature. The reaction mixture was filtered and evaporated to dryness. The residue was purified by flash column chromatography with 1:1 ethyl acetate and hexane to furnish the olefin **10** as oil (0.90 g, 45% yield). NMR (600 MHz; CDCl₃) δ 8.22 (m, 1H), 8.19 (d, *J* = 1.5 Hz, 1H), 7.06 (m, 1H), 5.85 (m, 3H, olefinic H), 5.30 (m, 2H), 4.84 (m, 1H), 4.14 (m, 2H), 4.12 (m, 2H), 3.36 (t, *J* = 6.36 Hz, 2H), 1.46 and 1.26 (2xs, 9H, ^tBu). MS, *m/z* 317 (77%, [M + H]⁺), 339 (100%, [M + Na]⁺).

2.1.3. 3-(1-*tert*-butoxycarbonyl-2-(*S*)-3,4-dehydropyrrolinylmethoxy)-5-(3'-hydroxypropyl)pyridine (11)—The olefin **10** (0.84 g, 2.67 mmol) in THF (5 mL) was treated with a solution of diborane.THF (3 mL, 4.4 mmol, 1.5 M) at 0°C with stirring. The reaction mixture was stirred at ice temperature for 45 min and room temperature for 45 min. The reaction mixture was cooled at 0°C and then treated with 3N sodium hydroxide (6 mL) followed by the addition of 30% hydrogen peroxide (300 μ L). The reaction mixture was stirred at room temperature for overnight. The reaction mixture was concentrated to dryness. The residue was extracted with ethyl acetate. The organic layer was dried over anhydrous MgSO₄ and concentrated to dryness. The residue was purified by flash column chromatography over silica gel using 50–75% ethyl acetate in hexane. The pure alcohol **11** was isolated as colorless oil (0.22 g, 25%). NMR (500 MHz; CDCl₃) δ 8.10-8.05 (m, 2H, ArH), 7.47 (m, 1H, ArH), 5.90 (m, 2H, olefinic H), 4.79 (m, 1H), 4.43-4.18 (m, 2H, CH₂-O), 4.10 (m, 2H, CH₂-N), 3.66 (brm, 2H, CH₂-OH), 2.78 (m, 2H, Ar-CH₂), 1.88 (m, 2H, CH₂-CH₂-CH₂), 1.48 (s, 9H, ^tBu). MS, *m/z* 335 (58%, [M + H]⁺), 357 (30%, [M + Na]⁺).

2.1.4. 3-(1-*tert*-butoxycarbonyl-2-(*S*)-3,4-dehydropyrrolinylmethoxy)-5-(3'-(4-methylsulfonyloxy)-propyl)pyridine (12)—The corresponding alcohol **11** (167 mg, 0.50 mmol) was treated with tosyl chloride (0.13 g, 0.69 mmol) in dichloromethane (5 mL) in the presence of triethylamine (0.2 mL, 1.43 mmol) at room temperature. The reaction solution was stirred at room temperature under nitrogen atmosphere for 16 hours. The reaction mixture was monitored by TLC. The reaction mixture was quenched with little water (1 mL) and extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was purified by flash column chromatography over silica gel using solvent system from 25% to 50% ethyl acetate in hexane to furnish the precursor **12** in pure form (98 mg, 42%). NMR (500 MHz; CDCl₃) δ 8.08 (m, 1H, ArH), 7.94 (m, 1H, ArH), 7.78 (d, *J* = 8 Hz, 2H, ArH), 7.37 (d, *J* = 8 Hz, 2H, ArH), 7.32 (m, 1H, ArH), 5.86 (m, 2H, olefinic H), 4.82 (m, 1H), 4.36-4.21 (m, 2H), 4.05 (m, 2H), 2.69 (m, 2H, Ar-CH₂), 2.46 (s, 3H), 1.97 (m, 2H, C-CH₂), 1.47 (s, 9H, ^tBu). MS, *m/z* 489 (98%, [M + H]⁺), 511 (92%, [M + Na]⁺).

2.1.5. 3-(1-*tert*-butoxycarbonyl-2-(*S*)-3,4-dehydropyrrolinylmethoxy)-5-(3'-Fluoropropyl)pyridine (13)—The above tosylate **12** (41 mg, 0.08 mmol) in THF (1 mL) was treated with a solution of tetrabutylammonium fluoride (TBAF 1.0 M in THF, 126 μ L, 0.12 mmol). The solution was heated at 75°C for 1.5 hrs. The reaction mixture was cooled to room temperature. The reaction mixture was concentrated to dryness. The residue was triturated with ether (3 $\times$ 5 mL) three times until the TLC showed no product in the residue. The ether solution was concentrated to dryness. The residue was purified by silica gel column chromatography (1:1 hexane and ethyl acetate) to furnish N-BOC-nifrolene **13** (16 mg, 57%). NMR (500 MHz; CDCl₃) δ 8.16 (m, 1H, ArH), 8.07 (m, 1H, ArH), 7.03 (m, 1H,

ArH), 5.90 (m, 2H, olefinic H), 4.84 (m, 1H), 4.49 (dt, $J = 47.10$ Hz, 5.85 Hz, 2H, $\text{CH}_2\text{-F}$), 4.36 (m, 2H), 4.13 (m, 2H), 2.72 (m, 2H, Ar- CH_2), 2.06 (m, 2H), 1.47 (s, 9H, ^tBu). MS, m/z 337 (70%, $[\text{M} + \text{H}]^+$), 359 (30%, $[\text{M} + \text{Na}]^+$).

