Supporting Information

Polyamine conjugation as a new strategy to target amyloid aggregation in the framework of Alzheimer’s disease

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Chemistry

**General Chemical methods.** Chemical reagents were purchased from Sigma Aldrich, Fluka and Lancaster (Italy). The course of the reactions was observed by thin layer chromatography (TLC) on 0.20 mm silica gel 60 F254 plates (Merck, Germany), then visualized with an UV lamp. Nuclear magnetic resonance spectra (NMR) were recorded at 400 MHz for $^1$H and 100 MHz for $^{13}$C on Varian VXR 400 spectrometer. Chemical shifts are reported in parts per millions (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Direct infusion ESI-MS mass spectra were recorded on a Waters ZQ 4000 apparatus. All final compounds 2−6 are >98% pure by HPLC analyses and were tested in biological assays using their TFA salt forms.

**Synthesis of 8-12**

Compounds 8-12 were prepared through acid catalysed crossed aldol condensation between piperidinone and the appropriate benzaldehyde according to a standard synthetic protocol,$^1$ which is easily amenable to parallel library synthesis. Hence, to set up the synthesis of 8-12, we adapted a reactor equipment for combinatorial chemistry (BUCHI Syncore line) to allow gaseous HCl injection following benzaldehyde addition. The resulting automated procedure gave the intermediates in good yield after an easy and efficient workup. Experimental data of 8-12 were in agreement with the literature and, $^1$H NMR spectra show that the compounds are stereoisomerically pure possessing an $E$ configuration as indicated by the region of absorbance of the two olefinic double bonds.$^2$

**General procedure.** To a solution of piperidin-4-one hydrochloride (1 equiv) in CH$_3$COOH (108 equiv) saturated with HCl(g) was added dropwise a solution of the appropriate benzaldehyde (3 equiv) in CH$_3$COOH (134 equiv). The reaction mixture was stirred for 48 hours at room temperature and worked up by filtration.
(3E,5E)-3,5-bis(3,4-dihydroxybenzylidene)piperidin-4-one hydrochloride (8). 7 was synthesized from piperidin-4-one hydrochloride (500 mg, 2.91 mmol) and 3,4-dihydroxybenzaldehyde (1210 mg, 8.73 mmol). The precipitate was filtered off and washed with petroleum ether, diethyl ether, ethanol and dichloromethane to give 8 as a green solid: 940 mg (86%), m.p. = 222-225°C. 1H NMR (400 MHz, DMSO-d6) δ 4.40 (s, 4H), 6.82-6.88 (m, 6H), 7.66 (s, 2H), 9.41 (br s, 4H), 9.78 (s, 2H).

(3E,5E)-3,5-bis(4-hydroxybenzylidene)piperidin-4-one hydrochloride (9). 9 was synthesized from piperidin-4-one hydrochloride (500 mg, 2.91 mmol) and 4-hydroxybenzaldehyde (1066 mg, 8.73 mmol). The precipitate was filtered off and washed with H2O, petroleum ether and dichloromethane to give 9 as a green solid: 680 mg (68%), m.p. = 248-251°C. 1H NMR (400 MHz, DMSO-d6) δ 4.40 (s, 4H), 6.87-6.93 (m, 4H), 7.31-7.36 (m, 4H), 7.74 (s, 2H), 9.83 (s, 2H), 10.31 (br s, 2H).

(3E,5E)-3,5-bis(3-hydroxybenzylidene)piperidin-4-one hydrochloride (10). 10 was synthesized from piperidin-4-one hydrochloride (468 mg, 2.73 mmol) and 3-hydroxybenzaldehyde (1000 mg, 8.19 mmol). The precipitate was filtered off and washed with petroleum ether and diethyl ether to afford 9 as a yellow solid: 630 mg (67%). 1H NMR (400 MHz, DMSO-d6) δ 4.45 (s, 4H), 6.91-6.94 (m, 6H), 7.30-7.34 (m, 2H), 7.78 (s, 2H), 9.90 (br s, 4H).

