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Synthesis and evaluation of 2-¹⁸F-fluoro-5-iodo-3-[2-(S)-3,4-dehydropyrrolinylmethoxy]pyridine (¹⁸F-Niofene) as a potential imaging agent for nicotinic $\alpha 4\beta 2$ receptors

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Abstract: Nicotinic $\alpha 4\beta 2$ acetylcholine receptors (nAChRs) have been implicated in various pathophysiologies including neurodegenerative diseases. Currently, 2-¹⁸F-A85380 (2-FA) and 5-¹²³I-A85380 (5-IA) are used separately in human PET and SPECT studies respectively and require >4-6 hours of scanning. We have developed 2-fluoro-5-iodo-3-[2-(S)-3,4-dehydropyrrolinylmethoxy]pyridine (niofene) as a potential PET/SPECT imaging agent for nAChRs with an aim to have rapid binding kinetics similar to that of ¹⁸F-nifene used in PET studies. Niofene exhibited a 10-fold better *in vitro* binding affinity in rat brain than that of nicotine. The relative binding of niofene was similar to that of niodene and twice as better as that of nifene. *In vitro* autoradiography in rat brain slices alongside niodene indicated selective binding of niofene to regions consistent with $\alpha 4\beta 2$ receptor distribution. Niofene, 10 nM, displaced >70% of ³H-cytisine bound to $\alpha 4\beta 2$ receptors in rat brain slices. Radiolabeling of ¹⁸F-niofene was achieved in 10-15% radiochemical yield in high specific activities >2 Ci/ μ mol and showed rapid *in vivo* kinetics similar to that of ¹⁸F-nifene and ¹⁸F-nifrolene. *In vivo* PET in rats showed rapid uptake in the brain and selective localization in receptor regions such as the thalamus (TH). Pseudoequilibrium with ¹⁸F-niofene was achieved in 30-40 minutes, which is similar to that of ¹⁸F-nifene. Further evaluation of ¹⁸F-niofene as a potential PET imaging agent is underway. Future studies will be conducted to radiolabel niofene with iodine-123 for use in SPECT imaging.

Keywords: Nicotine, nifene, niodene, nifrolene, fluorine-18

Introduction

Alterations of $\alpha 4\beta 2$ nicotinic acetylcholine receptors (nAChRs) in the brain have shown some significance in the etiology of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease. More recently, this receptor has been found to be overexpressed in mice models of lung tumors [1]. Efforts on imaging $\alpha 4\beta 2$ receptors using positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are currently underway with 2-¹⁸F-A85380 (2-FA) and 5-¹²³I-A85380 (5-IA) used in human studies for PET and SPECT respectively [2-5]. Although successful, 2-FA and 5-IA both require a period >4-6 hours to work, which is not ideal for patient comfort.

We have developed several $\alpha 4\beta 2$ imaging agents in our laboratory of which the leading ones are the following: agonist ¹⁸F-nifene (for PET) and putative antagonists ¹²³I-niodene (for SPECT) and ¹⁸F-nifrolene (for PET). With a 3,4-dehydropyrrolidine ring system, ¹⁸F-nifene has shown rapid brain imaging kinetics with a reduced scan time of <1 hour, which is faster than the well-known 2-FA [6]. ¹²³I-Niodene exhibits similar binding behavior as that of nifene, such as its high affinity for $\alpha 4\beta 2$ receptors, and it also incorporates a 3,4-dehydropyrrolidine ring system in its structure. This is evident when niodene displaces specifically bound ¹⁸F-nifene in rat brain slices at low concentrations [7]. ¹⁸F-Nifrolene also has a 3,4-dehydropyrrolidine ring system that allows it to have rapid binding kinetics similar to that of ¹⁸F-nifene. All

Niofene PET

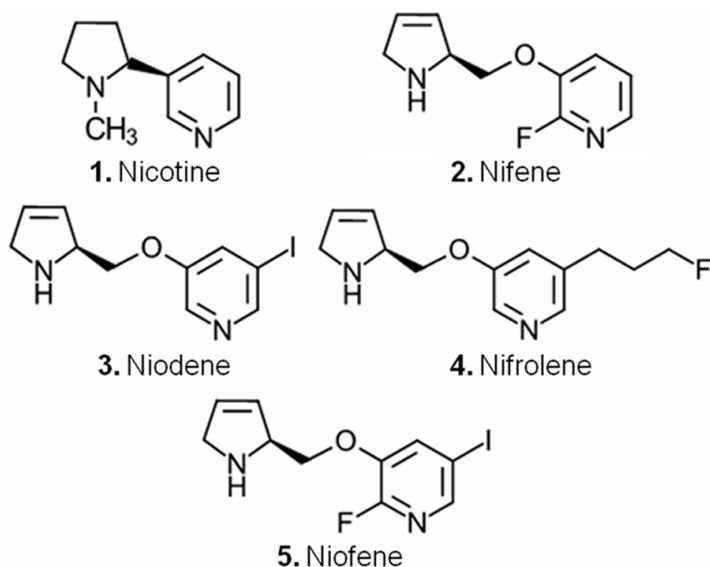


Figure 1. Chemical structures of $\alpha 4 \beta 2$ nAChRs binding agents. Nicotine **1**; Nifene **2**; Niodene **3**; Nifrolene **4**; Niofene **5**.

three agents express high *in vitro* binding affinity for $\alpha 4 \beta 2$ receptors in rat brain homogenate using ^3H -cytisine, with nifene's $K_i = 0.50$ nM, niodene's $K_i = 0.27$ nM, and nifrolene's $K_i = 0.36$ nM [8].

Due to the promising characteristics of nifene and niodene, we have decided to combine their molecular structures to develop a hybrid molecule, 2-fluoro-5-iodo-3-[2-(S)-3-dehydropyrrolylmethoxy]pyridine (niofene) as a potential PET/SPECT imaging agent for nAChRs (**Figure 1**). Our goal with incorporating the 3,4-dehydropyrroline ring is to have niofene exhibit similar rapid kinetics to that of nifene. The presence of the iodine at the 5-position causes niofene to become more of an antagonist and share similar binding characteristics as nifrolene. Furthermore, the addition of both fluorine and iodine is to allow niofene to be used in either PET or SPECT imaging, depending on the user's preference in imaging modalities.

