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Radiosynthesis of a ^{125}I analog of a highly selective alpha3beta4 nicotinic acetylcholine receptor antagonist ligand for use in autoradiography studies

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The $\alpha3\beta4$ subtype of the nicotinic acetylcholine receptors (nAChR) is present in limited but specific areas of the brain unlike the widely distributed $\alpha4\beta2$ nAChR subtype, known to be involved in the addictive effects of nicotine. Recently, the $\alpha3\beta4$ nAChR subtype has been linked to addiction to nicotine as well as other drugs of abuse. However, there have been no subtype-selective $\alpha3\beta4$ nAChR ligands available to study the role of this receptor in drug addiction. Our laboratory has discovered a series of very high affinity and highly selective ligands for the $\alpha3\beta4$ nAChR subtype. We now report the synthesis of a radiolabeled ^{125}I analog of *N*-(2-iodophenyl)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine (AT-1012), a subnanomolar affinity, highly selective $\alpha3\beta4$ nAChR ligand from this series. This analog, [^{125}I] AT-1012, was synthesized by a facile radio-iodination of a tributylstannylated precursor, which gave the radiolabeled compound with high specific activity and radiochemical purity. This high-affinity radioactive $\alpha3\beta4$ nAChR antagonist is very useful as a pharmacological tool in autoradiography studies, to elucidate the localization of the $\alpha3\beta4$ nAChR in the brain and study its pharmacology in the brain reward circuit. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: nicotinic acetylcholine receptor; $\alpha3\beta4$; $\alpha3\beta4$ antagonist; radio-iodinated analog; nAChR

Introduction

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that mediate cation flux. They consist of five subunits arranged around a central pore, allowing influx of cations. There are at least 14 different nAChR subtypes with different combinations of α and β subunits, although homomeric nAChR, particularly the prominent $\alpha7$ nAChR, are also present. nAChRs are widely distributed in the central nervous system (CNS) and the periphery, and are involved in a wide range of physiological processes. The most predominant nAChR subtypes in the CNS are the $\alpha4\beta2$ and the $\alpha7$ nAChR subtypes,[1,2] although other subtypes may be present in high concentrations in localized areas of the brain. One such subtype is the $\alpha3\beta4$ nAChR. Although the $\alpha3\beta4$ nAChR, also referred to as the 'ganglionic nAChR,' predominates in sensory and autonomic ganglia and in the adrenal gland, it is present in high concentrations in the medial habenula and the interpeduncular nucleus in the brain.[1,3] The $\alpha3\beta4$ subtype has recently drawn attention because genetic association studies linked the $\alpha3$, $\alpha5$ and $\beta4$ nAChR subunits to nicotine and alcohol dependence.[4–6] Consistent with these genetic studies, we recently showed that a highly selective $\alpha3\beta4$ nAChR antagonist AT-1001 significantly reduced nicotine self-administration in rats, without affecting food responding.[7] Previous studies also indicated that an antagonist of the $\alpha3\beta4$ nAChR subtype reduces self-administration and rewarding effects of several drugs of abuse, such as morphine, methamphetamine and ethanol,[8–10] suggesting that antagonism of $\alpha3\beta4$ nAChR may be an important mechanism for reducing the rewarding effects of several abused drugs. However, the involvement of the $\alpha3\beta4$ nAChR in the reward circuitry in the brain and in the rewarding effects of multiple abused drugs is not well understood, partly due to the lack of specific ligands for this subtype. We have discovered the first class of truly selective and high-affinity small-molecule antagonists for the $\alpha3\beta4$ subtype nAChR,[7,11] including an iodine-containing compound (AT-1012 in Figure 1). AT-1012 has high binding affinity ($K_i = 0.93$ nM) and very high selectivity for $\alpha3\beta4$ *versus* $\alpha4\beta2$ nAChR subtype (~144 fold). We developed a much needed imaging agent, by replacement of the ^{127}I with its radioactive isotope ^{125}I, without affecting its binding affinity and selectivity at the $\alpha3\beta4$ nAChR subtype. The ^{125}I-labeled AT-1012 can be used in autoradiography studies, to pinpoint the localization of the $\alpha3\beta4$ nAChR subtype in the brain and the reward circuitry. Here, we present the synthesis of the ^{125}I-labeled analog of AT-1012 in high

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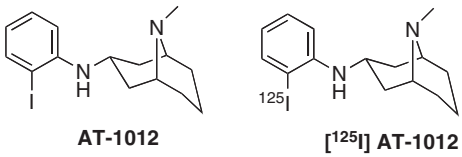


Figure 1. Structure of AT-1012 and [^{125}I] labeled AT-1012.

radiochemical purity and high specific activity, utilizing the stannylated analog (compound **3**) as a precursor to iodination.