(3E,5E)-3,5-bis(3,4-dimethoxybenzylidene)piperidin-4-one hydrochloride (11). 11 was synthesized from piperidin-4-one hydrochloride (500 mg, 2.91 mmol) and 3,4-dimethoxybenzaldehyde (1450 mg, 8.73 mmol). The precipitate was filtered off and washed with petroleum ether, diethyl ether, ethanol and dichloromethane to give 11 as a green solid: 650 mg (52%), m.p. = 233-236°C. 1H NMR (400 MHz, DMSO-d6) δ 4.00 (s, 6H), 4.02 (s, 6H), 4.68 (s, 4H), 7.26-7.31 (m, 4H), 7.34 (s, 2H), 8.02 (s, 2H), 10.27 (br s, 2H).

(3E,5E)-3,5-dibenzylidene piperidin-4-one hydrochloride (12). 12 was synthesized from piperidin-4-one hydrochloride (500 mg, 2.91 mmol) and benzaldehyde (889 µL, 8.73 mmol). The precipitate obtained was filtered off and washed with H2O, ethanol and petroleum ether to give 12...
as a yellow solid: 520 mg (57%), m.p. = 244-247°C. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 4.17 (s, 4H), 7.17-7.25 (m, 10H), 7.58 (s, 2H), 9.60 (s, 2H).

**General procedure for the intermediates 13-17.** To a solution of 7 (1 equiv) in dry DMF (2-3 mL) were added PPAA (2 equiv), Et$_3$N (3 equiv) and the appropriate intermediates (8-12) (1 equiv) under nitrogen atmosphere. After leaving the reaction to rt overnight, the solvent was evaporated and the crude product purified by column chromatography on silica gel to yield the desired compounds 13-17.

(3-{4-[3,5-Bis-(3,4-dihydroxybenzylidene)-4-oxo-piperidin-1-yl]-4-oxo-butanamido}propyl)-{4-[tert-butoxycarbonyl-(3-tert-butoxycarbonylamino-propyl)-amino]-butyl}-carbamic acid tert-butyl ester (13). 13 was synthesized from 8 (226 mg, 0.55 mmol). Elution with dichloromethane/MeOH (9:1) afforded 13 as a dark oil: 270 mg (54%). $^1$H NMR (400MHz, CD$_3$OD) δ 1.26-1.43 (m, 31H), 1.61-1.65 (m, 4H), 2.37 (t, $J = 6.0$ Hz, 2H), 2.56 (t, $J = 6.0$ Hz, 2H), 2.98-3.01 (m, 2H), 3.05-3.19 (m, 10H), 4.81 (s, 2H), 4.88 (s, 2H), 6.84-6.90 (m, 4H), 6.94-6.96 (m, 2H), 7.63 (s, 1H), 7.66 (s, 1H). MS [ESI]$m/z$ 922 [M+1].

(3-{4-[3,5-Bis-(4-hydroxybenzylidene)-4-oxo-piperidin-1-yl]-4-oxo-butanamido}propyl)-{4-[tert-butoxycarbonyl-(3-tert-butoxycarbonylamino-propyl)-amino]-butyl}-carbamic acid tert-butyl ester (14). 14 was synthesized from 9 (116 mg, 0.34 mmol). Elution with dichloromethane/MeOH (9:1) afforded 14 as a green foam solid: 170 mg (56%). $^1$H NMR (400MHz, CDCl$_3$) δ 1.43-1.45 (m, 27H), 1.63-1.66 (m, 6H), 2.39-2.43 (m, 2H), 2.58 (t, $J = 6.4$ Hz, 2H), 3.12-3.17 (m, 14H), 4.75 (s, 2H), 4.88 (s, 2H), 6.90-6.96 (m, 4H), 7.26-7.28 (m, 2H), 7.33-7.36 (m, 2H), 7.37 (s, 1H), 7.71 (s, 1H). MS [ESI]$m/z$ 890 [M+1].

(3-{4-[3,5-Bis-(3-hydroxybenzylidene)-4-oxo-piperidin-1-yl]-4-oxo-butanamido}propyl)-{4-[tert-butoxycarbonyl-(3-tert-butoxycarbonylamino-propyl)-amino]-butyl}-carbamic acid tert-butyl ester (15). 15 was synthesized from 10 (110 mg, 0.32 mmol). Elution with dichloromethane/MeOH (9:1) afforded 15 as a yellow oil: 160 mg (56%). $^1$H NMR (400MHz,
CD$_3$OD δ 1.40-1.42 (m, 27H), 1.57-1.64 (m, 6H), 2.33 (t, $J=6.6$ Hz, 2H), 2.50 (t, $J=6.6$ Hz, 2H), 2.99-3.19 (m, 14H), 4.83 (s, 2H), 4.88 (s, 2H), 6.51 (br s, exchangeable with D$_2$O, 1H), 6.82-6.94 (m, 6H), 7.23-7.30 (m, 2H), 7.68 (s, 1H), 7.72 (s, 1H), 7.79 (br s, exchangeable with D$_2$O, 1H). MS [ESI]$m/z$ 890 [M-1].