We report here the synthesis of niofene, *in vitro* binding affinity in rat brain homogenate, *in vitro* autoradiographic studies in rat brain slices, radiosynthesis of ^{18}F -niofene, and *in vivo* PET imaging studies in rat.

Materials and methods

General

All chemicals and solvents were of analytical or HPLC grade from Sigma-Aldrich and Fisher

Scientific. (S)-tert-butyl 2-(hydroxymethyl)-2,5-dihydro-1H-pyrrole-1-carboxylate was purchased from Aroma Circle (San Diego, CA, USA) and 2-Bromo-5-iodopyridin-3-ol was purchased from Sigma-Aldrich (St. Louis, MO, USA). ^3H -Cytisine (32.7 Ci/mmol specific activity) was purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA, USA). All water was deionized to specific resistance of <18 m Ω cm using a Millipore Milli-Q water purification system. Electrospray mass spectra were obtained on a Model 7250 mass spectrometer (Micromass LCT). Proton NMR spectra were recorded on a Bruker OMEGA 500-MHz spectrometer. Analytical thin layer chromatography (TLC) was carried out on silica-coated plates (Baker-

Flex, Phillipsburg, NJ, USA). Chromatographic separations were carried out on preparative TLC (silica gel GF 20_20 cm, 2000 Am thick; Alltech Assoc., Deerfield, IL, USA) or semi-preparative reverse-phase columns using the Gilson high-performance liquid chromatography (HPLC) systems. Tritium was assayed by using a Packard Tri-Carb Liquid scintillation counter with 65% efficiency. High specific activity ^{18}F -fluoride was produced in the MC-17 cyclotron or the GE cyclotron using oxygen-18 enriched water (^{18}O to ^{18}F using p, n reaction). The high specific activity ^{18}F -fluoride was used in subsequent reactions which were carried out in automated radiosynthesis units [either a chemistry processing control unit (CPCU) or a nuclear interface fluorine-18 module]. Fluorine-18 radioactivity was counted in a Capintec dose calibrator, whereas low-level counting was carried out in a well counter (Cobra quantum, Packard Instruments, Boston, MA, USA). Rat brain slices were obtained on a Leica 1850 cryotome. Tritiated cytisine autoradiographic studies were carried out by exposing tissue samples on storage phosphor screens. The apposed phosphor screens were read and analyzed by the OptiQuant acquisition and analysis program of the Cyclone Storage Phosphor System (Packard Instruments). All animal studies were approved by the Institutional Animal Care and Use Committee of University of California-Irvine and University of Wisconsin-Madison, Madison, WI.

Niofene PET

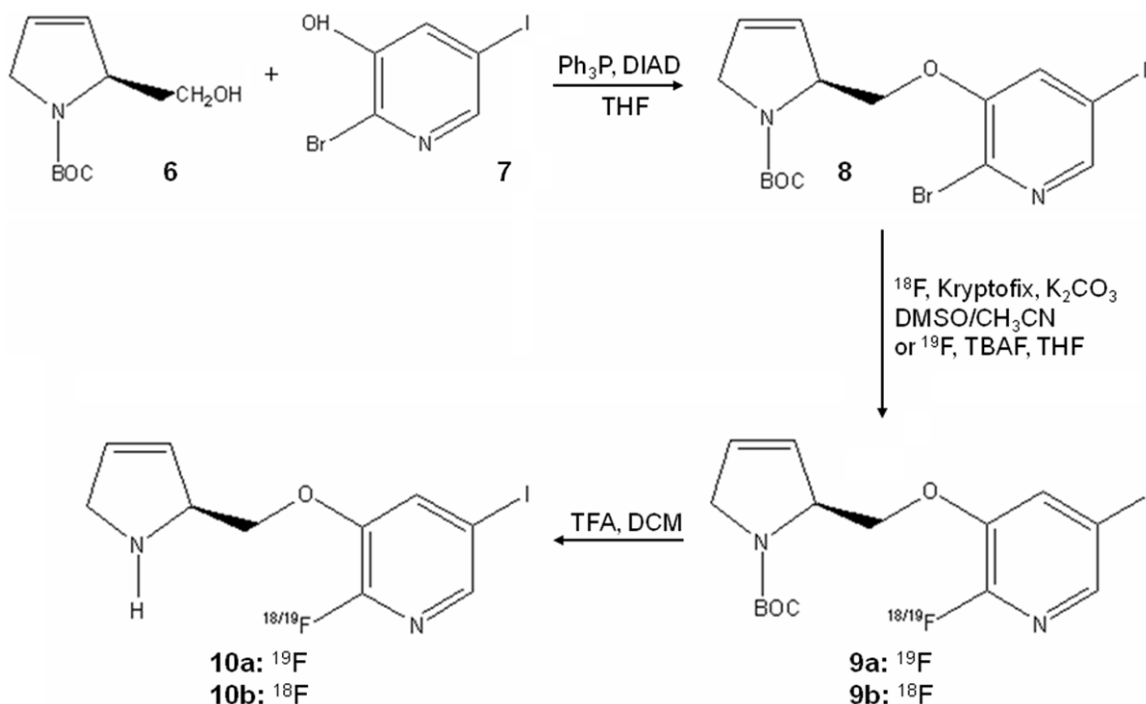


Figure 2. Synthesis for $^{18/19}\text{F}$ -niofene **10a** and **10b**. Diisopropyl azodicarboxylate (DIAD) was added to a solution of **6**, **7**, and triphenylphosphine (Ph_3P) in tetrahydrofuran (THF) that was stirred and cooled at 0°C . The reaction mixture came to room temperature over a period of 2 hours and was stirred overnight. Bromo-intermediate **8** served as a precursor for fluorination using tetrabutylammonium fluoride (TBAF) and radiofluorination using ^{18}F -fluoride, Kryptofix and potassium carbonate (K_2CO_3) in dimethylsulfoxide (DMSO)-acetonitrile (CH_3CN). Deprotection of **9a** and **9b** was carried out using trifluoroacetic acid (TFA) in dichloromethane (DCM) to yield niofene, **10a** or ^{18}F -niofene **10b**.