2.1.6. 3-(2-(S)-3,4-dehydropyrrolinylmethoxy)-5-(3'-Fluoropropyl)pyridine (nifrolene) (6)

—The above N-BOC-nifrolene **13** (12 mg, 0.035 mmol) was dissolved in dichloromethane (1 mL). The compound was treated with trifluoroacetic acid (100 μL). The solution was kept stirring at room temperature for 20 hours. The solution was concentrated to dryness. The residue was extracted with acetonitrile and the solution was dried over anhydrous K_2CO_3 followed by dryness. The residue was treated with 1 M HCl in diethyl ether (2 mL). The solution was stirred for 5 min and concentrated to dryness. The residue was triturated with chloroform repeatedly (3×3 mL) until no product was present in the residue (TLC). The chloroform solution was concentrated to dryness. The residue was purified by a short column of silica gel (DCM:MeOH 9:1) to furnish nifrolene hydrochloride salt **6** (6 mg, 61%). NMR (500 MHz; CDCl_3) δ 8.15 (d, $J = 2.65$ Hz, 1H, ArH), 8.09 (m, 1H, ArH), 7.06 (m, 1H, ArH), 6.01 (m, 2H, olefinic H), 4.69 (m, 1H), 4.40 (dt, $J = 47$ Hz, 5.75 Hz, 2H, $\text{CH}_2\text{-F}$), 4.19-4.0 (m, 2H), 4.10 (m, 2H), 2.72 (t, $J = 7.6$ Hz, 2H, Ar- CH_2), 1.99 (m, 2H). MS, m/z 237 (100%, $[\text{M} + \text{H}]^+$).

2.2. In Vitro Binding Affinity

Binding affinity of the compounds to $\alpha_4\beta_2$ receptors using ^3H -cytisine in rat brains was previously described [29,31]. Briefly, 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 twice and the supernatant discarded each time. The supernatant was then removed and the final pellet was taken up in the incubation buffer to a concentration of 120 – 150 mg/mL of tissue. Binding assays were carried out in 50 mM Tris buffer at pH 7.0 containing 120 mM NaCl, 5 mM KCl, 1mM MgCl_2 and 2.5 mM CaCl_2 in the presence of 1 nM ^3H -cytisine at 2°C for 75 min incubation. The total assay volume was 0.25 mL. Different concentrations of the assay compound, nifrolene in 0.025 mL (concentration range from 10^{-10} to 10^{-4}M) was taken in 0.1 mL of buffer along with 0.025 mL of 1 nM ^3H -cytisine. Nonspecific binding was measured using 300 μM 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 rapidly 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 the following procedure: (1) the non-specific binding of ^3H -cytisine was subtracted for all samples; (2) the specific binding was normalized to 100% (no competitive ligand) and (3) the binding isotherms were fit to the Hill equation (KELL BioSoft software (v 6), Cambridge, U.K.) and (4) affinity, K_i was computed using the reported K_d value of ^3H -cytisine, 0.59 nM to nicotine $\alpha_4\beta_2$ receptors [32].

2.3. Radiosynthesis

Using the tosylate precursor, **12**, radiosynthesis of ^{18}F -nifrolene [^{18}F]**6** was carried out in the chemistry-processing computer unit (CPCU) box. Fluorine-18, produced in the MC-17 Scanditronix cyclotron (^{18}O p,n ^{18}F) was passed through light QMA Sep-Pak (Waters Corp) pretreated with 3 mL of potassium carbonate, 140 mg/mL and 3 mL anhydrous acetonitrile. The trapped ^{18}F in QMA was eluted with Kryptofix and potassium carbonate solution (1 mL of stock solution containing 360 mg Kryptofix and 75 mg potassium carbonate in acetonitrile-water mixture 24:1) into the reaction vessel in the CPCU. The automated radiosynthesis steps involved drying of the ^{18}F solution with additional acetonitrile (2 mL)

at 120°C, reaction of the ^{18}F with precursor (1 to 2 mg, **12**) in acetonitrile (0.5 mL) at 96°C for 30 min.

The intermediate N-BOC- ^{18}F -nifrolene was transferred out of the CPCU reaction vessel using dichloromethane. This dichloromethane solution was used directly for deprotection. Deprotection of N-BOC- ^{18}F -nifrolene was carried out in dichloromethane (1 mL) and trifluoroacetic acid (0.2 mL). The solution was heated at 80°C for 20 min and evaporated to dryness. The residue was neutralized with 10% NaHCO_3 solution to a pH reading of 7.0. Semipreparative HPLC purification was performed using an Alltech C_{18} column (10 μm , 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, retention time 15 min (Fig-3). The collected fraction was taken to dryness in vacuo. The final formulation was carried out using 1–3 mL of saline (0.9% NaCl INJ) followed by sterile filtration through a membrane filter (0.22 μm), and the filtered solution was collected in a dose vial. The radiolabeled product ^{18}F -nifrolene (~10 mCi) was obtained in 99% radiochemical purity. Radiochemical yields of ^{18}F -nifrolene were 10–20% range decay corrected with specific activities > 2000Ci/mmol.

