Supporting Information

The Discovery and Structure-Activity Relationship of Quinuclidine Benzamides as α7 Nicotinic Acetylcholine Receptor (nAChR) Agonists.

Alice L. Bodnar, Luz A. Cortes-Burgos, Karen K. Cook, Dac M. Dinh, Vincent E. Groppi, Mihaly Hajos, Nicole R. Higdon, William E. Hoffmann, Raymond S. Hurst, Jason K. Myers,* Bruce N. Rogers, Theron M. Wall, Mark L. Wolfe, Erik Wong

Contents.

List of Abbreviations:

DCC	N,N'-dicyclohexylcarbodiimide
DIC	N,N'-diisopropyl carbodiimide
BOP	(Benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate
PyBOP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
BOPCl	Phosphoric acid bis(2-oxooxazolidide) chloride
FLIPR	Fluorescence Imaging Plate Reader
nAChR	Nicotinic acetylcholine receptors
DPPA	Diphenylphosphoryl azide
DPPC	Diphenylphosphoryl chloride
MLA	Methyllycaconitine
TFA	Trifluoroacetic acid
TEA	Triethylamine
HOBt	1-Hydroxybenzotriazole

General procedure for parallel libraries using DPPA and DPPC as the coupling regent. Amide forming reactions were conducted either in vials or in Robbins blocks. TEA (50μL, 0.35mmol) was added to a suspension of carboxylic acid (0.35mmol) in CH_2Cl_2 (1mL) followed by DPPA (65μL, 0.3mmol, or DPPC). The resulting solution was vortexed briefly and allowed to sit for 30 minutes. The appropriate amine was added via syringe (for amines 1,2,3, and 7) or as a solution in toluene (1M, 0.2mL, 0.2mmol, for all quinuclidine isomers). The resulting solution was vortexed and allowed to sit overnight. Methanol (2mL) was added and if a precipitate was present, it was filtered. The resulting solution was poured onto a column of AG50Wx2 (H^+ form, 400-500mg). The column was washed with methanol (20mL) then methanol/acetonitrile (3:1, 10mL). The product was eluted with methanol/acetonitrile/TEA (3:1:0.2, 10mL) into a pre-tared vial. The solvents were removed *in vacuo* to yield the desired product. All products were identified as the protonated molecular ion by electrospray mass spectrometry (ESMS). Purity was assessed by HPLC using a Vydac C18 column and a mobile phase consisting of a mixture of water (+0.1% TFA) and CH_3CN (+0.06%TFA) using UV detection. The average purity for all compound reported was 98% with a range from 78-100%. The average yield was 81% with a range from 31-100%.

N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride (PNU-282987). TEA (56mL, 0.4mol) was added to a solution of (R)-3-aminoquinuclidine dihydrochloride (40g, 0.2mol) in water (200mL). The resulting mixture was diluted with CH_3CN (1L) then 4-chlorobenzoic acid (37.7g, 0.24mol) and HOBt (32.4g, 0.24mol) were added. DIC (37.5mL, 0.24mol) was then added over 5 minutes. The resulting mixture was allowed to stir overnight. The reaction volume was reduced by approximately one half *in vacuo*. Aqueous HCl (1N, 0.5L) was added and a majority of the remaining organic solvents were removed *in vacuo* to yield an aqueous suspension. This was diluted with water (1L), filtered, and washed with CH_2Cl_2 (3x300mL, discarded). The aqueous layer was basified with aqueous sodium hydroxide (50%) to pH>12. The resulting precipitated product