Chemistry

3-[2-(S)-N-tert-butoxycarbonyl-2-pyrrolinemethoxy]-2-bromo-5-iodopyridine (8**):** Niofene precursor **8** was produced under Mitsunobu conditions as previously reported (**Figure 2**) [7]. Diisopropyl azodicarboxylate (DIAD; 0.2 mL) was added to a solution of 2-bromo-5-iodopyridin-3-ol (0.16 g, 0.55 mmol), (S)-tert-butyl 2-(hydroxymethyl)-2,5-dihydro-1Hpyrrole-1-carboxylate (0.1 g; 0.50 mmol) and triphenylphosphine (0.19 g) in tetrahydrofuran (THF; 10 mL), stirred under argon and cooled at 0°C . The reaction mixture was allowed to come up to ambient temperature over a period of 2 hours and was stirred overnight. The reaction mixture was evaporated to dryness and the residue was purified by preparative TLC (1:4, EtOAc in hexane) to give pure product 3-[2-(S)-N-tert-butoxycarbonyl-2-pyrrolinemethoxy]-2-bromo-5-iodopyridine as an oil with a modest yield of 42%. $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ ppm: 8.18 (d, 1H, PyH), 7.42 (d, 1H, PyH), 5.91 (m, 2H, olefinic), 4.80 (m, 1H, CH-CH_2), 4.35-3.95 (m, 4H, O-CH_2 , N-CH_2), 1.47 (s, 9H, Boc). MS, m/z , 481, 483 (50%, $[\text{M}+\text{H}]^+$), 503, 505 (100%, $[\text{M}+\text{Na}]^+$).

2-fluoro-5-iodo-3-[2-(S)-3-dehydropyrrolinylmethoxy]pyridine (10a** Niofene):** In order to prepare **9a**, two fluorination methods were carried out as described below under methods A and B.

Method A: 3-[2-(S)-N-tert-butoxycarbonyl-2-pyrrolinemethoxy]-2-bromo-5-iodopyridine, **8** (46 mg; 0.095 mmol) was taken in 2 mL of THF and tetra-*n*-butylammonium fluoride (TBAF; 1 mL; 1 mmol) in a round-bottom flask. The mixture was refluxed at $75\text{--}85^\circ\text{C}$ for 24 hours. Solution was purified by preparative TLC (9:1 dichloromethane (DCM): MeOH). Product yield was 6.9 mg (0.017 mmol; 18% yield).

Method B: 3-[2-(S)-N-tert-butoxycarbonyl-2-pyrrolinemethoxy]-2-bromo-5-iodopyridine, **8** (46 mg; 0.095 mmol) was dissolved in anhydrous acetonitrile. Kryptofix (36.5 mg; 0.10 mmol), potassium fluoride (9 mg; 0.16 mmol), and dimethyl sulfoxide (1 mL) were added to the solution. The flask was heated in a $120\text{--}125^\circ\text{C}$ oil bath for 5 hours and then dried. The product was extracted from the reaction mixture using DCM (the aqueous layer removed the Kryptofix

and inorganics). The DCM solution was purified by preparative TLC (9:1 DCM: MeOH) to provide pure 3-[2-(S)-N-tertbutoxycarbonyl-2-pyrroline-methoxy]-2-fluoro-5-iodopyridine, **9a** (34.6 mg; 0.083 mmol; 87% yield). ¹H-NMR (500 MHz, CDCl₃) δ ppm: 8.32 (d, 1H, PyH), 7.63 (d, 1H, PyH), 5.88 (m, 2H, olefinic), 4.80 (m, 1H, CH-CH₂), 4.34-4.05 (m, 4H, O-CH₂, N-CH₂), 1.45 (s, 9H, Boc). MS, m/z, 443 (90%, [M+Na]⁺).

Deprotection of the N-tertbutoxycarbonyl group in **9a** using trifluoroacetic acid (TFA) provided niofene **10a** in high yields. 3-[2-(S)-N-tertbutoxycarbonyl-2-pyrroline-methoxy]-2-fluoro-5-iodopyridine, **9a** (3.5 mg; 0.008 mmol) was taken in DCM (1 mL) and TFA was then added to the solution (0.1 mL) and stirred for 1 hour. DCM (3 mL) and saturated sodium bicarbonate (1 mL) was added to the reaction mixture, and the DCM was extracted and dried over magnesium sulfate. A yield of 2.6 mg (0.008 mmol, quantitative yield) of 2-fluoro-5-iodo-3-[2-(S)-3-dehydropyrroline(methoxy)pyridine (**10a** Niofene) was obtained. ¹H-NMR (500 MHz, CDCl₃) δ ppm: 8.24 (d, 1H, PyH), 7.54 (d, 1H, PyH), 5.86 (dd, 2H, olefinic), 4.27 (m, 1H, CH-CH₂), 3.94-3.75 (m, 4H, O-CH₂, N-CH₂), 1.73 (br, 1H, NH). MS, m/z, 321 (100%, [M+H]⁺).