2.4. In Vitro Autoradiographic Studies of ^{18}F -Nifrolene

Sets of brain slices (horizontal, 10 μm thick from male Sprague-Dawley rats) were preincubated in buffer (50 mmol/L Tris HCl containing 120 mmol/L NaCl, 5mmol/L KCl, 2.5 mmol/L CaCl_2 , 1 mmol/L MgCl_2 , pH 7.4) for 10 min. Subsequently, the preincubation buffer was discarded and the slices were incubated with ^{18}F -nifrolene (1–2 $\mu\text{Ci/cc}$) at 37°C for 60 min. Binding was measured in the presence of 0.1 μM , 1 μM and 300 μM (for nonspecific binding) of nicotine. After incubation, slices were washed twice (1 min each) with ice-cold Tris HCl buffer, pH 7.4, followed by a quick rinse in cold (0–5°C) 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 ^{18}F -nifrolene in the autoradiograms was evaluated in various brain regions (as digital lights units (DLU/ mm^2) using the OptiQuant acquisition and analysis program (Packard Instruments Co.).

2.5 In Vivo Rat PET Imaging

Male Sprague-Dawley rats were fasted 24 hours prior to time of scan. Rats were anesthetized using 4.0% isoflurane on the day of the study. The rat was placed on the scanner bed, on a warm-water circulating heating pad, and anesthesia was applied using a nose-cone. A transmission scan was subsequently acquired. Preparation of dose injection was as follows: 0.6 ± 0.2 mCi of ^{18}F -nifrolene was drawn into a 1 mL syringe and diluted with sterile saline to a final volume of 0.3 mL. The dose was injected into the tail vein of the rat intravenously. Isoflurane was reduced and maintained at 2.5% following the injection. Scans were carried out for 150 minutes and acquired by the Inveon MicroPET in full list mode. List mode data was collected dynamically which were rebinned using a Fourier Rebinning algorithm. The images were reconstructed using 2 dimensional Filter Back Projection using a Hanning Filter with a Nyquist cut off at 0.5, and corrected for attenuation using the Co-57 attenuation scan data. Calibration was conducted using a Germanium-68 phantom to Bq/cc units, which was scanned in the Inveon MicroPET and reconstructed under the same parameters as the subjects. Analyses of all data were done using Acquisition Sinogram Image Processing IDL's virtual machine (ASIPRO VM) and Pixelwise Modeling software (PMOD 3.0).

Nicotine challenge experiments were of two types. In order to demonstrate reversibility of bound ^{18}F -nifrolene, post-injection nicotine effects were measured. Sprague-Dawley rats were injected with ^{18}F -nifrolene and at approx. 30 mins postinjection of ^{18}F -nifrolene, 0.3

mg/kg of nicotine free base (administered as a ditartrate salt from Sigma Chem. Co) was administered intravenously. Total time of scan was 90 mins and was acquired in full list mode, similar to the protocol for the control scans described in section 2.3. Before and after images were analyzed using ASIPRO VM software and a time activity curve was generated.

2.6. Monkey PET Imaging

Subsequent to the rat PET studies, we then focused on PET study of ^{18}F -nifrolene on an anesthetized rhesus monkey using an ECAT EXACT HR+ scanner. Rhesus monkey (10 kg) was 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 the radiopharmaceutical 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. A transmission scan using a $^{68}\text{Ge}/^{68}\text{Ga}$ rod source was acquired before administration of the radiopharmaceutical to correct for tissue attenuation of the coincident radiation. A three-dimensional dynamic sequence of scans for a total of approximately 180 min was acquired in the 3-dimensional mode immediately after administering approximately 3–4 mCi (mass injected, $<0.2\text{ }\mu\text{g}$ of nifrolene) of ^{18}F -nifrolene intravenously. Data in the final form re-expressed in units of kilobecquerels per milliliter (kBq/mL). Areas showing maximal radioligands binding in the thalamus, lateral geniculate, subiculum and cerebellum were delineated in the images using a MR template. Time-activity curves were obtained for all these regions.

3. Results

3.1. Chemistry

The synthesis route to prepare nifrolene **6** is shown in Fig-2. Starting with 5-bromo-3-pyridinol **8** (prepared as reported [28]) the 5-bromo-3-pyridyl ether compound **9** was synthesized in 86% yield by the etherification of **8** with (*S*)-1-(*tert*-butoxycarbonyl)-2-hydroxymethyl-3-pyrroline **7** under Mitsunobu conditions (in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD)) [22]. The bromo compound **9** was heated under reflux with allyltributyltin in toluene in the presence of catalytic amount of tetrakis(triphenylphosphine)palladium [28] catalyst; the reaction went smoothly to give allylpyridyl ether derivative **10** in 45% yield. The olefin **10** was treated with BH_3 . THF and subsequently oxidized with $\text{NaOH}/\text{H}_2\text{O}_2$ to furnish the alcohol **11** in 25% yield [28]. This alcohol **11** was used for preparing the tosylate **12** necessary for preparing nifrolene **6** and radiolabeled ^{18}F -nifrolene [^{18}F]**6**. The tosylate was obtained in $>40\%$ yield starting from the alcohol. Fluorination of the tosylate **12** was carried out by TBAF rather than diethylaminosulfur trifluoride (DAST) and provided N-BOC-nifrolene **13** in 57% yield which was subsequently deprotected with TFA to provide nifrolene in 61% yield.

