Propidium-Based Polyamine Ligands as Potent Inhibitors of Acetylcholinesterase and Acetylcholinesterase-Induced Amyloid-β Aggregation

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Supporting Information

Contents of SI: Contains experimental details for the synthesis and for the determination of the biological activity, spectra data and elemental analysis data for all new compounds.

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Experimental Section

1. Chemistry

Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and direct infusion ESI-MS spectra were recorded on Nicolet Avatar 320 and Waters Micromass ZQ 4000 apparatus. ^{1}H NMR experiments were recorded on Varian VXR 200 and 300 MHz instruments. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), d (doublet), t (triplet), or m (multiplet). Although the IR spectra data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. When the elemental analysis is not included, crude compounds were used in the next step without further purification. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040-0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063-0.200 mm; Merck) chromatography. Compounds were named following IUPAC rules as applied by Beilstein-Institut AutoNom (version 2.1), a PC integrated software package for systematic names in organic chemistry.

***N*1-[3-(1,2,3,4-Tetrahydro-acridin-9-ylamino)-propyl]-propane-1,3-diamine (7).** *N*1-(3-aminopropyl)-propane-1,3-diamine (2.9 mL, 20.7 mmol) was added together with a catalytic amount of KI to a solution of **6** (0.15 g, 0.69 mmol) in 1-pentanol (3.5 mL). The reaction mixture was then heated at 160 °C for 24 h. After cooling to room temperature, the reaction mixture was diluted with water (10 mL) and extracted with CH_2Cl_2 (2 x 40 mL). The organic phases were dried and evaporated to give a residue that was purified by flash chromatography. Elution with a step gradient system of CH_2Cl_2/MeOH/aqueous 28% ammonia (8/2/0.2 to 7/3.5/0.35) afforded **7** as a clear oil: 42% yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (d, 1H, Ar*H*), 7.90 (d,1H, Ar*H*), 7.55 (t, 1H, Ar*H*), 7.25 (t, 1H, Ar*H*), 3.60 (t, 2H, Ar-NHCH_2), 3.00-3.15 (m, 2H, N=C-CH_2), 2.60-2.85 (complex m, 8H, ArCH_2 + NHCH_2+ CH_2NH$_2$), 1.50-2.00 (complex m, 8H + 3H exchangeable with D_2O, $CH_2CH_2CH_2$ + N*H* + NH_2).

3,8-Diamino-6-phenyl-5-(3-{3-[3-(1,2,3,4-tetrahydro-acridin-9-ylamino)-propylamino]-propylamino}-propyl)-phenanthridinium Chloride Trihydrocloride (4). Compound **8** (0.07 g, 0.12 mmol) was added to a solution of **7** (0.09 g, 0.29 mmol) in MeOH (4 mL) and the resulting solution was stirred under reflux for 7 h. After pouring the cooled mixture into water MeOH was evaporated to afford a yellow solid, which was washed with water and then dried (ESI-MS (*m*/*z*): 362 ($M + H^+/2$)). The crude material, dissolved in MeOH (4 mL), was added of 12 N HCl (0.4 mL) and the resulting mixture was refluxed under stirring for 2 h. After cooling, ether was added to precipitate **4** as hydrochloride salt (purple solid): 20% yield; mp 248 °C (dec.); ^{1}H NMR (200 MHz, CD_3OD): δ 8.50-8.65 (m, 2H), 8.79-8.83 (m, 2H), 7.50-8.02 (m, 10H), 7.35 (s, 1H), 4.75 (t, 2H), 4.05-4.14 (m, 2H), 2.82-3.41 (complex m, 12H), 1.91-2.62 (complex m, 10H); ESI-MS (*m*/*z*): 320 ($M + H^+/2$). Calcd. for $C_{41}H_{51}Cl_4N_7$: C 62.83, H 6.56, N 12.51. Found C 62.54, H 6.45, N 12.26.