In vitro binding affinity

Using previously described procedures, rat brain homogenate assays using ³H-cytisine were carried out to measure binding affinity of the compounds to α4β2 receptors [9]. The cerebrum of male Sprague-Dawley rats was isolated and homogenized using Tekmar tissumizer (15 seconds, half maximum speed) in the incubation buffer 1:100 (wt/vol). The rat brain homogenate was then centrifuged and the supernatant was discarded. The pellet was then resuspended in the same volume of buffer, homogenized, and centrifuged again. 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. All binding assays were carried out in duplicate. Binding assays were carried out in 50 mM Tris buffer at pH 7.4 containing 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂ and 2.5 mM CaCl₂ in the presence of 1 nM ³H-cytisine at 3-5°C for 75 minutes of incubation. The total assay volume was 0.25 mL. Different concentrations of the assay compound, 0.025 mL (nicotine and niofene, range

10⁻¹¹ to 10⁻⁴ M), were 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 minutes) 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 minutes in the scintillation counter.

In vitro autoradiographic studies of niofene

Horizontal brain slices, 10 μm thick from male Sprague-Dawley rats, were preincubated in buffer (50 mmol/L Tris HCl containing 120 mmol/L NaCl, 5 mmol/L KCl, 2.5 mmol/L CaCl₂, 1 mmol/L MgCl₂, pH 7.4) for 10 min. The preincubation buffer was then discarded and the slices were incubated with ³H-cytisine (2.5 nM, specific activity of 32.7 Ci/mmol) at an ambient temperature for 75 minutes. Binding was measured in the presence of 10 nM and 1 μM of niofene and 10 nM and 300 μM (for nonspecific binding) of nicotine. After incubation, slices were washed twice (1 minute each) with ice-cold Tris 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 and read by the Cyclone Phosphor Imaging System. The amount of bound ³H-cytisine in the autoradiograms was evaluated in various brain regions as digital lights units (DLU/mm²) using the OptiQuant acquisition and analysis program.

Radiosynthesis

Synthesis of ¹⁸F-niofene was carried out using modifications of our established procedures of ¹⁸F-nifene [6]. The radiosynthesis of ¹⁸F-niofene was carried out in the automated unit. Starting with 500-600 mCi of ¹⁸F and 2 mg of precursor **8**, ¹⁸F-niofene **10b** was obtained. An Alltech C₁₈ column (10 μm, 250 x 10 mm²) was used for reverse-phase HPLC purification (60% acetonitrile, 40% water, flowrate 5 mL/min) and specific activity of ¹⁸F-niofene was approximately 2000 Ci/mmol. The collected fraction was taken to near dryness *in vacuo*. The final formulation of ¹⁸F-niofene was carried out using

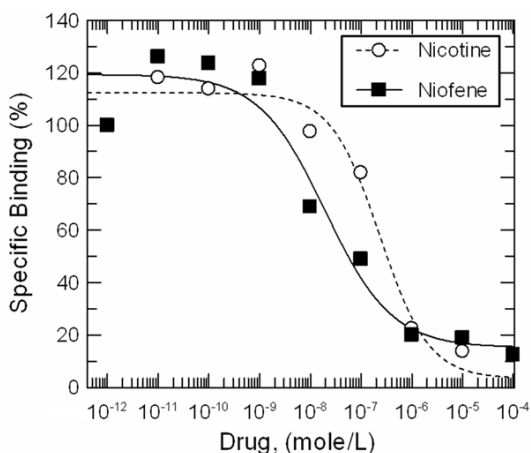


Figure 3. *In vitro* binding affinity curves of niofene and nicotine. Measured using rat brain homogenates labeled with ³H-cytisine. Measurements are an average on the assays that were carried out in duplicate.

approximately 2 to 5 mL of saline (0.9% NaCl INJ) followed by filtration through a membrane filter (0.22 μm) into a sterile dose vial for use in the PET studies.

In vivo rat PET imaging

Male Sprague-Dawley rats were fasted 24 hours prior to time of scan. Rats were initially anesthetized using 4.0% isoflurane prior to scanning procedures. 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 separate dose injections of ¹⁸F-niofene, ¹⁸F-nifene, and ¹⁸F-nifrolene was as follows: 0.6 ± 0.2 mCi 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. Emission data was collected beginning with bolus injection of radiotracer and was continued for 90-120 minutes after injection. List mode emission data were collected and rebinned using a Fourier Rebinning algorithm. The images were reconstructed using 2 dimensional Filtered 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).

Results

Chemistry

Mitsunobu coupling reaction of (*S*)-tert-butyl 2-(hydroxymethyl)-2,5-dihydro-1H-pyrrole-1-carboxylate **6** and 2-bromo-5-iodo-3-hydroxypyridine **7** gave the ether 2-bromo-5-iodo-3-[2-((*S*)-*N*-tertbutoxycarbonyl-3-pyrroline)methoxy]pyridine **8** in 42% yield. The exchange of bromide with fluoride using TBAF was carried out in 18% yield, while an alternate method of using Kryptofix and potassium fluoride provided 87% yield of 2-fluoro-5-iodo-3-[2-((*S*)-*N*-tertbutoxycarbonyl-3-pyrroline)methoxy]pyridine **9a**. Deprotection of the BOC group in **9a** proceeded efficiently using TFA in quantitative yields to provide 2-fluoro-5-iodo-3-[2-(*S*)-3-dehydropyrrolinylmethoxy]pyridine (niofene, **10a**).

In vitro binding affinity

Binding affinity at the α4β2 receptor subtype in rat brain homogenates was measured using ³H-cytisine. In rat brain homogenates, ³H-cytisine binds with high affinity ($K_d = 0.59$ nM). The competitive binding assays with ³H-cytisine were carried out for two different inhibitors: nicotine and niofene. The binding affinity curves for nicotine and niofene are shown in **Figure 3** (average of two measurements for each compound), and the calculated K_i values for these and other compounds are summarized in **Table 1**. Niofene was found to be slightly higher in affinity at the α4β2 receptor subtype than nicotine. Subnanomolar affinity of niofene results from its structural features resembling that of nifene and niodene [7].