was collected by filtration, washed with water, and dried at 60°C under vacuum. The solid was dissolved in methanolic HCl (1N, 500mL) then evaporated to dryness. The residue was dissolved in a minimum of hot methanol. Absolute ethanol (500mL) was added and the material was again evaporated to dryness. The resulting solid was dissolved in hot absolute ethanol (400mL). One volume of *t*-BuOH (400mL) was added and the mixture allowed to sit at room temperature overnight. The resulting crystals were collected by filtration, washed with a small amount of IPA and dried at 100°C under vacuum to give N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride as a white crystalline solid (42.5g, 71%). ^{1}H NMR (400MHz, DMSO-d_6): δ 1.70 (m, 1 H), 1.89 (m, 1 H), 2.09 (m, 2 H), 2.18 (m, 1 H), 3.18 (m, 4H), 3.3 (m), 3.60 (m, 1 H), 4.31 (m, 1 H), 7.56 (d, J=8.5Hz, 1 H), 7.98 (d, J=8.5Hz, 1 H), 8.89 (d, 6.1Hz, 1H), 10.60 (s, 1H). ^{13}C NMR (100MHz, DMSO-d_6): δ 165.5, 136.2, 132.5, 129.4, 128.2, 50.3, 45.3, 44.8, 44.7, 24.2, 21.3, 17.0. IR (diffuse reflectance) 2918, 2642, 2603, 2555, 2527, 2513, 2477, 1655, 1596, 1542, 1488, 1327, 1272, 837, 757 cm $^{-1}$ MS (EI) m/z (rel. intensity) 264 (M+, 32), 141 (52), 139 (99), 125 (46), 111 (68), 109 (78), 96 (50), 84 (41), 82 (41), 75 (45), 70 (55). HRMS (FAB) calculated for $C_{14}H_{17}ClN_2O$+H 265.1107, found 265.1115. % Water (KF): 2.36. $[\alpha]^{25}_{D}$ 12 (c 1.01, methanol). Anal. ($C_{14}H_{17}ClN_2O \cdot HCl$ + 2.36% water): C, H, N.

N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-methoxybenzamide. DPPA (675µL, 3.13mmol) was added to a solution of 4-methoxybenzoic acid (570mg, 3.75mmol) and TEA (700µL, 5mmol) in CH_2Cl_2 (5mL). The resulting solution was stirred at room temperature for 30 minutes. A solution of (*R*)-3-aminoquinuclidine (2.5mmol) in CH_2Cl_2 (5mL) was added. The reaction mixture was stirred for 24 hours then diluted with methanol (10mL). The resulting solution was poured through a column of Dowex 50W-X4 (8mL, H^+ form). The resin was washed with methanol then the product was eluted with MeOH/TEA/toluene (8:1:1). The solvents were removed *in vacuo* then the product was dried at 70 °C under high vacuum to give the desired product as a white solid (232mg, 36%). ^{1}H NMR (300MHz, MeOH-d_4): δ 1.54 (m, 1 H), 1.78 (m, 1 H), 1.90 (m, 2 H), 2.04 (m, 1 H), 2.84 (m, 4H), 3.00 (m, 1H), 3.29 (m), 3.86 (s, 3H), 4.12 (m, 1H), 6.99 (d, J=8.8Hz, 2 H), 7.82 (d, J= 8.9Hz, 2 H). ^{13}C NMR (100MHz,): δ 170.4, 164.0, 130.4, 128.0, 114.7, 56.0, 54.7, 48.8, 47.9, 47.5, 27.0, 26.4, 20.8. IR (diffuse reflectance) 3322, 2940, 2867, 1626, 1606, 1540, 1504, 1331, 1323, 1315, 1255, 1176, 847, 769, 613 cm $^{-1}$. MS (EI) m/z 260 (M+), 135, 125, 108, 96, 92, 83, 77, 70, 68, 64. Anal. ($C_{15}H_{20}N_2O_2$): C, H, N.