{3-[3-(2-Methoxy-benzylamino)-propylamino]-propyl}-carbamic Acid *tert*-Butyl Ester (10). A mixture of *N*1-BOC-3,3'-iminodipropylamine **9** (Fluka) (4.6 g, 20.0 mmol), molecular sieves (3 Å), and 2-methoxybenzaldehyde (2.5 mL, 21.0 mmol) in EtOH (100 mL) was stirred for 30 min at room temperature, then $NaBH_4$ (0.4 g, 10.5 mmol) was added, and the stirring was continued overnight. Following removal of molecular sieves, the solution was made acidic with 2 N $KHSO_4$ (4 mL). Removal of the solvent gave a residue that was dissolved in water (40 mL), then made basic with 2 N NaOH, and finally extracted with $CHCl_3$ (3 x 30 mL). The organic phases were dried and evaporated to give a residue that was purified by flash chromatography. Elution with $CHCl_3$/MeOH/aqueous 28% ammonia (8.2/1.8/0.18) gave **10** as an oil: 52% yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.15-7.20 (m, 2H, Ar*H*), 6.75-6.84 (m, 2H, Ar*H*), 5.43 (br s, 1H, exchangeable with D_2O, CON*H*), 3.75 (s, 3H, OCH_3), 3.70 (s, 2H, ArCH_2), 3.01-3.20 (m, 2H, CONHCH_2), 2.52-2.60 (m, 6H, NHCH_2), 1.45-1.65 (m, 4H + 2H exchangeable with D_2O, $CH_2CH_2CH_2$ + N*H*), 1.36 (s, 9H, CH_3C).

[3-({3-[(2-Methoxy-benzyl)-methyl-amino]-propyl}-methyl-amino)-propyl]-carbamic Acid *tert*-Butyl Ester (11). Formaldehyde (2.77 mL, 99.8 mmol), $NaBH_3CN$ (1.01 g, 16.1 mmol) and

CH_3COOH (0.69 mL, 11.97 mmol) were added to a solution of **10** (1.4 g, 3.99 mmol) in EtOH (40 mL) and the resulting mixture was stirred overnight. After cooling, it was made basic with 40% aqueous NaOH and evaporated to give a residue that was partitioned between water and $CHCl_3$. Removal of washed (brine) and dried organic phase gave **11** as an oil: 83% yield; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.20-7.35 (m, 2H, Ar*H*), 6.81-7.00 (m, 2H, Ar*H*), 5.43 (br s, 1H, exchangeable with D_2O, CON*H*), 3.80 (s, 3H, OCH_3), 3.50 (s, 2H, ArCH_2), 3.22 (t, 2H, CONHCH_2), 2.30-2.50 (m, 6H, CH_3NCH_2), 2.21 (s, 3H, NCH_3), 2.19 (s, 3H, NCH_3), 1.60-1.80 (m, 4H, CH_2C$H_2$$CH_2$), 1.45 (s, 9H, C$H_3$C).

***N*1-{3-[(2-Methoxy-benzyl)-methyl-amino]-propyl}-*N*1-methyl-propane-1,3-diamine (12).** A solution of **11** (0.27 g, 0.229 mmol) in $CHCl_3$ (40 mL) and CF_3COOH (18 mL) was stirred for 3 h. Removal of the solvents gave a residue that was purified by flash chromatography. Elution with CH_2Cl_2/MeOH/aqueous 28% ammonia (9/1/0.1) afforded **12** as an oil: 65% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.20-7.40 (m, 2H, Ar*H*), 6.80-7.00 (m, 2H, Ar*H*), 3.85 (s, 3H, OCH_3), 3.50 (s, 2H, ArCH_2), 2.75 (t, 2H, C$H_2$$NH_2$), 2.35-2.50 (m, 6H, CH_3NCH_2), 2.25 (s, 6H, NCH_3), 1.50-1.80 (m, 4H + 2H exch with D_2O, CH_2C$H_2$$CH_2$+ CH_2NH_2).