Results and discussion

The synthesis route used for the preparation of [^{125}I] AT-1012 is illustrated in Scheme 1. Although unlabeled AT-1012 can be directly synthesized by coupling 9-methyl-9-azabicyclo[3.3.1] nonan-3-amine (**1**) (Scheme 1) with 1,2-diiodobenzene under Buchwald conditions, or by a halogen exchange reaction between the 2-bromoaryl compound **2** and NaI, catalyzed by CuI in presence of N,N′-dimethylethylenediamine,[12] both methods are not practical for preparing the radio-iodinated analog of AT-1012 ([^{125}I] AT-1012) for several reasons. The requisite ^{125}I-labeled 1,2-diiodobenzene is not commercially available for the former method, whereas the dilution of I-125 with CuI in the latter method was unsuitable for obtaining high radiochemical purity and high specific activity due to concerns about the possibility of impurities in the catalyst and dilution with unlabeled CuI. Furthermore, purification problems were encountered in both cases, during the cold runs. Therefore, an alternate method for introducing the radiolabel ^{125}I was employed. The ^{125}I was introduced through a stannylated precursor **3**, shown in Scheme 1. Buchwald coupling of 9-methyl-9-azabicyclo[3.3.1]nonan-3-amine (**1**) (3B Scientific Corp., Libertyville, IL, USA) with excess 1,2-dibromobenzene gave the *N*-(2-bromoaryl) compound **2** in reasonable yield after purification. Compound **2** was then converted to a tributylstannyl derivative (**3**) by treating with 2 equivalents of n-butyllithium, followed by reaction with $^{n}Bu_3SnCl$. The unlabeled 2-iodoaryl compound AT-1012 was then obtained in good yield from the reaction of the stannylated derivative **3** with NaI in presence of H_2O_2 under acidic conditions at room temperature (RT) (Scheme 1). An alternate method of destannylation/iodination that was more suitable for introduction of the ^{125}I radiolabel was further investigated during the cold run. Two different oxidation methods were tried for the reaction with NaI: (i) pre-coated iodination beads and (ii) pre-coated iodination tube (both available from Pierce Biotechnology, Rockford, IL, USA). Although both oxidizing procedures gave the desired iodo compound, iodination with the pre-coated iodination tube was faster and gave a better yield. The radiolabeled ligand, [^{125}I]-AT-1012, was thus successfully obtained using an iodination tube and Na^{125}I to obtain a specific activity of 2200 Ci/mmol (Scheme 1). As with the cold run, purification was carried out on a reverse-phase HPLC column, providing 0.955 mCi of [^{125}I]AT-1012 with a final radiochemical purity of 98.2%. [^{125}I]AT-1012 was analyzed by reverse-phase HPLC, mass spectrometry (MS) and ^{1}H NMR.

Experimental procedures

General

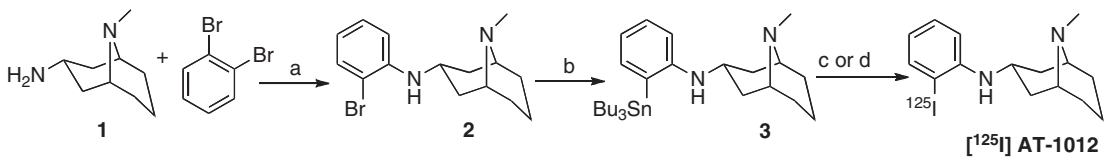
Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 300-MHz or 400-MHz spectrophotometer (Palo Alto, California, USA) using tetramethysilane as the internal standard. Chemical shifts are reported in ppm (parts per million) relative to tetramethylsilane as the internal standard, and signals are quoted as s (singlet), d (doublet), t (triplet) or m (multiplet), and br (broad). Reverse-phase HPLC data were obtained using a Waters 2690 Separations Module (Milford, MA, USA) with photodiode array detector and a Perkin Elmer radioactivity monitor (Waltham, MA, USA). Mass spectra were obtained on a Finnigan LCQ Duo LC-MS/MS (Waltham, MA, USA) atmospheric pressure chemical ionization (API) quadrupole ion trap MS system equipped with an electrospray (ESI) probe. Thin layer chromatography analyses were carried out on commercial pre-coated silica gel 60 F254 plates. Chromatography was carried out using 230–400 mesh silica gel 60 (E. Merck).