In vitro autoradiographic studies

Competition of unlabeled niofene and niodene with ³H-cytisine was carried out in rat brain slices. Thalamus to cerebellum (CB) ratio in the presence of 10 nM niofene was reduced from 11.1 to 2.9, suggesting a >70% decrease and 10 nM of niodene reduced the ratio to 1.7 (>80% decrease) (**Figure 4**). These results suggest that niofene binds at the same α4β2 receptor sites as ³H-cytisine, which reflect the

Table 1. Binding Affinity of Compounds at $\alpha 4\beta 2$ nAChR in Rat Brain Tissue

S.No.	Compound	Imaging Modality	Radioisotope to be Used	Binding Affinity, K_i nM ^a	Relative Affinity to Nicotine
1	Nicotine	PET	¹¹ C	2.83 ^b /30 ^c	1
2	Nifene	PET	¹⁸ F	0.50 ^b	5.7
3	Niodene	SPECT	¹²³ I-	0.27 ^b	10.5
4	Nifrolene	PET	¹⁸ F	0.36 ^b	7.9
5	Niofene	PET/SPECT	¹⁸ F-/ ¹²³ I-	2.90 ^c	10.3

^aBinding assay with ³H-cytisine in rat brain homogenates using reported methods [9]; K_i calculated from IC_{50} using Cheng-Prusoff equation, $K_i = IC_{50}/1 + ([^3H\text{-cytisine}]/K_d)$ (1973) and $K_d = 0.59$ nM for ³H-cytisine. ^bTaken from our previous works [7, 8]. ^c K_i measured using ³H-cytisine in rat brains.

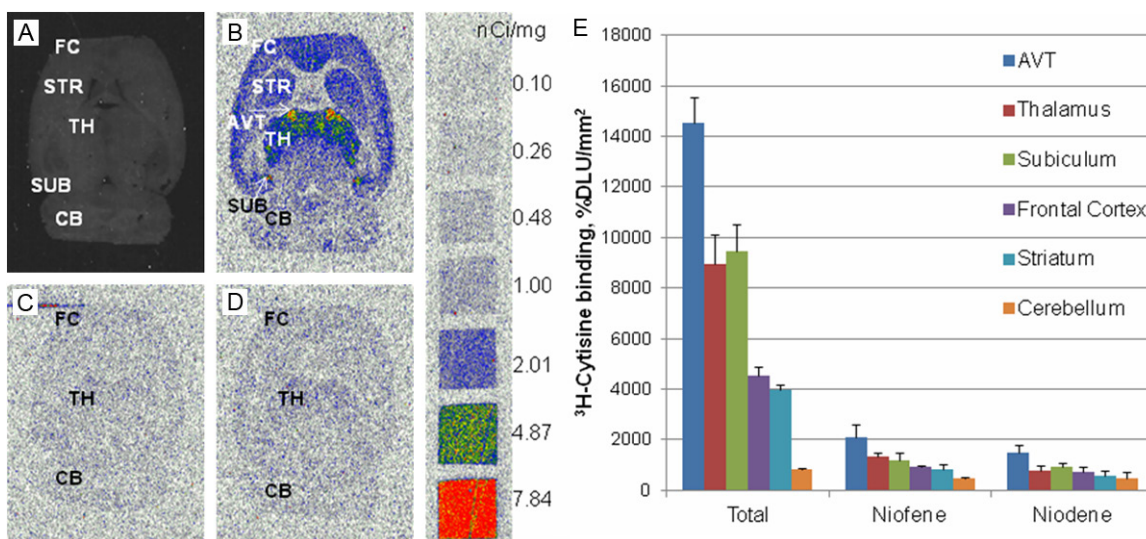


Figure 4. Rat brain ³H-cytisine autoradiography. (A) Scan of rat brain slice; (B) Total binding of ³H-cytisine in different brain regions (AVT, Anterior ventral thalamus; TH, thalamus; SUB, subiculum; FC, frontal cortex; STR, striatum; CB, cerebellum); (C) Binding of ³H-cytisine in the presence of 10 nM niodene; (D) Binding of ³H-cytisine in the presence of 10 nM niofene; (E) Graph of ³H-cytisine binding in brain.

concentrations of the $\alpha 4\beta 2$ receptors in the various brain regions [10].

Radiosynthesis

Radiosynthesis of ¹⁸F-niofene was carried out in two steps. In the first step, the BOC-protected bromo group **8** was reacted by ¹⁸F-fluoride (either from an MC-17 Scandotronix cyclotron or RDS 112 cyclotron) by aromatic nucleophilic substitution reaction using Kryptofix and potassium carbonate in DMSO-CH₃CN at 120°C for 30 minutes. The bromo-to-fluoride exchange proceeded in good yields of **9b**. The mixture was purified by HPLC (C18 Alltech reverse phase column) with a gradient of 0.1% aqueous triethylamine (40%) and acetonitrile (60%) with a flow rate of 5 mL/min. The retention time of ¹⁸F-N-BOC-niofene **9b** was found to be 19 minutes. The radioactive peak was collected and

solvents removed. Deprotection of the N-BOC protecting group was carried out with TFA at 80°C for 30 minutes. The radiosynthesis was accomplished in 2.5 hours with an overall radiochemical yield of 10-15% of ¹⁸F-niofene **10b**, decay corrected. The specific activity in several runs was estimated to be >2 Ci/ μ mol.

In vivo rat PET imaging

In vivo PET in rats showed rapid uptake in the brain and selective localization in receptor regions (Figure 5). Uptake of ¹⁸F-niofene in the brain reached levels of approximately 1% injected dose/cc. Thalamus exhibited the highest retention as it has a maximum amount of $\alpha 4\beta 2$ receptors. Significant levels of uptake were observed in other regions of the cortex while retention in the cerebellum was lower. Time activity curves of the thalamus and cerebellum

