SUPPORTING INFORMATION

Novel alpha3beta4 nicotinic acetylcholine receptor-selective ligands. Discovery, structure-activity studies and pharmacological evaluation.

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Contents:

EXPERIMENTAL DETAILS

General: ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer (300 MHz and 75 MHz, respectively) and are internally referenced to chloroform at δ 7.27. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (Hz), integration, and assignment. Data for ^{13}C are reported in terms of chemical shift. Melting points were obtained on a Thomas Hoover melting point apparatus. Mass spectra were obtained using a ThermoFinnigan LCQ Duo LC/MS/MS instrument and an electrospray ionization probe. Thin-layer chromoatgraphy was run on Analtech Uniplate silica gel TLC plates. Flash chromatography was carried out using silica gel, Merck grade 9385, 230-400 mesh. The purity of the final compounds reported was established using HPLC, using the following conditions: column: Phenomenex Synergi 4m Fusion RP, 250x4.60 mm; mobile phase: (A) MeCN (0.1% TFA):(B) H_2O (0.1% TFA) (gradient 20%A to 100%A, 15 mins); flow rate: 1 mL/min; detection: PDA 254 nm. The purity of all final compounds was greater than 95%.

General procedure for the synthesis of compounds 5, 12 and 13 (Scheme 1).

9-methyl-*N*-phenyl-9-azabicyclo[3.3.1]nonan-3-amine (15). A solution of pseudopelleteriene hydrochloride (**14)** (4.8 g, 31.4 mmol) and aniline (5.8 g, 5.7 mL) in benzene (25 mL) was treated with 5Å molecular sieves (1 g) and refluxed under argon for five days, using a Dean-Stark trap filled with 3Å molecular sieves. The mixture was cooled and filtered through Celite, evaporated to dryness and re-dissolved in methanol (20 mL). Sodium cyanoborohydride (1.97 g, 31.4 mmol) was then added and the solution was stirred under argon at room temperature 16 h. The reaction was then cooled to 5^{o}C, acidified with dilute hydrochloric acid and the methanol was evaporated. The pH of the resulting solution was brought to 11 with

potassium carbonate and the product extracted with chloroform (2X), dried (sodium sulfate) and evaporated to an oil. The mixture was purified by flash chromatography eluting with 0–8% methanol containing 5% of 28% ammonium hydroxide/methylene chloride to give 2.08 g recovered **14** and 1.80 g product **15** (44% based on consumed **14**).

2-chloro-*N*-(9-methyl-9-azabicyclo[3.3.1]nonan-3-yl)-*N*-phenylacetamide (16). To a solution of **15** (609 mg, 2.66 mmol) in methylene chloride (10 mL) was added triethylamine (807 mg, 1.11 mL) followed by chloroacetyl chloride (600 mg, 423 μL) dropwise under argon. The solution was refluxed 24 h, stirred at room temperature an additional 36 h and evaporated. The mixture was purified by flash chromatography eluting with 0–6% methanol containing 5% of 28% ammonium hydroxide/methylene chloride to give **16** as a white solid (576 mg, 71%).

1-(6-benzyl-6-azabicyclo[3.2.1]octan-3-yl)indolin-2-one (12) was obtained as a light yellow sticky oil, in 38% yield, starting from 6-benzyl-6-azabicyclo[3.2.1]octan-3-one (**19**).[1,2] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, J=7.8 Hz, 1H), 7.45 (d, J=7.2 Hz, 2H), 7.38-7.22 (m, 5H), 7.05 (td, J=7.5, 0.9 Hz, 1H), 4.75 (p, J=10.0 Hz, 1H), 3.97 (d, J=12.9 Hz, PhC**H**$_2$), 3.85 (d, J=12.9 Hz, PhC**H**$_2$), 3.49 (s, CO-C**H**$_2$), 3.22 (br, m, 1H), 2.90 (d, J=8.7 Hz, 1H), 2.64 (br, 1H), 2.52-2.44(m, 1H), 2.26-2.08(m, 2H), 2.02-1.86(m, 2H), 1.89 (d, J=11.7 Hz, 1H), 1.64 (br, 1H). MS *m/z* (ESI: 333.2 $[M+H]^+$.

1-(6-methyl-6-azabicyclo[3.2.1]octan-3-yl)indolin-2-one (13) was obtained as a brownish oil, in 3% overall yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (d, J=7.5 Hz, 2H), 7.04-6.95 (m, 2H), 4.60 (hept, J=6.0 Hz, C(3)**H**, 1H), 3.46 (s, CO-C**H**$_2$), 3.19 (br, t, J=4.7 Hz, 1H), 2.96-2.80 (m, 2H), 2.53 (s, C**H**$_3$), 2.58-2.50(m, 1H), 2.31 (td, J=12.3, 1.8 Hz, 1H), 2.20 (td, J=12.3, 1.5 Hz, 1H), 1.97-1.86(m, 2H), 1.72-1.62 (m, 1H), 1.64 (d, J=11.1 Hz, 1H). MS *m/z* (ESI: 257.1 $[M+H]^+$.