N-(2-bromophenyl)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine (2)

To a mixture of 1,2-dibromobenzene (2 eq), $Pd(OAc)_2$ (0.02 eq) and *rac*-BINAP (0.02 eq) in anhydrous toluene (2 mL/mmol), were added 9-methyl-9-azabicyclo[3.3.1]nonan-3-amine (pseudopelleteriene) (**1**) (1 eq) and NaO^tBu (4 eq) under argon. The resulting mixture was heated to 80 °C and stirred at that temperature until no starting material was observed on thin layer chromatography. The reaction mixture was partitioned between ethyl acetate and water. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was subjected to chromatography on silica gel, and eluted with dichloromethane/methanol (10% $cNH_3.H_2O$) (95:5), giving the desired product in 70% yield. Rf: 0.31. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.40 (dd, J = 7.8, 1.5 Hz, 1 H), 7.15 (ddd, J = 8.4, 6.9, 1.2 Hz, 1 H), 6.77 (dd, J = 8.1, 0.9 Hz, 1 H), 6.51 (ddd, J = 7.8, 7.5, 1.2 Hz, 1 H), 4.07 (d, J = 8.1 Hz, 1 H), 4.04–3.84 (m, 1 H), 3.08 (d, J = 10.8 Hz, 2 H), 2.64–2.56 (m, 2 H), 2.51 (s, 3 H), 2.06–1.91 (m, 3 H), 1.59–1.47 (m, 1 H), 1.24 (ddd, J = 12.3, 7.8, 2.7 Hz, 2 H), 1.06–0.94 (m, 2 H); MS (ESI) *m/z* 309, 311 $(M+H)^+$.

9-methyl-*N*-(2-(tributylstannyl)phenyl)-9-azabicyclo[3.3.1] nonan-3-amine (3)

To a solution of *N*-(2-bromophenyl)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine (1 eq) in anhydrous THF (10 mL/mmol) at −78 °C under an atmosphere of argon, was added dropwise, a *n*BuLi solution in hexane (1.6 M, 2 eq). After completion of addition, the mixture was stirred while the temperature was slowly allowed to rise to −30 °C and kept at this temperature for 30 min. Bu_3SnCl (2.0 eq) was then added dropwise and the resulting mixture was stirred at RT for 30 min. The reaction mixture was quenched with water and partitioned between $NaHCO_3$ (aq) and ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was subjected to chromatography on silica gel, eluting with dichloromethane/methanol (10% $cNH_3.H_2O$) (98:2–95:5), to give the desired product in 30% yield.



Scheme 1. Synthesis of the AT-1012 and [^{125}I]-AT-1012. Reagents and conditions: (a) $Pd(OAc)_2$, *rac*-BINAP, NaO^tBu, toluene; (b) $^{n}BuLi$, THF, -78 °C and then $^{n}Bu_3SnCl$; (c) For AT-1012, NaI, H_2O_2, HCl, H_2O-EtOH, RT *or* NaI, EtOH, iodogen tube; (d) For [^{125}I]-AT-1012, Na^{125}I, EtOH, Iodogen tube.

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.18 (d, J = 7.2 Hz, 1 H), 7.17 (td, J = 7.2, 2.0 Hz, 1 H), 6.70 (dd, J = 8.4 Hz, 1 H), 6.66 (td, J = 7.2, 1.2 Hz, 1 H), 3.96–3.82 (m, 1 H), 3.29 (d, J = 8.0 Hz, 1 H), 3.07 (d, J = 10.8 Hz, 2 H), 2.62–2.50 (m, 2 H), 2.50 (s, 3 H), 2.06–1.90 (m, 3 H), 1.58–1.50 (m, 7 H), 1.40–1.28 (m, 6 H), 1.18–0.95 (m, 10 H), 0.89 (t, J = 3.4 Hz, 9 H); MS (ESI) *m/z* 521 (100%), 519 (75%), 517 (50%) $(M+H)^+$.