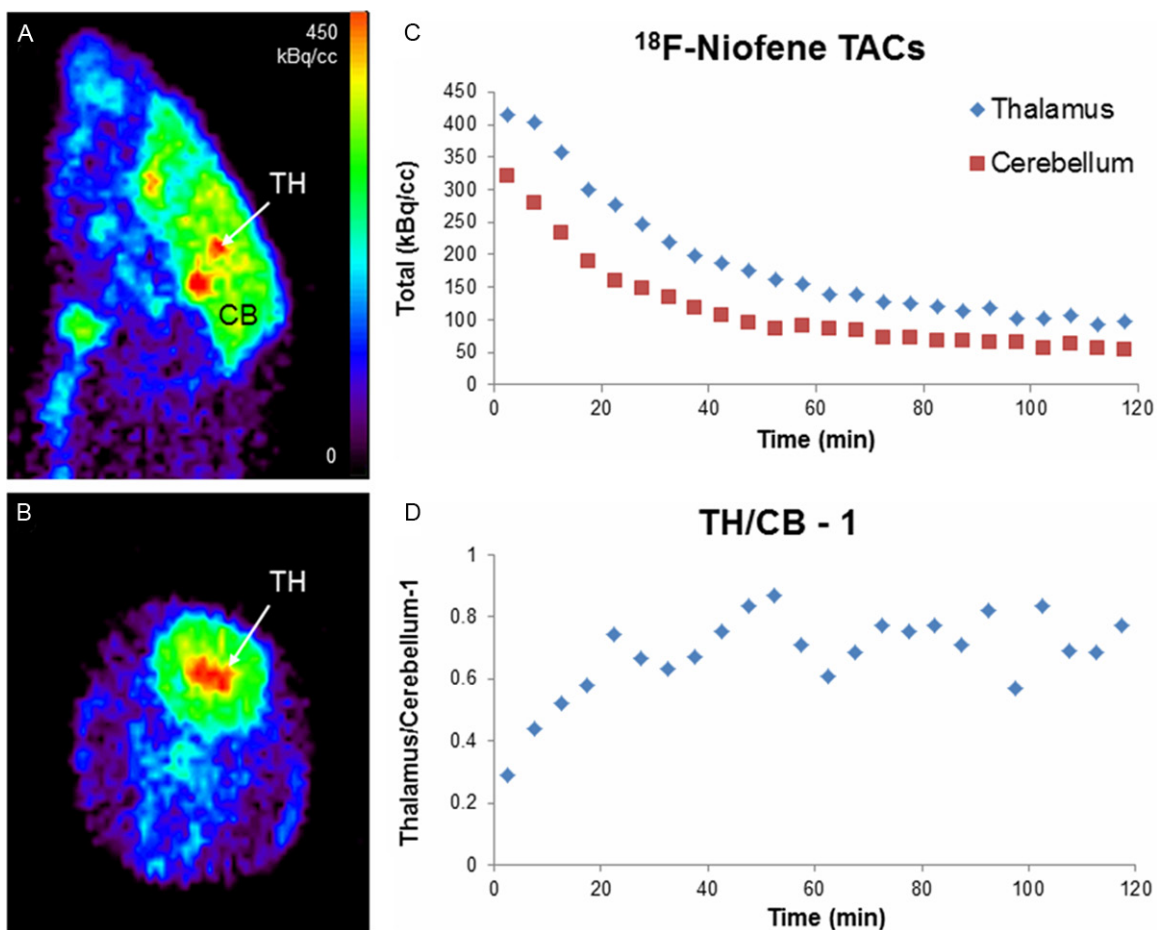


Figure 5. *In vivo* PET images of normal rat injected with ^{18}F -niofene. (A) Sagittal cross-section showing TH and CB; (B) Transverse cross-section showing TH. (C) Time activity curves of TH and CB that show initial rapid uptake in both regions. (D) Using CB as a reference region, plot of TH/CB-1 showing pseudoequilibrium achieved in 30-40 minutes with TH/CB >2.

in **Figure 5B** 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 approximately 30-40 minutes post-injection with a thalamus to cerebellum ratio >2 (**Figure 5C**).

Discussion

Following similar procedures as nifene, the synthesis of niofene was accomplished in two steps [6]. The first step, involving Mitsunobu coupling, led to a yield that was lower than expected when compared to that of nifene. This decrease in yield may have been due to bulky substituents present in the substituted pyridinol. The second step involved nucleophilic substitution of the 2-bromo with fluoride, which

was carried out using two methods: (1) Kryptofix carbonate, and (2) TBAF. Yield was greater and isolation of the product was easier in the Kryptofix method. Similar to deprotection methods of *N*-BOC nifene, TFA provided deprotection of the *N*-BOC niofene precursor at high yields.

Niofene was designed on the basis of incorporating the structures of nifene and niodene, which contain a 2-fluorine and 5-iodine respectively. Both nifene and niodene have a higher affinity compared to that of nicotine [7]. It was therefore anticipated that niofene would have a higher affinity compared to that of nicotine since the structure of niofene is the combined structures of nifene and niodene. Our results show that niofene has a higher affinity than that of nicotine (Niofene $K_i = 2.90$ nM; Nicotine $K_i = 30$ nM). **Table 1** summarizes the binding affinities of these drugs relative to nicotine for