3.2. In Vitro Binding Affinity

The agonist ^3H -cytisine binds to $\alpha 4\beta 2$ receptor sub-type and the displacement of ^3H -cytisine binding by nifrolene indicated specificity of nifrolene. The binding affinity K_i value for nifrolene was found to be 0.36 nM. The binding affinity of nifrolene is slightly better than our previously reported nifrolidine, nifzetidine and nifene (Table-1). The five-membered unsaturated ring structure in nifrolene contributes to the higher affinity.

3.3. Radiochemistry

Radiosynthesis of ^{18}F -nifrolene took two steps (Fig-3A). In the first step, the BOC-protected tosylate group was reacted by ^{18}F -fluoride (either from an MC-17 Scandotronix cyclotron or

RDS 112 cyclotron) by nucleophilic substitution reaction using Kryptofix and potassium carbonate in CH_3CN at 96°C for 30 min. The tosylate-to- ^{18}F -fluoride exchange proceeded in modest yields (20–30%, decay corrected). Deprotection of the N-BOC protecting group was carried out with TFA at 80°C for 30 min. The mixture was purified by HPLC (C18 Alltech reverse phase column) with a gradient of 0.1 M ammonium formate buffer (40%) and acetonitrile (60%) with a flow rate of 2.5 ml/min. The retention time of ^{18}F -nifrolene was found to be 15 min and is shown in Fig-3B. A large mass peak eluted just after the ^{18}F -nifrolene peak. We are currently in the process of characterizing the mass peak and investigating improved HPLC separation that will better separate the radioactive product. For this work, the purest fraction of the radioactive product eluting at 15 min was collected for biological studies. The radioactive peak was collected and solvents were removed. The radiosynthesis was accomplished in 2 hrs with an overall radiochemical yield of 5–10%, decay corrected. The specific activity in several runs was estimated to be $>2000\text{ Ci/mmol}$.

3.4. In Vitro Autoradiographic Studies

Consistent with $\alpha 4\beta 2$ receptor distribution in vitro autoradiography in rat brain slices indicated high binding of ^{18}F -nifrolene in the thalamus as well as extrathalamus regions such as cortex, striata, subiculum, cerebellum and other regions (Fig-4). The anteroventral thalamic nucleus (AVT) exhibited the highest amount of binding. Subiculum (Sub) also exhibited significant amounts of ^{18}F -nifrolene binding, which is known to contain $\alpha 4\beta 2$ receptors sites. Cerebellum showed some binding. Several experiments were tried for displacement studies. The experiments were carried out with different concentrations of nicotine. Nicotine at 10 nmol/L concentration was able to partially displace the selective binding in various brain regions. Specific binding of ^{18}F -nifrolene was completely abolished by 300 $\mu\text{mol/L}$ of nicotine in these brain regions. ^{18}F -nifrolene yielded higher region to cerebellum (Cer) ratios compared to ^{18}F -nifrolidine. The cerebellum was used as the reference region. The ratios of various brain regions with respect to the cerebellum are as follows: Th/Cer = 9.3; Sub/Cer = 5; Cor/Cer = 3.5 (Fig-4).

3.5 In Vivo Rat PET Imaging

Rapid uptake of ^{18}F -nifrolene was observed in the brain. Thalamic regions (Fig-5A) exhibited the highest retention since it has the maximum amount of $\alpha 4\beta 2$ receptors. Sagittal brain sections also reveal binding in the thalamus while very little binding was present in the cerebellum (Fig-5B). Some extracranial activity was also observed. Time activity curves of the thalamus and cerebellum in Fig-5C show initial rapid uptake in various brain regions followed by greater retention in the thalamus compared to the cerebellum. Ratio of uptake for the thalamus against the reference region cerebellum reached a plateau at approx. 60 min post-injection. Thalamus to cerebellum ratio was found to be >4 .

Administration of nicotine after ^{18}F -nifrolene was able to displace $>90\%$ of thalamic ^{18}F -nifrolene as seen in Fig-6A. Thalamus is clearly visualized prior to the injection of nicotine (Fig-6B) which is rapidly displaced (Fig-6C).

3.6. Monkey PET Imaging

PET studies of ^{18}F -nifrolene in rhesus monkeys were carried out. Vital signs were closely monitored; the monkey did not exhibit any unusual derivation from the baseline values. All images were co-registered with MRI template of monkey brain (Fig-7). The results indicated a significant amount of binding in the thalamic regions in all three sections—axial, sagittal and coronal. Brain uptake and distribution of ^{18}F -nifrolene in a rhesus monkey PET study demonstrated binding in thalamus and extrathalamic regions.

Several extrathalamic brain regions were also observed (Fig-8). Moderate binding was measured in lateral geniculate and temporal cortex. Within the temporal cortex, subiculum is expected to have significant receptor levels. Some binding was seen at the frontal cortex, striata occipital cortex and other regions. Cerebellum showed lowest levels of ^{18}F -nifrolene binding and as reported previously it was used as a reference region.