N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-methylthiobenzamide hydrochloride. DPPA (450µL, 2.1mmol) was added dropwise to a solution of 4-phenylthiobenzoic acid (421mg, 2.5mmol) and TEA (695µL, 5mmol). After stirring 30 minutes, a solution of (R)-3-aminoquinuclidine in DMF (0.5M, 3.3mL, 1.7mmol) was added. The resulting mixture was stirred for 24 hours. Methanol was added and the reaction mixture was poured through a column of AG50Wx2 resin (H^+ form). The column was washed with methanol and the product was eluted with 5% TEA in methanol. Toluene was added and the solvents were removed by evaporation. Methanolic HCl was added and the solvents were again removed by evaporation. Recrystallization from methanol-acetonitrile gave the product as a white solid (157mg, 30%). ^{1}H NMR (300MHz, DMSO-d_6): δ 1.69 (m, 1 H), 1.89(m, 2 H), 2.08(m, 1 H), 2.16 (m, 1 H), 2.52(s, 3 H), 3.17 (m, 4H), 3.25-3.35(m+H_2O), 3.58(m, 1 H), 4.29(m, 1 H), 7.33(d, J=8.5Hz, 2 H), 7.90(d, J=8.5Hz, 2 H), 8.72(d, J=6.2Hz, 1 H). ^{13}C NMR (100MHz, MeOH-d_4): δ 170.3, 146.0, 130.9, 129.1, 126.2, 53.3, 47.7, 47.3, 46.7, 25.8, 22.9, 18.6, 14.8. IR (diffuse reflectance) 3254, 2955, 2916, 2635, 2596, 2552, 2538, 2484, 1650, 1595, 1525, 1486, 1313, 835, 758 cm $^{-1}$. MS (EI) m/z 276 (M+), 151, 125, 109, 96, 84, 83, 82, 79, 77, 70. HRMS (FAB) Calculated for $C_{15}H_{20}N_2OS$+H 277.1375, found 277.1380. $[\alpha]^{25}_{D}$ 10 (c 0.93, methanol). Anal. ($C_{15}H_{20}N_2OS \cdot HCl$): C, H, N.

N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-bromobenzamide. DPPA (1.34mL, 6.25mmol) was added dropwise to a solution of 4-bromobenzoic acid (1.51g, 7.5mmol) and TEA (1mL, 7.5mmol). After stirring 20 minutes, this solution was transferred via cannula to a solution of (R)-3-aminoquinuclidine dihydrochloride made by dissolving the salt in water (3mL) and adding TEA (2.8mL) and DMF (25mL). The resulting mixture was stirred for 2 hours. Methanol was added and the reaction mixture was poured through a column of AG50Wx2 resin (H^+ form). The column was washed with methanol and the product was eluted with 5% TEA in methanol. Toluene was added and the solvents removed by evaporation to give the product as a white solid (1.21mg, 78%). ^{1}H NMR (400MHz, MeOH-d_4): δ 1.53 (m, 1 H), 1.77 (m, 2 H), 1.89 (m, 1 H), 2.01 (m, 1 H), 2.82, (m, 4H), 2.98 (m, 1H), 3.30 (m, 1H), 4.11 (m, 1 H), 7.63 (d, J=8.6, 2H), 7.74 (d, J=8.5, 2H). ^{13}C NMR (100MHz, MeOH-d_4): δ169.8, 135.0, 132.7, 130.4, 127.0, 54.5, 49.0, 47.9, 47.5, 26.9, 26.3, 20.8. IR (diffuse reflectance) 3294, 2956, 2935, 2868, 1629, 1592, 1540, 1481, 1331, 1321, 1058, 1011, 838, 761, 666 cm $^{-1}$ MS (EI) m/z 308 (M+), 184, 124, 109, 103, 96, 76, 75, 58, 56, 54. HRMS (FAB) Calculated for $C_{14}H_{17}BrN_2O$+H 309.0603, found 309.0602. $[\alpha]^{25}_{D}$ 29 (c 0.92, methanol). Anal. ($C_{14}H_{17}BrN_2O$): C, H, N.