General procedure for synthesis of compounds 8 and 9 (Scheme 2)

Ethyl 3-oxo-9-azabicyclo[3.3.1]nonane-9-carboxylate (17). A mixture of pseudopelletierine hydrochloride (**14)** (3.1 g, 20 mmol), ethyl chloroformate (4.0 mL, 20 mmol), K_2CO_3 (560 mg, 4 mmol), and toluene (40 mL) was stirred at 90–100°C for 8 h. The mixture was treated with hydrochloric acid (2 M, 30 mL) after cooling to room temperature. The aqueous phase was separated and extracted twice with ethyl acetate (30 mL). The combined organic phase was washed with saturated $NaHCO_3$ (30 mL) and brine, dried over sodium sulfate, and evaporated to a brownish oil (3.8 g, 83%). Compound **17** was obtained as a colorless semisolid (3.5 g, 76%) after filtration through a silica gel pad, eluting with ethyl acetate/hexanes (1:1).

Ethyl 3-(((trifluoromethyl)sulfonyl)oxy)-9-azabicyclo[3.3.1]non-2-ene-9-carboxylate (18). A solution of **17** (1.13 g, 5.00 mmol) in THF (10 mL) was added dropwise (1mL/min) to a solution of sodium bis(trimethylsilyl)amide [$NaN(TMS)_2$] (7.5 mL, 1.0 M in THF, 7.5 mmol) at –78°C, and the mixture was stirred at -78°C for 2 h. Then, a solution of N-(5-chloro-2-pyridyl) triflimide (2.9 g, 7.4 mmol) in THF (10 mL) was added in one portion at -78°C. The resultant mixture was stirred at the same temperature for 2 h, warmed to 0°C; ether (50 mL) was added, followed by addition of saturated sodium bicarbonate (15 mL). The aqueous was separated and extracted with ether (2 X 25 mL). The combined organic solution was dried over potassium carbonate and evaporated to give light yellow oil, which was subjected to chromatography on silica gel, eluting with a solvent mixture of ethyl acetate (20%) and hexanes, to give 0.71 g (78%) of **18** and 0.53 g of starting material **17**.

Ethyl 3-(pyridin-3-yl)-9-azabicyclo[3.3.1]non-2-ene-9-carboxylate (7). A mixture of **18** (343 mg, 1.00 mmol), 3-pyridineboronic acid (135 mg, 1.10 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol), K_3PO_4 (320 mg, 1.50 mmol), and dioxane (5 mL) was stirred at 85^{o}C overnight (14 h).

The mixture was treated with NaOH (2 M) to strong basic (pH > 12) and extracted with ethyl acetate (3 X 10 mL). The extract was washed with brine, dried over sodium sulfate, and evaporated to dryness. The residue was subjected to chromatography on silica gel, eluting with a solvent mixture of ethyl acetate (50%) and hexanes to afford 180 mg of desired product **7** as a colorless oil (67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (br, s, 1H), 8.50 (dd, J=4.6, 1.2 Hz, 1H), 7.67 (br, d, J=8.4 Hz, 1H), 7.27 (d, J=6.6 Hz, 1H), 6.16 (dd, J=17.6, 5.2 Hz, C=C**H,** 1H), 4.94-4.58 (m, 2H), 4.17 (q, J=7.2 Hz, MeC**H**$_2$, 2H), 3.02-2.84 (m, 1H), 2.30 (dd, J=17.6, 6.4Hz, 1H), 1.90-1.54(m, 6H), 1.23 (t, J=7.2Hz, C**H**$_3$, 3H).

3-(9-methyl-9-azabicyclo[3.3.1]non-2-en-3-yl)quinoline (9) was obtained as an oil, starting from 9-methyl-9-azabicyclo[3.3.1]nonan-3-one and quinolin-3-ylboronic acid, in 10% overall yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.08 (d, J=2.4 Hz, 1H), 8.08 (d, J=8.4 Hz, 1H), 8.05 (d, J=2.4 Hz, 1H), 7.81 (d, J=8.0 Hz, 1H), 7.68 (ddd, J=8.4, 6.8, 1.6 Hz, 1H), 7.54 (ddd, J=8.4, 6.8, 1.2, 1H), 6.33 (dt, J=4.2, 1.8 Hz, C=C**H,** 1H), 3.52 (br, s, 1H), 3.29 (br, m, 1H), 2.92 (dd, J=18.6, 6.6Hz, 1H), 2.49 (s, C**H**$_3$, 3H), 2.24 (dd, J=18.0, 1.0Hz, 1H), 2.08-1.96(m, 2H), 1.72-1.48 (m, 4H). MS *m/z* (ESI: 265.1 $[M+H]^+$.

