Published in final edited form as:

Bioorg Med Chem Lett. 2012 December 15; 22(24): 7610–7614. doi:10.1016/j.bmcl.2012.10.012.

Synthesis and evaluation of 3-¹²³I-iodo-5-[2-(S)-3-pyrrolinylmethoxy]-pyridine (Niodene) as a potential nicotinic $\alpha 4\beta 2$ receptor imaging agent

Suresh K. Pandey^{a,b}, Shawn Pan^a, Ritu Kant^c, Sharon A. Kuruvilla^a, Min-Liang Pan^a, and Jogeshwar Mukherjee^a

^aPreclinical Imaging, Department of Radiological Sciences, University of California-Irvine, Irvine, CA 92697

^cDepartment of Pharmacology, University of California-Irvine, Irvine, CA 92697

Abstract

Nicotinic acetylcholine receptors (nAChRs) are downregulated in disease conditions such as Alzheimer's and substance abuse. Presently, ¹²³I-5-IA-85380 is used in human studies and requires over 6 hrs of scanning time, thus increases patient discomfort. We have designed and synthesized 3-iodo-5-[2-(S)-3-pyrrolinylmethoxy]pyridine (Niodene) with the aim to have faster binding kinetics compared to ¹²³I-5-IA-85380, which may reduce scanning time and help in imaging studies. Binding affinity K_i of niodene for rat brain $\alpha 4\beta 2$ receptors in brain homogenate assays using ³H-cytisine was 0.27 nM. Niodene, 10 nM displaced >95% of ¹⁸F-nifene bound to $\alpha 4\beta 2$ receptors in rat brain slices. By using the iododestannylation method, ¹²³I-niodene was obtained in high radiochemical purity (>95%) but with low radiochemical yield (<5%) and low specific activity (~100 Ci/mmol). Autoradiograms show ¹²³I-niodene localized in the thalamus and cortex, which was displaced by nicotine (thalamus to cerebellum ratio = 4; cortex to cerebellum ratio = 1.6). Methods of radioiodination need to be further evaluated in order to obtain ¹²³I-niodene in higher radiochemical yields and higher specific activity of this potentially useful new SPECT imaging agent.

Keywords

Nicotine; Nifene; ¹²³I-5-IA-85380; SPECT; Imaging

Noninvasive imaging of nicotinic acetylcholine receptors of the $\alpha 4\beta 2$ subtype have been carried out using single photon emission computed tomography (SPECT). Iodinated A-85380 (5-IA-85380) has been developed for imaging studies of this receptor system.¹ In SPECT studies, ¹²³I-5-IA-85380 has been successfully used with an average study time of 6 hours.^{2,3} Since the four-membered azetidene ring found in 5-IA-85380 has been known to exhibit higher binding affinity than the five-membered pyrrolidine ring in this series of pyridylether compounds,⁴ efforts have been underway to optimize 5-IA-85380 in order to

© 2012 Elsevier Ltd. All rights reserved.

Address Correspondence to: Jogesh Mukherjee, Ph.D. Preclinical Imaging, B140 Medical Sciences, Department of Radiological Sciences, University of California – Irvine, Irvine, CA- 92697, Tel# (949) 824-2018, Fax# (949) 824-2344, j.mukherjee@uci.edu.

^bPresent address: Lantheus Medical Imaging, N. Billerica, MA

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

accelerate in vivo binding properties. Currently, ^{123}I -5-IA-85380 is the mainstay for SPECT imaging in order to study the downregulation of nAChRs in people with Alzheimer's disease,⁵ substance abuse,^{6,7} and other CNS studies.⁸ ^{123}I -5-IA-85380 has been developed as an agonist for the $\alpha_4\beta_2$ nAChR.⁹

It was anticipated that inclusion of a double bond in the five membered pyrrolidine ring (a) may reduce the basicity of the secondary pyrrolidine nitrogen and therefore assist *in vivo* binding kinetics; (b) may alter the planarity of the five-membered ring to assist in the binding. Conforming to the above concepts, 2-fluoro-3-[2-((*S*)-3-pyrrolinyl)methoxy]pyridine (Nifene), a potential $\alpha_4\beta_2$ nAChR agonist PET imaging agent with reduced scan time (<1 hour), was developed in our laboratory (Figure-1).¹⁰ ^{18}F -Nifene has faster brain imaging kinetics compared to the well-known ^{18}F -2-FA-85380.¹¹