N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-iodobenzamide. DPPA (1.34mL, 6.25mmol) was added dropwise to a solution of 4-iodobenzoic acid (1.86g, 7.5mmol) and TEA (1mL, 7.5mmol). After stirring 20 minutes, this solution was transferred via cannula to a solution of (R)-3-aminoquinuclidine dihydrochloride made by dissolving the salt in water (3mL) and adding TEA (2.8mL) and DMF (25mL). The resulting mixture was stirred for 2 hours. Methanol was added and the reaction mixture was poured through a column of AG50Wx2 resin (H^+ form). The column was washed with methanol and the product was eluted with 5% TEA in methanol. Toluene was added and the solvents removed by evaporation to give the product as a white solid (1.27g, 71%). ^{1}H NMR (400MHz, MeOH-d_4): δ 1.53 (m, 1 H), 1.76 (m, 2 H), 1.88 (m, 1 H), 2.02 (m, 1 H), 2.83, (m, 4H), 2.97 (m, 1H), 3.30 (m), 4.11 (m, 1 H), 7.60 (d, J=8.5, 2H), 7.74 (d, J=8.5, 2H). ^{13}C NMR (100MHz, MeOH-d_4): δ•170.0, 138.9, 135.5, 130.2, 99.1, 54.5, 48.9, 47.8, 47.5, 26.9, 26.3, 20.8. IR (diffuse reflectance) 3299, 2955, 2935, 2868, 1629, 1587, 1550, 1536, 1475, 1330, 1322, 1008, 836, 778, 759 cm $^{-1}$ MS (EI) m/z 356 (M+), 231, 96, 81, 79, 76, 70, 57, 56, 54, 51. HRMS (FAB) Calculated for $C_{14}H_{17}IN_2O$+H 357.0465, found 357.0471. $[\alpha]^{25}_{D}$ 26 (c 1.00, methanol). Anal. ($C_{14}H_{17}IN_2O$): C, H, N.

N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-fluorobenzamide. DPPA (1.34mL, 6.25mmol) was added dropwise to a solution of 4-fluorobenzoic acid (1.05g, 7.5mmol) and TEA (1mL, 7.5mmol). After stirring 20 minutes, this solution was transferred via cannula to a solution of (R)-3-aminoquinuclidine dihydrochloride made by dissolving the salt in water (3mL) and adding TEA (2.8mL) and DMF (25mL). The resulting mixture was stirred for 2 hours.

Methanol was added and the reaction mixture was poured through a column of AG50Wx2 resin (H^+ form). The column was washed with methanol and the product was eluted with 5% TEA in methanol. Toluene was added and the solvents removed by evaporation to give the product as a white solid (945mg, 76%). ^{1}H NMR (400MHz, MeOH-d_4): δ 1.54 (m, 1 H), 1.77 (m, 2 H), 1.89 (m, 1 H), 2.03 (m, 1 H), 2.79, (m, 4H), 2.99 (m, 1H), 3.30 (m), 4.12 (m, 1 H), 7.19 (dd, J=8.8, 8.8, 2H), 7.89 (dd, J=8.8, 5.3, 2H). ^{13}C NMR (100MHz, MeOH-d_4): δ 169.7, 166.2 (d, J=249Hz), 132.3 (d, J=3.1Hz), 131.1 (d, J=8.9Hz), 116.3 (d, J=22Hz), 54.5, 48.9, 47.9, 47.5, 26.9, 26.3, 20.7. IR (diffuse reflectance) 3265, 2945, 2931, 2871, 1644, 1628, 1602, 1544, 1502, 1327, 1318, 1229, 1221, 1158, 848 cm^{-1}. MS (EI) m/z 248 (M+), 123, 110, 97, 94, 84, 82, 80, 72, 69, 57. HRMS (FAB) calcd for $C_{14}H_{17}FN_2O$+H 249.1403, found 249.1406. % Water (KF): 1.31. $[\alpha]^{25}_D$ 36 (c 0.81, methanol). Anal. (C_{14} H_{17} F N_2 O + 1.31% H_2O): C, H, N.