Figure-9A shows a rapid uptake of ^{18}F -nifrolene into the various brain regions. Highest retention occurred in the thalamus and lateral geniculate. Temporal cortex and subiculum also exhibited significant levels of ^{18}F -nifrolene retention. Clearance from the cerebellum was the fastest indicative of few receptors in this brain region. Using cerebellum as the reference region, ratios (Fig-9B) in the thalamus exceeded 3 at 90 minutes postinjection. Ratios in the thalamus and lateral geniculate reached a plateau between 100–120 minutes postinjection.

4. Discussion

Several agonist-based radioligands have been explored in the literature for the imaging of $\alpha 4\beta 2$ receptors [33]. For over 10 years ^{18}F -2-F-A85380 has been the mainstay for human use [34]. It continues to be used successfully; however, the major limitations include the long scanning times. Additionally, there has been an interest in the development of agents that may be antagonists or partial agonists in order to enable human use with reduced toxicity issues. These factors have led to the exploration of agents with improved properties [35].

Nifene **3** is a new agonist PET agent for imaging $\alpha 4\beta 2$ receptors which exhibits promise of significantly enhanced in vivo kinetics. In the rhesus monkeys, ^{18}F -nifene demonstrated fast kinetics in receptor-rich regions (60 mins) [25]. These properties of ^{18}F -nifene make it a suitable $\alpha 4\beta 2$ agonist radiotracer for human studies which are now planned.

We focused our attention towards antagonist-based imaging agents by placing alkyl groups at 5 positions of 3-pyridyl ethers since the antagonist character increases [26–28]. A similar approach of placing larger alkyl or aryl groups has been considered in the epibatidine derivatives [35]. The 3-pyridyl ethers selectively bind to $\alpha 4\beta 2$ receptors unlike epibatidine analogs which may exhibit binding to $\alpha 3\beta 2$ and $\alpha 3\beta 4$ receptors.

In the fluoropropyl series, ^{18}F -nifrolidine was prepared which provided modest ratios between thalamus and cerebellum (approx. 2) in two hours of imaging time [28]. In a subsequent effort, ^{18}F -nifzetidine was prepared. However, the uptake of this radiotracer in the brain was very slow and would require longer imaging times compared to ^{18}F -nifrolidine [29]. Based on nifene kinetics, the presence of the unsaturated ring was considered essential to accelerate binding kinetics. Thus, nifrolene **6** which now contained the unsaturated ring, was evaluated for $\alpha 4\beta 2$ receptors.

We have accomplished synthesis of nifrolene using a similar approach for the synthesis of nifzetidine by taking (*S*)-*N*-*tert*-butoxycarbonyl-2-hydroxymethyl-3-pyrroline **7** for the Mitsunobu coupling. The alkyl group at 5 position was successfully introduced via allylation of 5-bromo-3-pyridyl ether compound with an allylating agent allyltri-*n*-butyltin followed by hydroboration of the corresponding double bond and conversion of alcohol. The corresponding alcohol is the key intermediate for making the precursor for ^{18}F -nifrolene. This was the first approach to make unlabeled nifrolene directly from the intermediate alcohol **7**. But the DAST reaction did not give satisfactory results. Finally, nifrolene was prepared from tosylate **12** by reacting with TBAF followed by deprotection.

Among the various compounds synthesized nifrolene showed the highest affinity for $\alpha 4\beta 2$ receptors (Table-1). Interestingly, the affinity of nifrolene is similar to 2-F-A85380 (using a correction factor of $10\text{nM}/2.83\text{nM} = 3.53$ for differences in reported affinities of nicotine in the two assays which results in $1.33\text{nM}/3.53 = 0.38\text{ nM}$ for 2-F-A85380 compared to 0.36 nM for nifrolene).

Rat studies, both in vitro and in vivo, exhibited selective binding to the various brain regions. Autoradiographic studies were consistent with our previously reported findings with ^{18}F -nifene [22]. In vivo binding kinetics was rapid similar to ^{18}F -nifene [23]. Thalamus to cerebellum ratio exceeded 4. Sensitivity to nicotine was confirmed both in vitro and in vivo.

Monkey studies confirmed the improved binding kinetics of ^{18}F -nifrolene, compared to ^{18}F -nifrolidine [28] and ^{18}F -nifzetidine [29]. The ratio between the thalamus and the cerebellum was approximately 3.3 at 90–100 min (point of pseudoequilibrium) and is higher than nifrolidine [28] and ^{18}F -nifzetidine [29]. However, the kinetics of binding of ^{18}F -nifrolene is a significant improvement and is indicative of the value of this radiotracer for further studies.

5. Conclusions

A new PET radiotracer, ^{18}F -nifrolene has been successfully prepared and evaluated as a putative antagonist for $\alpha 4\beta 2$ nAChR. It exhibits specific in vivo binding to receptor rich regions and therefore is worthy of further evaluation. ^{18}F -Nifene is one of the fast binding radiotracers in the rhesus monkey for this receptor and is twice as fast as ^{18}F -nifrolene [25]. This may be suggestive of the differences in their agonist/antagonist character at the receptor site.