(3-{4-[3,5-Bis-(3,4-dimethoxybenzylidene)-4-oxo-piperidin-1-yl]-4-oxo-butanamido}-propyl)\-{4-[tert-butoxycarbonyl-(3-tert-butoxycarbonylamino-propyl)-amino]-butyl}\-carbamic acid tert-butyl ester (16). 16 was synthesized from 11 (75 mg, 0.16 mmol). Elution with dichloromethane/MeOH (9.5:0.5) afforded 16 as a green foam solid: 80 mg (53%). $^1$H NMR (400MHz, CD$_3$OD) δ 1.39-1.43 (m, 31H), 1.57-1.63 (m, 4H), 2.35 (t, $J=6.4$ Hz, 2H), 2.54 (t, $J=6.4$ Hz, 2H), 3.04-3.18 (m, 12H), 3.83 (s, 12H), 4.81 (s, 2H), 4.83 (s, 2H), 6.93-7.02 (m, 6H), 7.63 (s, 1H), 7.67 (s, 1H). MS [ESI]$m/z$ 1014 [M+Cl].

(3-{4-[3,5-dibenzylidene-4-oxo-piperidin-1-yl]-4-oxo-butanamido}-propyl)\-{4-[tert-butoxycarbonyl-(3-tert-butoxycarbonylamino-propyl)amino]-butyl}\-carbamic acid tert-butyl ester (17). 17 was synthesized from 12 (74 mg, 0.21 mmol). Elution with petroleum ether/ethyl acetate/MeOH (6:3.5:0.7) afforded 17 as a yellow oil: 100 mg (55%). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 1.31-1.34 (m, 31H), 1.47-1.51 (m, 4H), 2.14 (t, $J=6.6$ Hz, 2H), 2.37 (t, $J=6.6$ Hz, 2H), 2.83-2.89 (m, 4H), 3.02-3.04(m, 8H), 4.80 (s, 2H), 4.82 (s, 2H), 7.43-7.55 (m, 10H), 7.67 (s, 2H). MS [ESI]$m/z$ 894 [M+Cl].

**General procedure for compounds 2-6.** To a stirred solution of the appropriate Boc-compound (13-17) (1 equiv) in CH$_2$Cl$_2$ (5 mL) was carefully added at 0°C trifluoroacetic acid (30% in DCM). After being stirred at room temperature for 4 hours, the solvent was evaporated under reduced pressure, adding heptane for the azeotropic removal of trifluoroacetic acid traces. The trifluoroacetate salts were washed with ether to obtain 2-6.

$N$-{3-[4-(3-Amino-propylamino)-butylamino]-propyl}-4-[3,5-bis-(3,4-dihydroxybenzylidene)-4-oxo-piperidin-1-yl]-4-oxo-butanamide tri(trifluoroacetate) (2). 2 was synthesized from 13
(270 mg, 0.30 mmol) as a dark solid: 250 mg (86%). $^1$H NMR (400MHz, CD$_3$OD) $\delta$ 1.65-1.79 (m, 6H), 2.09-2.11 (m, 2H), 2.36 (t, $J$= 6.0 Hz, 2H), 2.59 (t, $J$=6.0 Hz, 2H), 2.78 (t, $J$=7.2 Hz, 2H), 2.90 (t, $J$=6.6 Hz, 2H), 2.99-3.07 (m, 4H), 3.12 (t, $J$=7.2 Hz, 2H), 3.22 (t, $J$=6.6 Hz, 2H), 4.85 (s, 4H), 6.84-6.90 (m, 4H), 6.96 (d, $J$=10.8 Hz, 2H), 7.63 (s, 1H), 7.67 (s, 1H). $^{13}$C NMR (100MHz, CD$_3$OD) $\delta$ 22.75, 22.82 (2C), 23.95, 26.17, 27.54, 29.82, 35.05, 36.43, 43.20, 44.47, 44.58, 46.19 (2C), 115.47, 117.34, 117.42, 123.89, 124.21, 126.30, 126.62, 128.98, 129.19, 137.83, 138.05, 145.15, 145.25 (2C), 147.51, 147.60, 171.23, 174.90, 186.72. MS [ESI$^+$]m/z 624 [M+1]$^+$. 