Functional High-Throughput Screens for α7/5-HT$_3$ chimera, 5-HT$_3$, neuromuscular junction ($\alpha_1\beta_1\gamma\delta$) and ganglionic ($\alpha_3\beta_4$) nAChRs: The α 7/5-HT$_3$ chimera and the 5-HT$_3$ receptor were stably expressed in SH-EP1 cells. TE671 and SH-SY5Y cells were used as an endogenous source for neuromuscular junction and ganglionic nAChRs, respectively[1]. All functional high throughput screens were conducted as calcium flux assays using the Fluorescence Imaging Plate Reader (FLIPR, Molecular Devices). Transfected SH-EP1 cells were grown in minimal essential medium (MEM) containing nonessential amino acids supplemented with 10% fetal bovine serum, L-glutamine, 100 units/ml penicillin/streptomycin, 250 ng/ml fungizone, 400 µg/ml Hygromycin-B, and 800 µg/ml Geneticin. TE671 and SH-SY5Y cells were grown according to published methods. All cells were grown in a 37° C incubator with 6% CO2 . The cells were trypsinized and plated in 96 well plates with dark side walls and clear bottoms (Corning #3614) at density of 26×10^4 cells per well two days before analysis. Cells were loaded in a 1:1 mixture of 2 mM Calcium Green-1, AM (Molecular Probes) prepared in anhydrous dimethylsulfoxide and 20% pluronic F-127 (Molecular Probes). This reagent was added directly to the growth medium of each well to achieve a final concentration of 2 µM Calcium Green-1, AM. Cells were then incubated in the dye for one hour at 37° C and then washed with 4 cycles of Bio-Tek plate washer. Each cycle was programmed to wash each well four times with Mark's Modified Earle's Balanced Salt Solution (MMEBSS) composed of (in mM): $CaCl_2$ (4), $MgSO_4$ (0.8), NaCl (20), KCl (5.3), D-Glucose (5.6), Tris-HEPES (20), N- methyl-D-glucamine (120), pH 7.4. After the third cycle, the cells were allowed to incubate at 37° C for at least ten min. The final volume of MMEBSS in each well was 100 µl. FLIPR was set up to excite Calcium Green with at 488 nm using 500 mW of power and reading fluorescence emission above 525 nm. A 0.5 s exposure was used to illuminate each well. Fluorescence was detected using a F-stop set of either 2.0 or 1.2. Specifically, after 30 s of baseline recording, test compounds were added to each well of a 96 well plate using 50 µL from a 3× stock. In each experiment, 4 wells were used as solvent controls.

Data from cell-based FLIPR assays for PNU-282987:

α7/5-HT$_3$ receptor chimera (EC_{50} = 178 ± 5 nM (n=70)).

5-HT3 receptor functional antagonist (IC_{50} = 4,541 ± 413 nM, n=46)).

α3: no detectable agonist activity at concentrations up to 100 µM (n = 69), for antagonist activity, $IC_{50} \geq 60\mu M$ (n = 70)

α1 no detectable agonist activity at concentrations up to 100 µM (n = 69), for antagonist activity, $IC_{50} \geq 60\mu M$ (n = 10)