Niofene PET

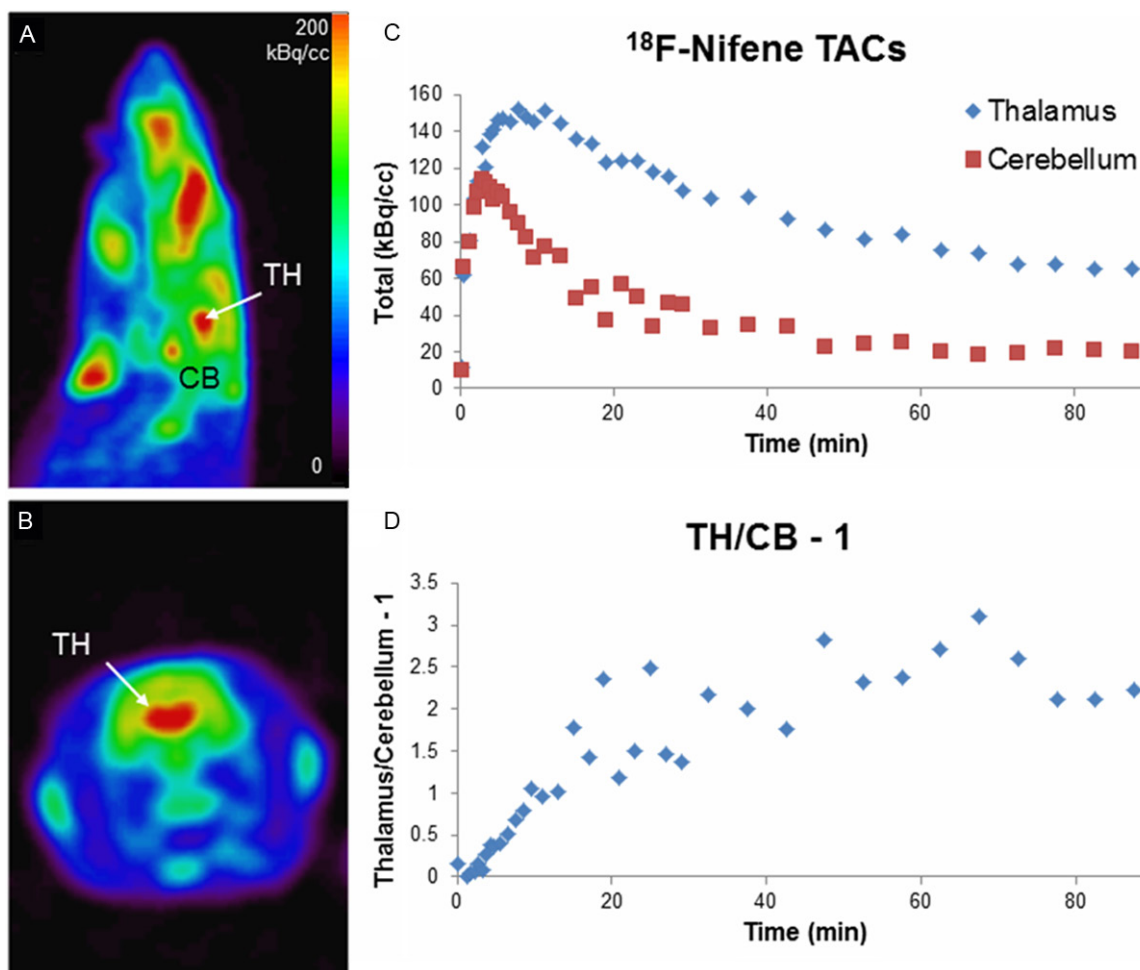


Figure 6. *In vivo* PET images of normal rat injected with ^{18}F -niofene. (A) Sagittal cross-section showing TH and CB; (B) Transverse cross-section showing TH. (C) Time activity curves of TH and CB that show initial rapid uptake and increased retention in the TH. (D) Using CB as a reference region, plot of TH/CB-1 showing pseudoequilibrium achieved in 30-40 minutes with TH/CB >3.

cross-comparison between studies done at different times. The relative affinities suggest the order of binding affinity to the $\alpha 4\beta 2$ receptors among the derivatives studied thus far is niodene > niofene > nifrolene > nifene > nicotine (all of which contain the dehydroproline ring, with the exception of nicotine).

Binding of ^3H -cytisine to $\alpha 4\beta 2$ receptors in rat brain slices was consistent with our observations of ^{18}F -niofene binding and reflected the concentrations of the $\alpha 4\beta 2$ receptors in the various brain regions [10]. The order of ^3H -cytisine binding was anterior ventral thalamus (AVT), thalamus, subiculum, frontal cortex, striatum, and cerebellum. Niofene was able to compete with ^3H -cytisine labeled $\alpha 4\beta 2$ receptors in rat brain slices effectively. Binding in the

thalamus was reduced >70% by niofene while niodene was able to displace slightly greater amounts of ^3H -cytisine (refer to **Figure 4**). These findings suggest that niofene is a selective ligand for $\alpha 4\beta 2$ receptors.

For the radiosynthesis of ^{18}F -niofene, a bromide-for-fluoride substitution was used instead of the nitro-for-fluoride reaction in the radiosynthesis of ^{18}F -niofene [6]. This bromide substitution for the synthesis of ^{18}F -niofene worked equally well with radiochemical yields of >25%, decay-corrected. ^{18}F -niofene was found to be stable for use in *in vivo* experiments.

^{18}F -Niofene uptake in the rat brain was rapid and was consistent with our previous observations with ^{18}F -niofene [11]. Retention of ^{18}F -