Acknowledgments

This research was financially supported by a grant from National Institutes of Health R01 AG029479. We like to thank Drs. Min-Liang Pan, Evgeni Sevrioukov and Cristian Constantinescu and Daphne Collins for technical assistance and Mr. Said Shokair at the Undergraduate Research Opportunities Program at University of California, Irvine for summer support (SAK) through the ID-SURE program.

References

1. Romanelli MN, Gualtieri F. Cholinergic nicotinic receptors: competitive ligands, allosteric modulators, and their potential applications. *Med Res Rev.* 2003; 23:393–426. [PubMed: 12710018]
2. Easwaramoorthy B, Pichika R, Collins D, Potkin SG, Leslie FM, Mukherjee J. Effect of acetylcholinesterase inhibitors on the binding of nicotinic $\alpha 4\beta 2$ receptor PET radiotracer, ^{18}F -nifene: A measure of acetylcholine competition. *Synapse.* 2007; 61:29–36. [PubMed: 17068780]
3. Benowitz NL. Pharmacology of nicotine: addiction and therapeutics. *Annu Rev Pharmacol Toxicol.* 1996; 36:597–613. [PubMed: 8725403]
4. Brody AL, Mandelkern MA, London ED, Olmstead RE, Farahi J, Scheibal D, et al. Cigarette smoking saturates brain $\alpha 4\beta 2$ nicotinic acetylcholine receptors. *Arch Gen Psychiatry.* 2006; 63:907–915. [PubMed: 16894067]
5. Brody AL, Mandelkern MA, Costello MR, Abrams AL, Scheibal D, Farahi J, et al. Brain nicotinic acetylcholine receptor occupancy: effect of smoking a denicotinized cigarette. *Int J Neuropsychopharmacol.* 2008; 12:305–316. [PubMed: 18706128]
6. Dwoskin LP, Smith AM, Wooters TE, Zhang Z, Crooks PA, Bardo MT. Nicotine receptor-based therapeutics and candidates for smoking cessation. *Biochem. Pharmacol.* 2009; 78:732–743. [PubMed: 19523455]
7. Levin ED. Nicotine receptor subtypes and cognitive function. *J Neurobiol.* 2002; 53:633–640. [PubMed: 12436426]

8. Paterson D, Nordberg A. Neuronal nicotinic receptors in the human brain. *Prog Neurobiol.* 2000; 61:75–111. [PubMed: 10759066]
9. Kuwabara H, Wong DF, Gao Y, Valentine H, Holt DP, Ravert HT, et al. PET imaging of nicotinic acetylcholine receptors in baboons with 18F-AZAN, a radioligand with improved brain kinetics. *J Nucl Med.* 2012; 53:121–129. [PubMed: 22173841]
10. Chefer SI, London ED, Koren AO, Pavlova OA, Kurian V, Kimes AS, et al. Graphical analysis of 2-[18F]FA binding to nicotinic acetylcholine receptors in rhesus monkey brain. *Synapse.* 2003; 48:25–34. [PubMed: 12557269]
11. Valette H, Xiao Y, Peyronneau M-A, Damont A, Kozikowski AP, Wei Z-L, et al. 18F-ZW-104: A new radioligand for imaging neuronal nicotinic acetylcholine receptors-In vitro binding properties and PET studies in baboons. *J Nucl Med.* 2009; 50:1349–1355. [PubMed: 19652217]
12. Abreo MA, Lin NH, Garvey DS, Gunn DE, Hettinger AM, Wasicak JT, et al. Novel 3-pyridylethers with subnanomolar affinity for central neuronal nicotinic acetylcholine receptors. *J Med Chem.* 1996; 39:817–825. [PubMed: 8632405]
13. Brown LL, Kulkarni S, Pavlova O, Koren A, Mukhin AG. Synthesis and evaluation of a series of 2-chloro-5-((1-methyl-2-(S)-pyrrolidinyl)-methoxy)-3-(2-(4-pyridinyl)vinyl)pyridine analogues as potential PET imaging agents for nicotinic acetylcholine receptors. *J Med Chem.* 2002; 45:2841–2849. [PubMed: 12061886]
14. Iida Y, Ogawa M, Ueda M, Tominaga A, Kawashima H, Magata Y, et al. Evaluation of 5-(11)C-methyl-A-85380 as an imaging agent for PET investigations of brain nicotinic acetylcholine receptors. *J Nucl Med.* 2004; 45:878–884. [PubMed: 15136639]
15. Roger G, Lagnel B, Rouden J, Besret L, Valette H, Demphel S, et al. Synthesis of a 2-pyridinyl-18F-labelled fluoro derivative of (-)-cytisine as a candidate radioligand for brain nicotinic alpha4beta2 receptor imaging with PET. *Bioorg Med Chem.* 2003; 11:5333–5343. [PubMed: 14642577]
16. Fujita M, Seibyl JP, Vaupel BD, Tamagnan G, Early M, Zoghbi SS, et al. Whole body distribution, radiation absorbed dose, and brain SPET imaging with 123I- 5-I-A85380 in healthy human subjects. *Eur J Nucl Med.* 2002; 29:183–190.
17. Kimes AS, Horti AG, London ED, Chefer SI, Contoreggi C, Ernst M, et al. 2-[18F]FA85380: PET imaging of brain nicotinic acetylcholine receptors and whole body distribution in humans. *FASEB Journal.* 2003; 17:1331–1333. [PubMed: 12759330]
18. Bottlaender M, Valette H, Roumenov D, Dolle F, Coulon C, Ottaviani M, et al. Biodistribution and radiation dosimetry of 18F-fluoro-A-85380 in healthy volunteers. *J Nucl Med.* 2003; 44:596–601. [PubMed: 12679405]
19. Ding YS, Fowler J, Logan J, Wang GJ, Telang F, Garza V, et al. 6-[18F]Fluoro-A-85380, a new PET tracer for the nicotinic acetylcholine receptor: studies in the human brain and in vivo demonstration of specific binding in the white matter. *Synapse.* 2004; 53:184–189. [PubMed: 15236351]
20. Loftipour S, Mandelkern M, Brody AL. Quantitative molecular imaging of neuronal nicotinic acetylcholine receptors in the human brain with A-85380 radiotracers. *Curr Med Imaging Rev.* 2011; 7:107–112. [PubMed: 22773924]
21. Gallezot JD, Bottlaender M, Gregoire MC, Roumenov D, Deverre JR, Coulon C, et al. In vivo imaging of human cerebral nicotinic acetylcholine receptors with 2-18F-fluoro-A-85380 and PET. *J Nucl Med.* 2005; 46:240–246. [PubMed: 15695782]
22. Pichika R, Easwaramoorthy B, Collins D, Christian BT, Shi B, Narayanan TK, et al. Nicotine ?4?2 receptor imaging agents. Part II. Synthesis and biological evaluation of 2-[18F]fluoro-3-2-(S)-pyrrolinyl-3,4-dehydromethoxy.pyridine (¹⁸F-Nifene) in rodents and imaging by PET in non-human primate. *Nucl Med Biol.* 2006; 33:294–304.
23. Kant R, Constantinescu CC, Parekh P, Pandey SK, Pan M-L, Easwaramoorthy B, et al. Evaluation of 18F-nifene binding to a4b2 nicotinic receptors in the rat brain using microPET imaging. *EJNMMI Research.* 2011; 1:6. [PubMed: 22039577]
24. Bieszczad KM, Kant R, Constantinescu CC, Pandey SK, Kawai HD, Metherate R, et al. Nicotinic acetylcholine receptors in rat forebrain that bind 18F-nifene: Relating PET imaging, autoradiography, and behavior. *Synapse.* 2012; 66:418–434. [PubMed: 22213342]