Based on our findings with ^{18}F -nifene, ^{123}I -5-iodo-3-[2-((*S*)-3-pyrrolinyl)methoxy]pyridine (^{123}I -niodene) has been designed as a new $\alpha_4\beta_2$ nAChR iodinated agonist tracer for potential use in SPECT imaging (Figure-1). This new derivative is similar to 5-IA-85380 except that the 4-membered azetidine ring has been replaced with the 5-membered unsaturated pyrrolinyl ring. Lipophilicity of ^{18}F -nifene was found to be -0.52 ,¹² and with the replacement of fluorine (XLOGP = 0.493) in nifene with iodine (XLOGP = 1.489) in niodene, an additive effect is expected to raise the log P of niodene to 0.52. Following the differences in the in vivo kinetic properties of ^{18}F -nifene compared to that of ^{18}F -2-FA-85380, our goal in this work is an attempt to reduce the scan time required for ^{123}I -5-IA-85380 while maintaining the effectiveness and quality of the human brain scans. Reported here is the synthesis and preliminary evaluation of ^{123}I -niodene in rat brain tissue.

Synthesis of niodene and the precursor to prepare radioiodinated niodene was carried out by using our previously reported procedures (Figure-2).^{12,13} Mitsunobu coupling of 3-iodo and 3-bromo pyridinols¹⁴ with (*S*)-*N*-tertbutoxycarbonyl-2-hydroxymethyl-3-pyrroline provided modest yields of 3-[2-((*S*)-*N*-tertbutoxycarbonyl-2-pyrrolinemethoxy)-5-iodopyridine (50%)¹⁵ and 3-[2-((*S*)-*N*-tertbutoxycarbonyl-2-pyrrolinemethoxy)-5-bromopyridine (64%).¹⁷ Deprotection of the *N*-tertbutoxycarbonyl group in **12** using trifluoroacetic acid provided niodene **5** in high yields.¹⁶ In order to prepare radioiodinated niodene, the tributyltin precursor **9** was prepared in low to modest yields using modifications of published procedures.^{1,18}

Binding affinity of niodene was measured in rat brain homogenates using ^3H -cytisine using our previously published methods.^{19,20} Niodene has higher binding affinity ($K_i = 0.27$ nM) compared to nicotine ($K_i = 2.8$ nM) (Figure-3). The affinity of niodene is more similar to the affinity of nifene ($K_i = 0.50$ nM) (Table-1). These findings suggest that the five-membered unsaturated ring system has generally favorable binding characteristics for the $\alpha_4\beta_2$ receptor system in vitro. In the case of ^{18}F -nifene, this in vitro affinity enables in vivo visualization of the $\alpha_4\beta_2$ receptors in rats¹⁰ and monkeys¹¹. Consistent with the observation of niodene and nifene binding affinities, the reported binding affinity of 5-IA-85380 is higher than 2-FA-85380 (Table-1). However, 5-IA-85380 is about 22-fold better than 2-FA-85380,²¹ while niodene is 2-fold better than nifene. Structurally both nifene and niodene are very similar to 2-FA-85380 and 5-IA-85380, respectively (Figure-1). Energy-minimized structures using Alchemy of the four-membered ring structure of the azetidine ring in 2-FA-85380 and 5-IA-85380 render their energies at approx 25–30 kcal/mol compared to 7–9 kcal/mol for the five-membered unsaturated ring structures of nifene and niodene.

Radiolabeling was carried out under different reaction conditions.²² This included using oxidants, chloramine-T as well as hydrogen peroxide/acetic acid (peracetic acid, PAA) mixture. Using sodium iodide-123 in the presence of chloramine-T, radiolabeling of the tin

precursor was carried out under no-carrier and carrier added conditions. Radiolabeling of the precursor did not occur to any significant extent in the absence of added iodine. With added iodine, radiolabeling proceeded in low radiochemical yields (approx 5%) and the product was obtained in low specific activity as shown in the HPLC chromatogram (Figure-4). The specific activity of ^{123}I -niodene was approx. 100 Ci/mmol. In the presence of PAA no radiolabeling was observed. Reasons for the low efficiency of the radioiodination is unclear, since ^{123}I -I-5-A83850 has been prepared in good radiochemical yields and high specific activity.¹ A major difference between ^{123}I -I-5-A83850 and ^{123}I -niodene synthesis procedures is the presence of the olefin in the pyrrolinyl ring of niodene. Although no other major radiolabeled side products were observed, it is possible that there may be interference by the olefin in the radiolabeling process under electrophilic reaction conditions. Additionally, although every effort was made to remove any residual bistrityl tin, it is unclear if this impurity may have been a source of interference. Since it is essential to produce ^{123}I -niodene in high radiochemical yield and high specific activity for in vivo studies, alternative trimethyltin substituted precursors are being considered in order to further examine the efficiency of radioiodination.