$N$-[3-[4-(3-Amino-propylamino)-butylamino]-propyl]-4-[3,5-bis-(4-hydroxybenzylidene)-4-oxo-piperidin-1-yl]-4-oxo-butanamide tri(trifluoroacetate) (3). 3 was synthesized from 14 (170 mg, 0.19 mmol) as a green solid: 65 mg (77%). $^1$H NMR (400MHz, CD$_3$OD) $\delta$ 1.70-1.82 (m, 6H), 2.14-2.20 (m, 2H), 2.37-2.39 (m, 2H), 2.58-2.61 (m, 2H), 2.70-2.81 (m, 2H), 2.91-2.94 (m, 2H), 3.05-3.15 (m, 6H), 3.23-3.29 (m, 2H), 4.87 (s, 4H), 6.88-6.91 (m, 4H), 7.39-7.41 (m, 4H), 7.71 (s, 1H), 7.75 (s, 1H). $^{13}$C NMR (100MHz, CD$_3$OD) $\delta$ 22.87, 22.97 (2C), 24.01, 26.30, 27.41, 29.69, 34.93, 36.55, 43.23, 44.56, 44.60, 46.25 (2C), 115.57, 125.68, 125.98, 129.10, 129.28 (2C), 132.61 (2C), 132.75 (4C), 137.52, 137.73, 159.37, 159.44, 171.29, 175.09, 186.88. MS [ESI$^+$]m/z 592 [M+1]$^+$. 

$N$-[3-[4-(3-Amino-propylamino)-butylamino]-propyl]-4-[3,5-bis-(3-hydroxybenzylidene)-4-oxo-piperidin-1-yl]-4-oxo-butanamide tri(trifluoroacetate) (4). 4 was synthesized from 15 (60 mg, 0.07 mmol) as a yellow solid: 65 mg (77%). $^1$H NMR (400MHz, CD$_3$OD) $\delta$ 1.74-1.82 (m, 6H), 2.12-2.20 (m, 2H), 2.36-2.40 (m, 2H), 2.57-2.60 (m, 2H), 2.86-2.90 (m, 2H), 2.95-2.97 (m, 2H), 3.05-3.11 (m, 4H), 3.16-3.18 (m, 2H), 3.25-3.27 (m, 2H), 4.91 (s, 4H), 6.90-6.98 (m, 6H), 7.31-7.33 (m, 2H), 7.72 (s, 1H), 7.77 (s, 1H). $^{13}$C NMR (100MHz, DMSO-$d_6$) $\delta$ 23.10 (2C), 24.23, 26.51, 27.53, 30.14, 35.90, 36.64, 42.93, 44.34, 44.92, 46.50, 46.59 (2C), 117.27 (2C), 121.91 (2C), 129.29 (2C), 130.37 (2C), 132.96 (2C), 135.75 (2C), 136.75 (2C), 157.97 (2C), 170.65, 172.29, 186.50. MS [ESI$^+$]m/z 592 [M+1]$^+$. 