25. Hillmer AT, Wooten DW, Moirano JM, Slesarev M, Barnhart TE, Engle JW, et al. Specific $\alpha 4\beta 2$ nicotinic acetylcholine receptor binding of 18F-nifene in the rhesus monkey. *Synapse*. 2011; 65:1309–1318. [PubMed: 21674627]
26. Lin NH, Gunn DE, Li Y, He Y, Bai H, Ryther KB, et al. Synthesis and structure-activity relationships of pyridine modified analogs of A-84543: A potent nicotine receptor ligand. *Bioorg Med Chem Lett*. 1998; 8:249–254. [PubMed: 9871663]
27. Lin NH, Li Y, He Y, Holladay MW, Kuntzweiler T, Anderson DJ, et al. Synthesis and structure-activity relationships of 5-substituted pyridine analogs of A-84543: A potent nicotine receptor ligand. *Bioorg Med Chem Lett*. 2001; 11:631–633. [PubMed: 11266158]
28. Chattopadhyay S, Xue B, Pichika R, Collins D, Bagnara R, Leslie FM, et al. Synthesis and evaluation of nicotine $\alpha 4\beta 2$ receptor ligand, 5-(3'-fluoropropyl)-3-(2-(S)-pyrrolidinyl)methoxy)pyridine in rodents and imaging by PET in non-human primate. *J Nucl Med*. 2005; 46:130–140. [PubMed: 15632043]
29. Pichika R, Easwaramoorthy B, Christian BT, Shi B, Narayanan TK, Collins D, et al. Nicotinic $\alpha 4\beta 2$ receptor imaging agents. Part III Synthesis and biological evaluation of 3-(2-(S)-azetidylmethoxy)-5-(3'-18F-fluoropropyl)pyridine (18F-nifzetidine). *Nucl Med Biol*. 2011; 38:1183–1192. [PubMed: 21831652]
30. Constantinescu C, Mukherjee J. Performance evaluation of an Inveon PET preclinical scanner. *Phys. Med. Biol*. 2009; 54:2885–2899. [PubMed: 19384008]
31. Pabreza LA, Dhawan S, Kellar KJ. [3H]Cytisine binding to nicotinic cholinergic receptor in brain. *Mol Pharmacol*. 1991; 39:9–12. [PubMed: 1987453]
32. Smulders CJGM, Awart R, Bermudez I, van Kleef RGDM, Groot-Kormelink PJ, Vijverberg HPM. Cholinergic drugs potentiate human nicotinic $\alpha 4\beta 2$ acetylcholine receptors by a competitive mechanism. *Eur J Pharmacol*. 2005; 509:97–108. [PubMed: 15733544]
33. Koren AO, Horti AG, Mukhin AG, Gundisch D, Kimes AS. 2-, 5-, and 6-Halo-3-(2(S)-azetidylmethoxy)pyridines: synthesis, affinity for nicotinic acetylcholine receptors, and molecular modeling. *J Med Chem*. 1998; 41:3698.
34. Dollé F, Dolci L, Valette H, Hinnen F, Vaufrey F, Guenther I, et al. Synthesis and nicotinic acetylcholine receptor in vivo binding properties of 2-fluoro-3-[2(S)-2-azetidylmethoxy.pyridine: a new positron emission tomography ligand for nicotinic receptors. *J Med Chem*. 1999; 42:2251–2299. [PubMed: 10377231]
35. Horti AG, Gao Y, Kuwabara H, Dannals RE. Developments of radioligands with optimized imaging properties for quantification of nicotinic acetylcholine receptors by positron emission tomography. *Life Sci*. 2010; 86:575–584. [PubMed: 19303028]