Competition of unlabeled niodene and nicotine with ^{18}F -nifene was carried out in rat brain slices.²³ Thalamus to cerebellum ratio in the presence of 10 nM niodene was reduced from 7.3 to 2.5, suggesting a >65% decrease while 10 nM nicotine reduced the ratio to 5.6 (24% decrease) (Figure-5). Higher concentrations of niodene (1 μM) and nicotine (300 μM) displaced over 80% of ^{18}F -nifene. Similar effect was seen in the other receptor regions of frontal cortex, striatum and subiculum. This extent of displacement of ^{18}F -nifene by niodene was significantly greater than nicotine, reflective of the significantly higher affinity of niodene. Nicotine at a high concentration (300 μM) was able to displace ^{18}F -nifene to the same extent as niodene. These experiments suggest that niodene binds at the same $\alpha 4\beta 2$ receptor site as ^{18}F -nifene.

Autoradiography of ^{123}I -niodene in rat brain sections revealed binding of ^{123}I -niodene predominantly to the thalamus region of the brain which is the major target brain region for $\alpha 4\beta 2$ receptors (Figure-6).²⁴ Binding of ^{123}I -niodene was also observed in the cortex with the least amount of binding in the cerebellum. This is consistent with observations with ^{18}F -nifene (Figure-5). With the use of low specific activity ^{123}I -niodene, the binding ratio for thalamus to cerebellum was 4 while cortex to cerebellum ratio was 1.6. The thalamus to cerebellum ratio is lower for ^{123}I -niodene compared to that observed for ^{18}F -nifene, possibly due to the higher concentration of ^{123}I -niodene (5 nM)²⁴ compared to ^{18}F -nifene (2.5 nM)²³ in the incubation. The intensity of binding of ^{123}I -niodene is weak due to the lower specific activity. Improvements in specific activity of ^{123}I -niodene may provide higher target to cerebellum ratios of this potentially useful new SPECT imaging agent.

Based on the in vitro binding experiments niodene demonstrates similar binding behavior like its derivative, nifene, as a potential new radiotracer for SPECT imaging. Niodene exhibits high affinity for the $\alpha 4\beta 2$ receptor and is similar to nifene. Niodene displaces specifically bound ^{18}F -nifene in rat brain slices at low concentrations, indicative of its high affinity. Radiolabeled ^{123}I -niodene has been synthesized and binds to $\alpha 4\beta 2$ receptor rich regions, suggesting its potential use as an in vivo SPECT radiotracer. Further investigations are needed for improvements in reaction conditions in order to increase the radiochemical yield and specific activity of ^{123}I -niodene for in vivo experiments.

Acknowledgments

This research was financially supported by a grant from National Institutes of Health R01 AG029479.