3,8-Diamino-5-{3-[3-({3-[(2-methoxy-benzyl)-methyl-amino]-propyl}-methyl-amino)propylamino]-propyl}-6-phenyl-phenanthridinium Chloride Trihydrocloride (5). It was synthesized as a foam from **8** and **12**, following the procedure described for **4**. ^{1}H NMR (300 MHz, CD_3OD): δ 8.80-9.01 (m, 2H), 7.79-7.82 (m, 3H), 7.35-7.72 (m, 7H), 7.00-7.20 (m, 3H), 4.75 (t, 2H), 4.42 (s, 2H), 4.05-4.14 (m, 2H), 3.98 (s, 3H), 3.04-3.41 (complex m, 10H), 2.12-2.60 (complex m, 6H); ESI-MS (*m*/*z*): 605 (M^+), 302 (M + H^+/2). Calcd. for $C_{38}H_{52}Cl_4N_6O$: C 60.80, H 6.98, N 11.20. Found C 60.50, H 6.75, N 11.00.

2. Biology

Inhibition of AChE and BChE. The method of Ellman et al. was followed. Five different concentrations of each compound were used in order to obtain inhibition of AChE or BChE activity

comprised between 20-80%. The assay solution consisted of a 0.1 M phosphate buffer pH 8.0, with the addition of 340 μM 5,5'-dithio-bis(2-nitrobenzoic acid), 0.02 unit/mL of human recombinant AChE or human serum BChE (Sigma Chemical), and 550 μM of substrate (acetylthiocholine iodide or butyrylthiocholine iodide). Test compounds were added to the assay solution and preincubated at 37 °C with the enzyme for 20 min followed by the addition of substrate. Assays were done with a blank containing all components except AChE or BChE in order to account for non-enzymatic reaction. The reaction rates were compared and the percent inhibition due to the presence of test compounds was calculated. Each concentration was analyzed in triplicate, and IC_{50} values were determined graphically from log concentration–inhibition curves.

Determination of Steady State Inhibition Constant. To obtain estimates of the competitive inhibition constant K_i, reciprocal plots of 1/V versus 1/[S] were constructed at relatively low concentration of substrate (below 0.5 mM). The plots were assessed by a weighted least square analysis that assumed the variance of V to be a constant percentage of V for the entire data set. Slopes of these reciprocal plots were then plotted against the concentration of the inhibitors (range: 0 – 30 μM for **2**, 0 – 2.0 nM for **4**, and 0 – 2.0 μM for **5**) in a weighted analysis and K_i was determined as the ratio of the replot intercept to the replot slope.

Inhibition of AChE-induced Aβ aggregation. Aliquots of 2 μL Aβ peptide, lyophilized from 2 mg mL^{-1} 1,1,1,3,3,3-hexafluoro-2-propanol solution and dissolved in DMSO, were incubated for 24 h at room temperature in 0.215 M sodium phosphate buffer (pH 8.0) at a final concentration of 230 μM. For co-incubation experiments aliquots (16 μL) of AChE (final concentration 2.30 μM, Aβ/AChE molar ratio 100:1) and AChE in the presence of 2 μL of the tested inhibitor in 0.215 M sodium phosphate buffer pH 8.0 solution (final inhibitors concentration ranging between 5 and 100 μM) were added.

Blanks containing Aβ, AChE, and Aβ plus inhibitors at various concentrations, in 0.215 M sodium phosphate buffer (pH 8.0) were prepared. The final volume of each vial was 20 μL. Each assay was run in duplicate. To quantify amyloid fibril formation, the thioflavin T (ThT)

fluorescence method was then applied. After dilution with glycine-NaOH buffer (pH 8.5), containing 1.5 μM ThT, the fluorescence intensities due to β-sheet conformation was monitored for 300 s at λ_{em} = 490 nm (λ_{ex} = 446 nm). The percent inhibition of the AChE induced aggregation due to the presence of the test compound was calculated by the following expression: 100-(IF_i/IF_o x 100) where IF_i and IF_o are the fluorescence intensities obtained for Aβ plus AChE in the presence and in the absence of inhibitor, respectively, minus the fluorescent intensities due to the respective blanks. Inhibition curves were obtained for each compound by plotting the percentage inhibition versus the logarithm of inhibitor concentration in the assay sample. The linear regression parameters were determined and the IC_{50} extrapolated, when possible (GraphPad Prism 3.0 GraphPad Software Inc.)