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N-[3-[4-(3-Amino-propylamino)-butylamino]-propyl]-4-[3,5-bis-(3,4-dimethoxybenzylidene)-4-oxo-piperidin-1-yl]-4-oxo-butanamide tri(trifluoroacetate) (5). 5 was synthesized from 16 (80 mg, 0.08 mmol) as a green solid: 80 mg (97%). $^1$H NMR (400MHz,CD$_3$OD) δ 1.74-1.86 (m, 6H), 2.13-2.15 (m, 2H), 2.42-2.44 (m, 2H), 2.64 (t, J=6.0 Hz, 2H), 2.88 (t, J=6.0 Hz, 2H), 2.97 (t, J=6.2 Hz, 2H), 3.08-3.14 (m, 4H), 3.18-3.21 (m, 2H), 3.28 (t, J=6.2 Hz, 2H), 3.94 (s, 12H), 4.95 (s, 2H), 4.99 (s, 2H), 7.13-7.20 (m, 4H), 7.80 (s, 2H), 7.80 (s, 1H), 7.85 (s, 1H). $^{13}$C NMR (100MHz, CD$_3$OD) δ 23.08, 24.21 (2C), 26.46, 27.52, 30.30, 35.93, 36.62 (2C), 42.72, 44.32, 44.94, 46.49 (2C), 56.03 (4C), 112.19, 114.72, 124.36, 127.38, 128.92, 129.07 (2C), 129.25 (2C), 130.80, 134.44, 136.76, 149.07 (2C), 150.58, 170.67, 172.22, 186.09. MS [ESI$^+$]m/z 680 [M+1]$^+$. 

N-[3-[4-(3-Amino-propylamino)-butylamino]-propyl]-4-[3,5-dibenzylidene-4-oxo-piperidin-1-yl]-4-oxo-butanamide tri(trifluoroacetate) (6). 6 was synthesized from 17 (100 mg, 0.12 mmol) as a green solid: 90 mg (83%). $^1$H NMR (400MHz, DMSO-d$_6$, T 31°C) δ 1.55-1.64 (m, 6H), 1.84-1.88 (m, 2H), 2.18 (t, J=6.2 Hz, 2H), 2.40 (t, J=6.2 Hz, 2H), 2.79-2.99 (m, 12H), 4.81 (s, 4H), 7.46-7.53 (m, 10H), 7.67 (s, 2H), 7.88 (br s, exchangeable with D$_2$O, 4H), 8.42 (br s, exchangeable with D$_2$O, 2H), 8.71(br s, exchangeable with D$_2$O, 2H). $^{13}$C NMR (100MHz, DMSO-d$_6$) δ 23.07 (2C), 24.23 (2C), 26.50, 27.54, 30.15, 35.89, 36.64, 42.58, 44.34, 44.91, 46.49, 46.58, 110.00, 129.28 (2C), 129.36 (2C), 130.05, 130.94 (2C), 131.06 (2C), 132.81 (2C), 134.55 (2C), 136.64 (2C), 170.72, 172.29, 186.57. MS [ESI$^+$] m/z 560 [M+1]$^+$. 

**Purity determination: HPLC analyses of 2-6.** HPLC analysis was performed under reversed-phase conditions on a Phenomenex Jupiter C18 (150x4.6 mm I.D.) column, using a binary mixture (A/B) of 0.1% TFA in H2O/acetonitrile (v/v) as mobile phase, UV detection at $\lambda$ = 250 nm and a flow-rate of 0.8 mL/min. A loop valve of 20 µL volume was used. The liquid chromatograph was by Jasco Corporation (Tokyo, Japan), model PU-1585 UV.
| Compounds | A/B   | Purity % |
|-----------|-------|----------|
| 2         | 78/22 | > 98     |
| 3         | 78/22 | >98      |
| 4         | 78/22 | >98      |
| 5         | 75/25 | >98      |
| 6         | 75/25 | >98      |

**Biological methods**

**Sample preparation for Aβ_{42} self-aggregation.** 1,1,1,3,3,3-(Hexafluoro-2-propanol (HFIP)-pretreated Aβ_{42} samples (Bachem AG, Switzerland) were resolubilized with a CH$_3$CN/0.3 mM Na$_2$CO$_3$/250 mM NaOH (48.4:48.4:3.2) mixture to have a stable stock solution ([Aβ$_{42}$]=500 µM).$^7$ Tested inhibitors were dissolved in MeOH and diluted in the assay buffer. Experiments were performed by incubating the peptide diluted in 10 mM phosphate buffer (pH 8.0) containing 10 mM NaCl at 30°C (Thermomixer Comfort, Eppendorf, Italy) for 24 h (final Aβ concentration=50 µM) with and without inhibitor.