References

1. Musachio JL, Villemagne VL, Scheffel UA, Dannals RF, Dogan AS, Yokoi F, Wong DF. Nucl Med Biol. 1999; 26:201. [PubMed: 10100220]
2. Saji H, Ogawa M, Ueda M, Iida Y, Magata Y, Tominaga A, Kawashima H, Kitamura Y, Nakagawa M, Kiyono Y, Mukai T. Ann Nucl Med. 2002; 16:189. [PubMed: 12126044]
3. Fujita M, Seibyl JP, Vaupel DB, Tamagnan G, Early M, Zoghbi SS, Baldwin RM, Horti AG, Koren AO, Mukhin AG, Khan S, Bozkurt A, Kimes AS, London ED, Innis RB. Eur J Nucl Med Mol Imaging. 2002; 29:183. [PubMed: 11926380]
4. Fan H, Scheffel UA, Rauseo P, Xiao Y, Dogan AS, Yokoi F, Hilton J, Kellar KJ, Wong DF, Musachio JL. Nucl Med Biol. 2001; 28:911. [PubMed: 11711310]
5. Terrière E, Sharman M, Donaghey C, Herrmann L, Lonie J, Strachan M, Dougall N, Best J, Ebmeier KP, Pimlott S, Patterson J, Wyper D. Neurochem Res. 2008; 33:643. [PubMed: 17960479]
6. Brasi JR, Zhou Y, Musachio JL, Hilton J, Fan H, Crabb A, Endres CJ, Reinhardt MJ, Dogan AS, Alexander M, Rousset O, Maris MA, Galecki J, Nandi A, Wong DF. Synapse. 2009; 63:339. [PubMed: 19140167]
7. Cosgrove KP, Mitsis EM, Bois F, Frohlich E, Tamagnan GD, Krantzler E, Perry E, Maciejewski PK, Epperson CN, Allen S, O'Malley S, Mazure CM, Seibyl JP, van Dyck CH, Staley JK. J Nucl Med. 2007; 48:1633. [PubMed: 17873128]
8. Mitsis EM, Cosgrove KP, Staley JK, Bois F, Frohlich EB, Tamagnan GD, Estok KM, Seibyl JP, van Dyck CH. Neurobiol Aging. 2009; 30:1490. [PubMed: 18242781]
9. Caldarone BJ, Wang D, Paterson NE, Manzano M, Fedolak A, Cavino K, Kwan M, Hanania T, Chellapan SK, Kozikowski AP, Olivier B, Picciotto MR, Ghavami A. Psychopharmacology. 2011; 217:199. [PubMed: 21487659]
10. Kant R, Constantinescu CC, Parekh P, Pandey SK, Pan ML, Easwaramoorthy B, Mukherjee J. EJNMMI Research. 2011; 1:6. [PubMed: 22039577]
11. Hillmer AT, Wooten DW, Moirano JM, Slesarev M, Barnhart TE, Engle JW, Nickles RJ, Murali D, Schneider ML, Mukherjee J, Christian BT. Synapse. 2011; 65:1309. [PubMed: 21674627]
12. Pichika R, Easwaramoorthy B, Collins D, Christian BT, Shi B, Narayanan TK, Potkin SG, Mukherjee J. Nucl Med Biol. 2006; 33:295. [PubMed: 16631077]
13. All chemicals and solvents were of analytical or HPLC grade from Aldrich Chemical Co. and Fisher Scientific. *N*-Boc-L-3,4-dehydroproline was purchased from EMD Biosciences, Inc. (San Diego, CA) and 3-Iodo-5-methoxypyridine was purchased from Adesis, Inc. (New Castle, DE). ³H-Cytisine (0.99 GBq/μmol specific activity) was purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA). All water was deionized to specific resistance of 12 mΩ.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). Chromatographic separations were carried out on preparative TLC (silica gel GF 20×20 cm 2000 micron thick; Alltech Assoc. Inc., Deerfield, IL) or silica gel flash column 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 ¹²³I-Sodium Iodide was purchased from MDS Nordion, Canada. Iodine-123 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. Iodine-123 autoradiography studies were carried out by exposing tissue samples on storage phosphor screens. The apposed phosphor screens were read and analyzed by OptiQuant acquisition and analysis program of the Cyclone Storage Phosphor System (Packard Instruments Co., Boston, MA). All animal studies were approved by the Institutional Animal Care and Use Committee of the University of California-Irvine, Irvine, CA.
14. (*S*)-*N*-tert-butoxycarbonyl-2-hydroxymethyl-3-pyrroline (**6**) and 3-bromo-5-hydroxypyridine (**7**) (Figure-2) were prepared as previously reported.