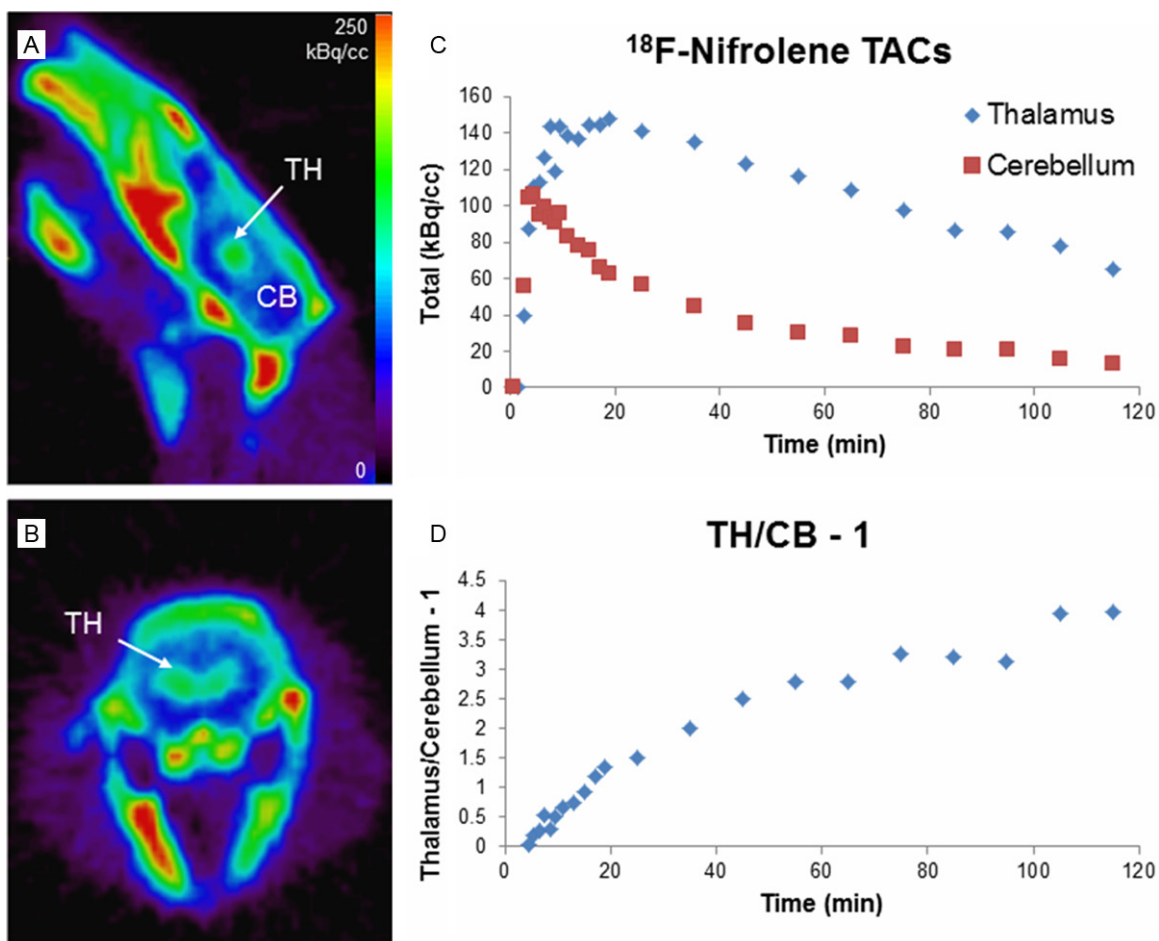


Figure 7. *In vivo* PET images of normal rat injected with ^{18}F -nifrolene. (A) Sagittal cross-section showing TH and CB; (B) Transverse cross-section showing TH. (C) Time activity curves of TH and CB that shows greater retention in the TH. (D) Using CB as a reference region, plot of TH/CB-1 showing pseudo-equilibrium achieved in >80 minutes with TH/CB >4.

niofene was evident in the thalamus while it cleared more from the cerebellum. Using the cerebellum as the reference region, pseudo-equilibrium was achieved in 30-40 minutes (similar to that of ^{18}F -nifene) with TH/CB >2.

Consistent with the lower affinity of niofene compared to that of nifene, the binding kinetics of ^{18}F -nifene showed greater retention in the thalamus compared to that of niofene, thus resulting in slightly greater TH/CB ratios (Figure 6). This is also evident in the sagittal and transverse images of the rat brain in Figure 6A and 6B. Maximum binding was found in the thalamus while moderate binding is seen in the cortex and minimal binding in the cerebellum. Time activity curves for thalamus and cerebellum show ^{18}F -nifene peaks early into the scan (Figure 6B) and nonspecific binding in the cer-

ebellum cleared rapidly. Thalamus to cerebellum ratios were >3 (Figure 6C).

Based on the structures of niofene and nifene, the former is expected to be an antagonist while the latter is an agonist. It is possible that this difference between the two agents play a factor in their binding kinetics. Therefore, we have also compared the behavior of niofene with our previously reported putative antagonist, nifrolene, shown in Figure 7. Sagittal brain sections also reveal binding in the thalamus while very little binding was present in the cerebellum (Figure 7A). Some extracranial activity was also observed. Time activity curves of the thalamus and cerebellum in Figure 7C show initial rapid uptake in brain regions followed by greater retention in the thalamus compared to the cerebellum. Ratio of uptake for the thala-

mus against the reference region cerebellum reached a plateau at approximately 60 minutes post-injection. Thalamus to cerebellum ratio was found to be >4 (Figure 7D).

As can be seen in the time activity curves, retention of nifrolene was greater in the thalamus compared to that of both niofene and nifene. It is therefore likely that the binding kinetics of the three related dehydroproline derivatives may be dictated by their binding affinities to the $\alpha 4\beta 2$ receptors.

The presence of a substituent at the 5-position in this class of compounds changes an agonist to an antagonist [12]. Therefore, niofene-which contains an iodine-and nifrolene-which contains a fluoropropyl group-are rendered as antagonists [8]. However, with our preliminary results for niofene reported here, it appears that the nature of the substituent at the 5-position may dictate *in vivo* kinetics. This may be due to the large spherical iodine atom in niofene versus the carbon chain in the fluoropropyl group in nifrolene. This aspect warrants further investigation.

When radiolabeled with iodine-123, ^{123}I -niofene may have similar *in vivo* kinetics like that of ^{18}F -niofene and is likely to have faster kinetics compared to that of 5- ^{123}I -IA, a SPECT imaging agent currently used in human studies, which requires long imaging times (>4 to 6 hours) [13].

Conclusion

The goal of this study was to combine the molecular structures of two imaging agents previously developed in our lab, nifene and niofene. This was done in order to produce a novel imaging agent, niofene, which has potential use in both PET and SPECT studies whilst exhibiting similar binding kinetics as those of nifene and niodene. Our preliminary studies were focused on niofene's characteristics and its use solely in PET applications after being radiolabeled with fluorine-18. Results from this study have suggested that niofene is a selective ligand for $\alpha 4\beta 2$ receptors and uptake of ^{18}F -niofene in rat brain was consistent with our previous observations with ^{18}F -nifene. Further investigation will be done to study niofene's potential use in SPECT applications once radiolabeled with iodine-123.

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Disclosure of conflict of interest

The authors have no conflict of interest.

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