**Inhibition of Aβ$_{42}$ self-aggregation as determined by the ThT assay.** Inhibition studies were performed by incubating Aβ$_{42}$ samples under the assay conditions reported above, with and without tested inhibitors. Inhibitors were screened at 10 µM in a 0.2:1 ratio with Aβ$_{42}$. To quantify amyloid fibril formation, the ThT fluorescence method was used.$^8, 9$ After incubation, samples were diluted to a final volume of 2.0 mL with 50 mM glycine–NaOH buffer (pH 8.5) containing 1.5 µM ThT. A 300 s time scan of fluorescence intensity was performed ($\lambda_{exc}$=446 nm; $\lambda_{em}$=490 nm), and values at plateau were averaged after subtracting the background fluorescence of 1.5 µM ThT solution. Blanks containing inhibitor and ThT were also prepared and evaluated to account for quenching and
fluorescence properties. The fluorescence intensities were compared and the percentage inhibition was calculated.

**Inhibition of Aβ_{42} self-aggregation as determined by flow injection ESI-MS.** Inhibition studies were performed by incubating Aβ_{42} samples under the assay conditions reported above, with and without the tested inhibitor. All selected compounds were first screened at 10 µM in a 0.2:1 ratio with Aβ_{42}. Concentration dependence of the inhibition of Aβ_{42} aggregation was assessed for 2 and 8 by assaying compound 2 at 50 µM and 2 µM (1:Aβ_{42} ratio of 1:1 and 0.04:1, respectively), while compound 8 was also tested at 50 µM (8:Aβ_{42} = 1:1). At t=0 and t=24 h, aliquots with and without inhibitor were analyzed by flow injection (FIA)-ESI-IT-MS. FIA–ESI-IT-MS analyses were performed, as described by Fiori *et al.* Briefly, the Aβ_{42} samples were analyzed by 10 µL loop injection after previous addition of reserpine as internal standard. Analyses were performed on a Jasco PU-1585 Liquid Chromatograph (Jasco, Tokyo, Japan) interfaced with a LCQ Duo Mass Spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source operating with an ion trap analyzer. The mobile phase consisted of 0.1% (v/v) formic acid in CH₃CN/H₂O (30/70). The ESI system employed a 4.5 kV spray voltage and a capillary temperature of 200°C. Mass spectra were operated in positive polarity, in the scan range of 200-2000 m/z and at a scan rate of three microscans per second. Single-ion monitoring (SIM) chromatograms for the quantitative analysis were reconstructed at the base peaks corresponding to the differently charged amyloid monomer ions (Native, N) and oxidized ions (Ox). The ratio between the total monomer area and the IS area was used for Aβ_{42} monomer determination. The Area_{total monomer}/Area_{IS} ratio at t=0 was considered as 100% of the monomer content.

The percentage of inhibition was calculated as follow:

\[
\text{% inhibition} = 100 \times \left( \frac{x_{t=24} - x_{t=0}}{x_{t=0} - x_{t=24}} \right)
\]

where \(x_{t=0}\) is the Area Aβ42m/Area IS ratio at \(t=0\), \(x_{t=24}\) is the Area Aβ42m/Area IS ratio after 24h in the absence of inhibitor and \(x_{t=24}\) is the Area Aβ42m/Area IS ratio after 24h in the presence of
inhibitor. The results are expressed as mean ±SD of three independent experiments, and p<0.05 was considered statistically significant (Dunnett’s multiple comparison test).

Cultures of mixed cortical neurons and assessment of neuronal injury.

Cultures of pure cortical neurons were obtained from rats at embryonic day 17 (Envigo Laboratories, Italy). Briefly, cortices were dissected in Ca²⁺/Mg²⁺ free buffer and mechanically dissociated. Cortical cells were plated at a density of 2 x 10⁶/dish on 35 mm dishes (Nunc, Rochester, NY, USA) pre-coated with 0.1 mg/mL poly-D-lysine (Sigma-Aldrich Chimica srl, Milan, Italy) in DMEM/F12 (1:1) supplemented with 10% horse serum, 10% foetal calf serum (FCS), 2 mM glutamine, 6 mg/mL glucose. After 7-10 days in vitro, glia cell division was halted by exposure to 10 µM cytosine-D-arabinoside for 3 days and cells were shifted into a maintenance serum-free medium. Mature cultures contained about 35-40% neurons.

Neuronal injury was measured in all experiments by examination of cultures with phase-contrast microscopy at 20×2 days after Aβ42 treatment, when the process of cell death was largely complete. Neuronal injury was assessed by Trypan Blue staining in mixed neuronal cultures 72h after Aβ42 treatment. Neuronal damage was quantitatively assessed by counting dead neurons stained with Trypan blue. Stained neurons were counted in three random microscopic fields/well.