15. *3-[2-(S)-N-tert-butoxycarbonyl-2-pyrrolinemethoxy]-5-iodopyridine (12)*. Boron tribromide (BBr₃ 12 ml; 1M in CH₂Cl₂) was slowly added to a stirred solution of 3-Iodo-5-methoxypyridine (**10**, 471 mg; 2 mmol) in CH₂Cl₂ (10 ml) at -40°C (dry ice bath) and reaction mixture was left overnight stirring with gradual warming to ambient temperature. MeOH (7 ml) was slowly added and the reaction mixture was refluxed for 2 hrs. After removal of solvent, water was added and pH was adjusted to 7–8 by adding Na₂CO₃. The reaction mixture was extracted with EtOAc, dried over Na₂SO₄ and concentrated to yield crude product which was purified by prep-TLC using EtOAc/Hexanes (1:3) to furnish 3-Iodo-5-hydroxypyridine, **11** (300 mg, 68%) as a solid compound. To a solution of **6** (0.20 g, 1.0 mmol), 3-iodo-5-hydroxypyridine (**11**, 0.24 g, 1.1 mmol) and triphenylphosphine (0.40 g, 1.5 mmol) in THF (10 mL), stirred under argon and cooled at 0 °C, was added diisopropyl azodicarboxylate (DIAD; 0.3 mL, 1.5 mmol). The reaction mixture was allowed to come up to ambient temperature over a period of 2 hrs and was stirred overnight. The reaction mixture was evaporated to dryness and the residue was purified by preparative TLC (30% EtOAc in hexane) to give pure product **12** (0.20 g, 50%) as an oil. ¹H-NMR (500 MHz, CDCl₃) δ ppm: 8.41(d, J=15.0, 1H, PyH), 8.25 (d, J=2.0, 1H, PyH), 7.56 (d, J=18.5, 1H, PyH), 5.88 (m, 2H, olefinic), 4.80 (m, 1H, CH-CH₂), 4.34-3.95 (m, 4H, O-CH₂, N-CH₂), 1.48 (s, 9H, Boc). MS, m/z, 403 (100%, [M+H]⁺).
16. *3-[2-(S)-2-pyrrolinemethoxy]-5-iodopyridine (5)*. The BOC protected **12** (90 mg; 0.22 mmol) in dichloromethane (5.0 mL) was treated with trifluoroacetic acid (TFA; 0.5 mL). The solution was stirred for 3 hrs at ambient temperature and concentrated to dryness at 50 °C. Aqueous NaOH (10 mL, 1N) was added and the free base was extracted with dichloromethane (3 × 10 mL). The organic extract was dried over anhydrous MgSO₄, filtered and concentrated to yield **5** as a free base (64 mg, 95%) and was found to be quite pure as per TLC, HPLC, NMR and Mass. ¹H-NMR (500 MHz, CDCl₃) δ ppm: 8.41(d, J=1.5, 1H, PyH), 8.26 (d, J=2.5, 1H, PyH), 7.55 (dd, J=1.5, 2.5, 1H, PyH), 6.02 (m, 1H, olefinic), 5.83 (m, 1H, olefinic), 4.44 (m, 1H, CH-CH₂), 3.94-3.75 (m, 4H, O-CH₂, N-CH₂), 2.26 (brs, 1H, NH). MS, m/z, 303 (78%, [M+H]⁺).
17. *3-[2-(S)-N-tert-butoxycarbonyl-2-pyrrolinemethoxy]-5-bromopyridine (8)*. To a solution of **6** (0.25 g, 1.25 mmol), 3-bromo-5-hydroxypyridine (**7**, 0.24 g, 1.38 mmol; prepared from 3-bromo-5-methoxypyridine) and triphenylphosphine (0.40 g, 1.5 mmol) in THF (10 mL), stirred under argon at 0 °C, was added diisopropyl azodicarboxylate (DIAD; 0.3 mL, 1.5 mmol). The reaction mixture was allowed to come up to ambient temperature over a period of 2 hrs and was stirred overnight. The reaction mixture was evaporated to dryness and the residue was purified by preparative TLC (30% EtOAc in hexane) to give pure product **8** (0.28 g, 64%) as a white solid. ¹H-NMR (500 MHz, CDCl₃) δ ppm: 8.25 (m, 2H, PyH), 7.39 (m, 1H), 5.90 (m, 2H, olefinic), 4.82 (m, 1H, CH-CH₂), 4.35-3.95 (m, 4H, O-CH₂, N-CH₂), 1.48 (s, 9H, Boc). MS, m/z, 355 (100%, [M+H]⁺), 377 (20%, [M+Na]⁺).
18. *5-tributyltin-3-[2-((S)-N-tert-butoxycarbonyl-3-pyrrolinyl)methoxy]pyridine (9)*. To a solution of **8** (20 mg; 0.06 mmol) in anhydrous triethylamine (1 mL) under Argon, bistrabutyltin (100 mg; 0.17 mmol) and Tetrakis(triphenylphosphine)palladium(0) (5 mg) were added and the reaction mixture was refluxed overnight. Dark yellow crude reaction mixture was purified over prep silica gel TLC plate two times using 1/3:EtOAc/Hexanes as a solvent to yield **9** (5 mg; 0.009 mmol) as an oil. ¹H-NMR (500 MHz, CDCl₃) δ ppm: 8.20(m, 1H, PyH), 8.18 (m, 1H, PyH), 7.32 (m, 1H, PyH), 5.88 (m, 2H, olefinic), 4.80 (m, 1H, CH-CH₂), 4.34-3.95 (m, 4H, O-CH₂, N-CH₂), 1.53 (t, J=8.0, 6H, (CH₂CH₂CH₂CH₃)₃), 1.48 (s, 9H, Boc), 1.33(q, J=7.5, 6H, (CH₂CH₂CH₂CH₃)₃), 1.10(t, J=8.0, 6H, (CH₂CH₂CH₂CH₃)₃), 0.90(t, J=7.5, 9H, (CH₂CH₂CH₂CH₃)₃), MS, m/z, 403 (100%, [M+H]⁺).
19. Easwaramoorthy B, Pichika R, Collins D, Potkin SG, Leslie FM, Mukherjee J. Synapse. 2007; 61:29. [PubMed: 17068780]
20. *In Vitro Binding Affinity*: Rat brain homogenate assays using ³H-cytisine were carried out to measure binding affinity of the compounds to α₄β₂ receptors using previously described procedures.¹³ The cerebrum of male Sprague-Dawley rats was isolated and homogenized using Tekmar tissumizer (15 secs, half maximum 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. Binding assays were carried out