Brain homogenate binding assays ([^{3}H]-MLA, [^{3}H]-cytisine, [^{3}H]-GR65630): Male Sprague-Dawley rats (300-350 g) were sacrificed by decapitation and the brains (whole brain minus cerebellum) were dissected quickly, weighed and homogenized in 9 volumes/g wet weight of ice-cold 0.32 M sucrose using a rotating pestle on setting 50 (10 up and down strokes). The homogenate was centrifuged at 1,000 x g for 10 minutes at 40° C. The supernatant was collected and centrifuged at 20,000 x g for 20 minutes at 40° C. The resulting pellet was resuspended to a protein concentration of 1 - 8 mg/ml. Aliquots of 5 ml homogenate were frozen at -80° C until needed for the assay. On the day of the assay, aliquots were thawed at room temperature and diluted with Kreb's - 20 mM HEPES buffer pH 7.0 (at room temperature) containing 4.16 mM $NaHCO_3$, 0.44 mM KH_2PO_4, 127 mM NaCl, 5.36 mM KCl, 1.26 mM $CaCl_2$, and 0.98 mM $MgCl_2$, so that 25 - 150 mg protein are added per test tube. Protein concentration was determined by the Bradford method using bovine serum albumin as the standard. For α7, nonspecific binding was determined in tissues incubated in parallel in the presence of 1 µM MLA, added before the radioligand, and in competition studies, compounds were added in increasing concentrations to the test tubes before addition of approximately 3 nM [^{3}H]-MLA (25 Ci/mmol). For α4, nonspecific binding was determined in tissues incubated in parallel in the presence of 1 mM (-)-nicotine, added before the radioligand, and in competition studies, compounds were added in increasing concentrations to the test tubes before addition of approximately 1.0 nM [^{3}H]-cytisine. For 5-HT$_3$, nonspecific binding was determined in tissues incubated in parallel in the presence of 1 µM ICS-205930, added before the radioligand, and in competition studies, compounds were added in increasing concentrations to the test tubes before addition of approximately 0.45 nM [^{3}H]-GR65630. For all binding assays, 0.4 ml homogenate was added to test tubes containing buffer, test compound and radioligand, and was incubated in a final volume of 0.5 ml for 1 h at 25°. The incubations were terminated by rapid vacuum filtration through Whatman GF/B glass filter paper mounted on a 48 well Brandel cell harvester. Filters were pre-soaked in 50 mM Tris HCl pH 7.0 - 0.05 % polyethylenimine. The filters were washed two times with 5 ml aliquots of cold 0.9% saline and then counted for radioactivity by liquid scintillation spectrometry. The inhibition constant (Ki) was calculated from the concentration dependent inhibition of radioligand binding obtained by fitting the data to the Cheng-Prusoff equation.

PNU-282987 had a Ki in this assay of 27 ± 1 nM (n = 48).

---

[1] Fitch RW, Xiao Y, Kellar KJ, Daly JW. Membrane potential fluorescence: a rapid and highly sensitive assay for nicotinic receptor channel function. *Proc. Natl. Acad. Sci. U S A.* **2003**; *100*:4909-4914.

PNU-282987 did not significantly displace tritiated cytisine from rat brain homogenates at 1 μM (inhibition = 14 ± 4 %, n=13)

With respect to the 5-HT3 receptor, PNU-282987 displaced tritiated GR-65630 with a Ki of 1,662 ± 331 nM (n=10)

Patch-clamp electrophysiology: Cultured neurons were prepared according to Brewer[2]. Briefly, Sprague-Dawley rats (postnatal day 3) were killed by decapitation and their brains were removed and placed in ice cold Hibernate-A medium. Hippocampal regions were gently removed, cut into small pieces and placed in Hibernate-A medium with 1 mg/ml papain for 60 min at 35°C. After digestion, the tissues were washed several times in Hibernate-A media and transferred to a 50 ml conical tube containing 6 ml Hibernate-A medium with 2% B-27 supplement. Neurons were dissociated by gentle trituration and plated onto poly-D-lysine/laminin coated coverslips at a density of 300 – 700 cells/mm^2, and transferred to 24-well tissue culture plates containing warmed culture medium composed of Neurobasal-A medium, B-27 supplement (2%), L-glutamine (0.5 mM), 100 U/ml penicillin, 100 mg/ml streptomycin, and 0.25 mg/ml Fungizone. Cells were maintained in a humidified incubator at 37°C and 6% CO_2 for 1 – 2 weeks. The medium was changed after 24 h and then approximately every three days thereafter. Patch pipettes were pulled from borosilicate capillary glass using a Flaming/Brown micropipette puller (P97, Sutter Instrument, Novato, CA) and filled with an internal pipette solution composed of (in mM): $CsCH_3SO_3$ (126), CsCl (10), NaCl (4), $MgCl_2$ (1), $CaCl_2$ (0.5), EGTA (5), HEPES (10), ATP-Mg (3), GTP-Na (0.3), phosphocreatin (4), pH 7.2. The resistances of the patch pipettes when filled with internal solution ranged between 3 – 6 M•. All experiments were conducted at room temperature. Cultured cells were continuously superfused with an external bath solution containing (in mM): NaCl (140), KCl (5), $CaCl_2$ (2), $MgCl_2$ (1), HEPES (10), glucose (10), bicuculline (0.01), CNQX (0.005), D-AP-5 (0.005) tetrodotoxin (0.0005), pH 7.4. Compounds were dissolved in water or DMSO and diluted into the external bath solution containing a final DMSO concentration of 0.1% and delivered via a multibarrel fast perfusion system (Warner Instrument, Hamden, CT). Whole-cell currents were recorded using an Axopatch 200B amplifier (Axon Instruments, Union City, CA). Analog signals were filtered at 1/5 the sampling frequency, digitized, stored, and measured using pCLAMP software (Axon Instruments). All data are reported as mean ± SEM. Cell culture reagents were purchased from Invitrogen Corp. (Carlsbad, CA).