General procedure for synthesis of compounds 10 and 11 (Scheme 3)

6-benzyl-6-azabicyclo[3.2.1]oct-2-en-3-yl trifluoromethanesulfonate (20). A solution of 6-benzyl-6-azabicyclo[3.2.1]octan-3-one (**19**)[39,40] (1.06 g, 5.00 mmol) in THF (10 mL) was added dropwise (1mL/min) to a solution of sodium bis(trimethylsilyl)amide [$NaN(SiMe_3)_2$] (7.5 mL, 1.0 M in THF, 7.5 mmol) at -78°C, and the mixture was stirred at -78°C for 2 h. Then, a solution of N-(5-chloro-2-pyridyl) triflimide (2.9 g, 7.4 mmol) in THF (10 mL) was added in one portion at -78°C. The resultant mixture was stirred at the same temperature for 2 h, warmed to 0°C; ether (50 mL) was added, followed by addition of saturated sodium bicarbonate (15 mL). The aqueous

was separated and extracted with ether (2 X 25 mL). The combined organic solution was dried over potassium carbonate and evaporated to give light yellow oil, which was subjected to chromatography on silica gel, eluting with a mixture solvent of ethyl acetate (20%) and hexanes, to give two fractions; **21** (0.31 g, 18%) was obtained from fraction 1 and its isomer (0.62 g, 36%) from fraction 2.

3-(6-benzyl-6-azabicyclo[3.2.1]oct-3-en-3-yl)quinoline (11) was obtained as a light yellow oil, starting from **20** and quinolin-3-ylboronic acid, in 32% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.05 (d, J=2.0 Hz, 1H), 8.06 (d, J=8.4 Hz, 1H), 8.00 (d, J=1.6 Hz, 1H), 7.78 (dd, J=8.0, 1.2 Hz, 1H), 7.70-7.28(m, 7H), 6.75 (d, J=6.8 Hz, C=C**H,** 1H), 4.01 (d, J=10.8 Hz, PhC**H**$_2$, 1H), 3.86 (d, J=10.8 Hz, PhC**H**$_2$, 1H), 3.61 (s, 1H), 3.16 (d, J=9.2 Hz, 1H), 2.97 (dd, J=9.0, 4.6 Hz, 1H), 2.89-2.82 (m, 1H), 2.80 (d, J=17.2 Hz, 1H), 2.64 (d, J=17.6 Hz, 1H), 2.08-2.00 (m, 1H), 1.85 (d, J=10.4 Hz, 1H).

In vitro Testing of Ligands

Cell Culture. KXα3β4R2 and KXα4β2R2 cells, obtained from Dr. Kenneth Kellar, were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 0.5% penicillin/streptomycin, and 0.4 mg/mL of geneticin. The cells are maintained in an atmosphere of 7.5% CO_2 in a humidified incubator at 37ºC. For binding assays, cells are plated on 100-mm dishes. For functional assays, the cells are seeded into 96-well collagen-coated plates (Becton Dickinson Biocoat) at a density of approximately 50,000 cells/well. Cells seeded at this density grow into a confluent monolayer in 24 to 30 h.

Binding Assays. Cells were harvested by scraping the plates with a rubber policeman and then centrifuged at 500 x *g* (2200 rpm) for 10 min. The cell pellet was suspended in Tris buffer, homogenized in a Polytron Homogenizer, and centrifugation repeated twice at 20,000 x *g*

(13,500 rpm) for 20 min. Cell membranes were finally suspended in 5 mL of Tris buffer to determine their protein content. For binding, the cell membrane was incubated with the test compounds at concentrations ranging from 10^{-5} to 10^{-10} M in the presence of 0.3 nM of [^{3}H]epibatidine. After 3 h of incubation at room temperature, samples were filtered through glass fiber filters, presoaked in 0.1% polyethyleneimine (PEI), by using a Tomtec cell harvester. Filters were counted on a betaplate reader (Wallac). Nonspecific binding was determined by using 0.1 μM of the unlabeled epibatidine.

Binding to the α7 nAChR was conducted on rat brain membranes. Rats were decapitated and brains quickly removed. The brains were homogenized and centrifuged twice as described above. Membranes were suspended in buffer containing 1.4 mM NaCl, 1.5 mM KCl, 2.0 mM, $CaCl_2$, 1.0 mM $MgCl_2$, 25 mM HEPES, using 40 mL per gram original wet weight. Binding was conducted for 2 h with 1.0 nM [^{125}I] α-bungarotoxin. Samples were filtered as described above. IC_{50} values and Hill coefficients were determined by using the program PRISM. Ki values were calculated using the Cheng Prusoff transformation:

$$Ki = \frac{IC50}{1+L/Kd}$$

Where, L is radioligand concentration and Kd is the binding affinity of the radioligand, as determined previously by saturation analysis.

REFERENCES

1. Bartlett, P. A.; McQuaid, L. A. Total synthesis of (.+-.)-methyl shikimate and (.+-.)-3-phosphoshikimic acid. *J. Amer. Chem. Soc.* **1984**, *106*, 7854-7860.

2. Pitner, J. B. P.; Abraham, P.; Joo, Y. J.; Triggle, D. J.; Carroll, F. I. Synthesis and stereoselective reduction of (±)−, (+)− and (-)-6-substituted-6-azabicyclo[3.2.1]octan-3-one. *J. Chem. Soc. Perkin Trans. 1* **1991**, 1375-1381.