in 50 mM Tris buffer at pH 7.0 containing 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂ and 2.5 mM CaCl₂ 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, 0.025 mL (nicotine, nifene and niodene, range 10⁻¹⁰ to 10⁻³M) 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 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 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.): $B = B_0(B_{\max}[L]^n/IC_{50} + [L]^n)$, where B is the total amount of specific binding at a given concentration of unknown compounds as competitor [L], B₀ and B_{max} are the minimum and maximum levels of specific binding, respectively, IC₅₀ is the inflection point of the isotherm, and n is the Hill number, used to determine the number of binding sites. The K_i (inhibition binding constant) of various compounds were calculated using the equation, $K_i = IC_{50}/1 + ([^3H\text{-cytisine}]/K_d)$ using dissociation constant, K_d for ³H-cytisine of 0.59 nM.

21. Valette H, Xiao Y, Peyronneau MA, Damont A, Kozikowski AP, Wei ZL. J Nucl Med. 2009; 50:1349. [PubMed: 19652217]
22. *Radioiodination*: No-carrier-added Na¹²³I (MDS Nordion®, supplied in 0.1N NaOH) (5.0 mCi, approx 100μL) was divided into four different vials. NaI (carrier) 25μg, 10μg, 1μg and 0 (no-carrier) was added in the 4 vials respectively. 1N HCl (20μL), tributyltin precursor **9** (500μg dissolved in 200μL EtOH), and chloramine-T trihydrate (50μL of 1mg/mL aq. stock) were added in sequence and the reaction mixture was incubated at room temp for about 2, 2.5, 3 and 4 hrs respectively. The reaction was quenched by adding NaHSO₃ (100μL of 1mg/mL aq. stock) and saturated NaHCO₃ solution (200μL) was added. The reaction mixture was then extracted with CH₂Cl₂ (2x 400μL), dried (MgSO₄), evaporated (N₂ gas stream) and the crude ¹²³I-N-Boc-Niodene, **13** was purified on HPLC (Econosil C18; 10μm; 250 × 10mm) using CH₃CN/H₂O (0.1%Et₃N): 60/40 at a flow rate of 2.5mL/min and UV detector setting at 287nm (Fig-3). The desired radioactive product **13** was collected at 25 min (Fig-3), evaporated to dryness and deprotected using 20% TFA in CH₂Cl₂ (1 mL) at 80°C (external temperature of heating block) for 30 min. Solvents were subsequently evaporated to dryness, neutralized by NaHCO₃, formulated in saline and passed through 0.22μm filter for biological experiments.
23. *In Vitro ¹⁸F-Nifene Autoradiographic Studies*: 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, 5mmol/L KCl, 2.5 mmol/L CaCl₂, 1 mmol/L MgCl₂, pH 7.4) for 10 min. Subsequently, the preincubation buffer was discarded and the slices were incubated with ¹⁸F-nifene (2.5 nM, specific activity of 2 Ci/μmol) at 37°C for 60 min. Binding was measured in the presence of 10 nM and 1 μM niodene and 10 nM 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 ¹⁸F-nifene in the autoradiograms was evaluated in various brain regions (as digital lights units (DLU/mm²) using the OptiQuant acquisition and analysis program (Packard Instruments Co.).
24. *In Vitro ¹²³I-Niodene Autoradiographic Studies*: Male Sprague-Dawley rats (200 – 250 g) were decapitated, and the brain was rapidly removed and frozen in isopentane at –20° C. Horizontal sections (10 μm thick) containing the cortex, striatum, thalamus, hippocampus and cerebellum were prepared using LEICA CM 1850 cryotome. The temperature for harvesting the brain sections was –20° C; sections were stored at –20° C until use. For competitive binding studies, slides were thawed for approx. 15 mins at ambient temperature and were subsequently pre-incubated for 10 mins at ambient temperature in buffer (120 mmol/L Tris HCl containing 5 mmol/L NaCl, 5mmol/L KCl, 2.5 mmol/L CaCl₂, 1 mmol/L MgCl₂, pH 7.4). The preincubation buffer was then discarded. Subsequently, the slices were treated with incubation buffer containing ¹²³I-niodene (5 nM, specific activity of 0.1 Ci/μmol) at 37°C for 60 min. Nonspecific binding was measured in the