Auditory gating assay. Experiments were performed on Male Sprague-Dawley rats (Harlan, Indianapolis, IN; weighing 250 to 300 gm) under chloral hydrate anesthesia (400 mg/kg, IP). The femoral artery and vein were cannulated for monitoring arterial blood pressure and administration of drugs or additional doses of anaesthetic, respectively. Unilateral hippocampal field potential (EEG) was recorded by a metal monopolar macroelectrode placed into the CA3 region (co-ordinates: 3.0 – 3.5 mm posterior from the bregma, 2.6 –3.0 mm lateral and 3.8 – 4.0 mm ventral; Paxinos and Watson, 1986[3]). Field potentials were amplified, filtered (0.1 – 100 Hz), displayed and recorded for on-line and off-line analysis (Spike3). Quantitative EEG analysis was performed by means of Fast Fourier Transformation (Spike3). The auditory stimulus consisted of a pair of 10 ms, 5 KHz tone bursts with a 0.5 s delay between the first "conditioning" stimulus and second "test" stimulus. Auditory evoked responses were computed by averaging of responses to 50 pairs of stimuli presented with an interstimulus interval of 10 s. Percentage of gating was determined by the formula: (1 - test amplitude/conditioning amplitude) x 100. Amphetamine (D-amphetamine sulfate, 0.3-1 mg/kg, IV) was administered in order to disrupt sensory gating. Recordings of evoke potentials commenced 5 min after amphetamine administration, and only rats showing gating deficit exceeding 20 % were used for subsequent evaluation of α7 nAChR agonists or vehicle. Statistical significance was determined by means of two-tailed paired Student's t-test.

---

[2]Brewer G.J. Isolation and culture of adult rat hippocampal neurons. *J Neurosci Methods*. **1997;** *71*:143-55.

[3] Paxinos G and Watson C **1986**, *The Rat Brain in Stereotaxic Coordinates*. A cademic

Press, Sydney.

Table A. Effects of PNU-282987 on specific radioligand binding to 29 receptors and channels.

Receptor	% Inhibition
A_1 (h)	–
A_2 (h)	–
β_2 (h)	–
β_1 (h)	11
BZD (central)	–
BZD (peripheral)	–
CB1 (h)	–
DA uptake (h)	–
GABA (non-selective)	–
NMDA	–
H1 (central)	–
H2	–
I2 (central)	–
ML1	–
M (non-selective)	13
NK1 (h)	–
Y (non-selective)	–
N (neuronal)	–
Opiate (non-selective)	–
PCP	15
5-HT3 (h)	56
5-HT4	23
5-HT uptake	–
σ (non-selective)	15
Ca channel (L, DHP site)	–
Ca channel (N)	–
Kv channel	–
Na channel (site 1)	–
Na channel (site 2)	17

Results are expressed as a percent inhibition of control specific binding (mean values; n=2). The symbol "–" indicates an inhibition of less than 10%.

Table B. Effects of PNU-282987 on the activity of 6 enzymes.

Enzyme	% Inhibition
MAO-A (h)	–
MAO-B	–
NOS inducible	–
NOS constitutive (h)	14
Tyrosine hydroxylase	–
Xanthine oxidase	–

Results are expressed as a percent inhibition of control activity (mean values; n=2). The symbol "–" indicates an inhibition of less than 10%.