N-(2-iodophenyl)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine (AT-1012). Synthesis of non-radioactive AT-1012

A Pierce Iodogen tube (Pierce Chemicals, cat# 28601) was pre-wetted with 1 mL of 25 mM Tris·HCl (pH 7.5), decanted and then 20 μL of 25 mM Tris·HCl was added. Next, 5 μL of 9.5 mM NaI in 0.01 M NaOH (0.0475 μmol of NaI) was added. The iodide (I^-) was allowed to oxidize to (I^+) for 6 min at RT, swirling the tube every 30 s. The solution of activated iodine was transferred via syringe (rinsing with 25 μL of Tris buffer) to a vial containing a solution of 9-methyl-*N*-(2-(tributylstannyl)phenyl)-9-azabicyclo[3.3.1]nonan-3-amine (**3**) (0.058 mg, 0.112 μmol) in 50 μL of ethanol. The vial was sealed under argon with a Teflon-lined cap and the reaction solution stirred slowly for 45 min at RT and then diluted with 1 mL of ethyl acetate. The resulting solution was stirred for 2 min and then 165 mg of anhydrous sodium sulfate was added. The mixture was stirred for 10 min, and then filtered, and the solvent evaporated with a slow stream of argon. The crude product was dissolved in 50 μL of ethanol and purified in one injection by reverse-phase HPLC. (In the cold runs, because of the extremely small amounts of reactants and product, only characterization and identification by HPLC and MS and comparison with an authentic standard were performed). The purity was determined by HPLC to be 98%. Column: Varian Pursuit C_{18} (5 micron), 150 × 4.6 mm; solvent: acetonitrile/0.01 M HCl (pH 3) (35/65); flow: 1 mL/min; detection: UV: 245 nm; R_t 6.5 min. MS (ESI): *m/z* (rel. intensity) 357 (M + H, 100).

^{125}I-labeled *N*-(2-iodophenyl)-9-methyl-9-azabicyclo[3.3.1]nonan-3-amine ([^{125}I]AT-1012)

A Pierce Iodogen tube (cat# 28601) was pre-wetted with 1 mL of 25 mM Tris·HCl (pH 7), decanted and then 25 μL of 25 mM Tris·HCl was added. Next, 4.46 mCi (185 mBq) of carrier free $Na^{125}I$ (American Radiolabeled Chemicals; St. Louis, MO, USA) (in 15 μL of 0.01 M NaOH) was added. The radioiodide was allowed to activate for 6 min at RT, swirling the tube every 30 s. The solution of activated radioiodide was transferred via syringe to a vial containing a solution of 9-methyl-*N*-(2-(tributylstannyl)phenyl)-9-azabicyclo[3.3.1]nonan-3-amine (**3**) (0.058 mg, 0.112 μmol) in 50 μL of ethanol. The vial was sealed under argon with a Teflon-lined cap and the reaction solution stirred slowly for 45 min at RT, and then diluted with 1 mL of ethyl acetate. The resulting solution was stirred for 2 min and then 165 mg of anhydrous sodium sulfate was added. The mixture was stirred for 10 min, was then filtered, and the solvent evaporated with a slow stream of argon. The crude radio-iodinated product was dissolved in 50 μL of ethanol and purified in 1 injection by reverse-phase HPLC, using the same column conditions as with the cold run. The total activity (0.955 mCi, 21.4% radiochemical yield) was determined by counting an aliquot using a Packard Cobra Gamma Counter. The radiochemical purity and specific activity were determined by HPLC with a Perkin Elmer Radiomatic Pro FSA 610TR detector, and were found to be 98.23% and 2200 Ci/mmol, respectively.

Conclusions

An efficient and concise route to the synthesis of a useful α3β4 nAChR radioligand [^{125}I]-AT-1012 has been developed. The high radiochemical purity enables this radioligand to be used to successfully label and localize the populations of the α3β4 nAChR subtype in the brain using autoradiography techniques. The results of these labeling studies are to be reported elsewhere shortly.

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Conflict of Interest

The authors did not report any conflict of interest.

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