presence of 300 μmol of nicotine. Competitive binding assay with different concentrations of nicotine was also carried out. For each drug concentration, several adjacent brain sections ($n=5$ or more) were used. After incubation, slides were washed twice (2 mins each) with ice-cold incubation buffer, followed by a quick rinse in cold ($0-5\text{ }^{\circ}\text{C}$) deionized water. The slides were then air dried and apposed to phosphor screens (Perkin Elmer medium type, MS, films) for at least 16 hours and read by the Cyclone Phosphor Imaging System (Packard Instruments Co). The amount of bound ^{123}I -niodene 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.). Data from sets of brain sections were pooled to provide average values and standard deviation of ^{123}I -niodene binding.

Figure 1.
Chemical structures of nAChR $\alpha 4 \beta 2$ agonists: Nicotine **1**; 2-F-A85380 **2**; Nifene **3**; 5-I-A-85380 **4**; Niodene **5**.

Figure 2.
Synthesis scheme of niodene **5** and radiolabeling tributyl tin precursor **9**.

Figure 3.
In vitro binding affinity curves of niodene and nicotine measured using rat brain homogenates labeled with ^3H -cytisine.

Figure 4.

A. Radiolabeling of ^{123}I -niodene using sodium ^{123}I -iodide in the presence of chloramine-T.
 B. HPLC purification of ^{123}I -N-BOC-niodene of the reaction carried out in the presence of added 25 μg of sodium iodide. The UV peak corresponding to ^{123}I -N-BOC-niodene is the unlabeled N-BOC-niodene.

Figure 5. Rat brain ^{18}F -nifene autoradiography. A. Total binding of ^{18}F -nifene in different brain regions (FC: frontal cortex; STR: striatum; TH: thalamus; SUB: subiculum; CB: cerebellum); B. Binding of ^{18}F -nifene in the presence of 10 nM nicotine; C. Binding of ^{18}F -nifene in the presence of 10 nM niodene; D. Cerebellum ratio plot shows decrease in ^{18}F -nifene binding in the presence of different concentrations of nicotine (10 nM, 300 μM) and niodene (10 nM, 1 μM).

Figure 6. Rat brain autoradiography of ^{123}I -niodene. A. Rat brain slice showing cortex (COR), thalamus (TH) and cerebellum (CB); B. Same brain slice showing binding of ^{123}I -niodene; C. Displacement of ^{123}I -niodene in the presence of 300 μM nicotine; D. Ratio of ^{123}I -niodene binding in thalamus and cortex versus cerebellum.

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

Compound	Nitrogen Containing Aliphatic ring	Binding affinity, K_i , nM	Reference
Nicotine (1)	5-membered, Saturated, <i>N</i> -CH ₃	2.83	Vs. ³ H-cytisine This work
2-F-A85380 (2)	4-membered, saturated, <i>N</i> -H	1.33	Vs. ³ H-epibatidine ²¹
Nifene (3)	5-membered, unsaturated, <i>N</i> -H	0.50	Vs. ³ H-cytisine ¹⁹
5-I-A85380 (4)	4-membered, saturated, <i>N</i> -H	0.06	Vs. ³ H-epibatidine ²¹
Niodene (5)	5-membered, unsaturated, <i>N</i> -H	0.27	Vs. ³ H-cytisine This work