1. Nicotine **4. Nifrolidine** **2. 2-F-A85380** **5. Nifzetidine** **3. Nifene** **6. Nifrolene**

Figure 1.
Chemical Structures of $\alpha_4\beta_2$ radioligands: (a) Nicotine **1**; (b) 2-F-A85380 **2**; (c) Nifene **3**;
(d) Nifrolidine **4**; (e) Nifzetidine **5**; (f) Nifrolene **6**.

Figure 2.
Synthesis scheme for nifrolene **6** synthesis.

Figure 3.

(A) Reaction scheme showing the radiosynthesis of ^{18}F -*N*-BOC-nifrolene **[^{18}F]13** and ^{18}F -nifrolene **[^{18}F]6**. (B) HPLC purification of ^{18}F -nifrolene using C_{18} reverse-phase semi-preparative column eluted with 60% acetonitrile-0.1M ammonium formate at a flow rate of 2.5 mL/min. Retention of ^{18}F -nifrolene was found to be 15 min.

Figure 4.

In vitro autoradiographic studies of ¹⁸F-nifrolene [¹⁸F]6 in rat brain slices. Binding of ¹⁸F-nifrolene in 20 μm horizontal slices (1.6 μCi/cc at 37°C. Horizontal brain slices of rat brain showing binding of ¹⁸F-nifrolene (red = highest binding and white = lowest binding). (A) Total binding (COR: frontal cortex; STR: striatum; TH: thalamus; SUB: subiculum; CB: cerebellum). (B) Binding in the presence of 1 μM nicotine showing partial displacement of ¹⁸F-nifrolene [¹⁸F]6. (C) Binding in the presence of 300 μM nicotine showing >80 displacement of ¹⁸F-nifrolene [¹⁸F]6. (D). Plot showing amount of ¹⁸F-nifrolene binding in brain regions in total, 10⁻⁷ and 10⁻⁶ nicotine.

Figure 5.

In vivo MicroPET rat brain slices of ¹⁸F-nifrolene: (A) coronal, (B) sagittal. The thalamus (TH) shows the highest binding while the cerebellum (CB) exhibits little binding. (C) Time-activity curves of the thalamus and the cerebellum show initial rapid uptake in both regions, and greater retention is seen in the thalamus compared to the cerebellum.

Figure 6.

In vivo MicroPET rat brain slices (coronal) of ^{18}F -nifrolene before and after the administration of nicotine. (A) Time-activity curves for the thalamus and cerebellum show initial rapid uptake of ^{18}F -nifrolene and a lack of retention in the thalamus post-injection of nicotine. (B) Pre-nicotine scans show uptake in the thalamus. (C) Post-nicotine scans exhibit displacement of selective binding of ^{18}F -nifrolene in the thalamus.

Figure 7. MR-PET Coregistered Monkey Images of ^{18}F -nifrolene. Distribution of ^{18}F -nifrolene in the rhesus monkey brain: (A–C) MRI, (D–F) PET. Male rhesus monkey anesthetized with ketamine/isoflurane. Approximately 4 mCi of ^{18}F -nifrolene injected iv. Summed PET images (100–200 mins) showing binding of ^{18}F -nifrolene in select brain slices.

Figure 8.

PET images of the distribution of ^{18}F -nifrolene in the rhesus monkey brain (G-R). Brain regions include TH: thalamus; STR: striatum; LG: lateral geniculate; TC: temporal cortex; SB: subiculum; OC: occipital cortex; CB: cerebellum.

Figure 9.

(A) Time-activity curves of the binding of ^{18}F -nifrolene in select areas of the monkey brain corresponding to the regions identified in Figure-7, 8. (B) Ratio plot of brain regions to cerebellum.

S.No.	Compound	R	X	R'	Binding Affinity, Ki nM ^a
1	Nicotine	a	H	H	2.83/10 ^b
2	2-F-A83580	d	F	H	1.33 ^b
3	Nifene	b	F	H	0.50
4	Nifrolidine	c	H	(CH ₂) ₃ F	0.80
5	Nifzetidine	d	H	(CH ₂) ₃ F	0.67
6	Nifrolene	b	H	(CH ₂) ₃ F	0.36

^bK_i measured using ³H-epibatidine in rat brains [11]. [