Sample preparation of monomeric and oligomeric Aβ42. Monomeric Aβ42 was prepared using a Zagorsky modified procedure. The Aβ42 lyophilized peptide, purchased from Bachem, was dissolved in TFA (1 mg/mL) and sonicated in a water bath sonicator for 10 min. Then, the TFA was evaporated under a gentle stream of argon and 1 mL HFIP was added to the peptide. After 1h incubation at 37 °C, the peptide solution was dried under a stream of argon, and then solubilized again by adding 2 mL of HFIP. Finally, HFIP was removed by argon streaming followed by further drying in a lyophilizer for 1h and Aβ42 then resuspended in 5 mM anhydrous dimethyl sulfoxide (DMSO) before dilution to 100 µM in ice-cold cell culture medium DMEM-F12. 100µM Aβ42
samples were incubated for 72h at 4 °C. Human Aβ$_{42}$ oligomers were then used at a final concentration of 1 µM in the presence of the glutamate receptor antagonists MK-801 (10 µM) and DNQX (30 µM) to avoid the potentiation of endogenous glutamate toxicity.

In co-incubation experiments samples of 100 µM Aβ$_{42}$ monomers were incubated for 72 h at 4 °C in the absence or in the presence of a 5-fold molar excess of compounds 2 or 8 and then added to mixed neuronal cultures for other 72 h.

**ROS quantification**

**DCFDA.** T67 cells were seeded in 24-well plates at 30000 cells/well and incubated overnight to allow adhesion. Then, cells were incubated for 4 hours with 10µM of compounds or equal amounts of vehicle (DMSO) dissolved in complete DMEM. After this time, cells were washed with PBS and treated with 10µM DCFDA (2,7-Dichlorofluorescin diacetate) dissolved in DMEM for 1 hour. Then, the cells were washed with PBS and exposed to 100µM TBHP (tert-butyl hydroperoxide) dissolved in DMEM for 30 minutes. Finally, cells were washed with PBS and the fluorescence emission from each well was measured ($\lambda_{exc} = 485$ nm; $\lambda_{em} = 535$ nm) with a multiplate reader (Enspire, PerkinElmer).

**MitoSOX.** T67 cells were seeded in 96-well plate (Viewplate-96 F TC) at 6000 cells/well and incubated overnight to allow adhesion. After this time, cells were treated for 30 minutes with (5µM) MitoSOX dissolved in KRB saline (135 mM NaCl, 5 mM KCl, 0.4 mM KH$_2$PO$_4$, 1 mM MgSO$_4$, 20 mM HEPES, glucose 1 g/L and 1 mM CaCl$_2$ pH 7.4). Then, cells were washed twice with KRB and incubated for 4h with different concentrations of 2 and 3 dissolved in KRB or equal amount of vehicle (DMSO) dissolved in KRB. 10µM Antimycin A was used as a positive control. Finally, cells were washed with KRB and the fluorescence emission from each well was measured ($\lambda_{exc} = 510$ nm; $\lambda_{em} = 580$ nm) with a multiplate reader (Enspire, PerkinElmer).
Computational Methods

Modeling of helical and fibril Aβ42-ligand systems. The ligand structures were built using the Schrödinger Software (v.2.1, Maestro 9.5.0.14) and minimized by semi-empirical NDDO Module PM3. The force field parameters of ligands were obtained using ParamChem server employing CGenFF (CHARMM General Force Field) for small molecules. In order to study binding of ligands to Aβ42 we used helical conformations of amyloid as well as its oligomeric form. The helical structures of Aβ42 were taken from Protein Data Bank NMR structure (PDB id:1IYT). The first five from ten models deposited in 1IYT were selected to our study. They were distributed circularly with ligand in the center. Helical structures of Aβ42 were rotated randomly around their long axis, including upside down flipping, to simulate random distribution. The structure of Aβ42 in oligomeric fibril form, involving five monomers, was also taken as NMR structure from Protein Data Bank (PDB id:2BEG). Since only part of the structure is visible (residues 17-42) the lacking residues were added by incorporating 16-residue fragment from NMR structure of Aβ(1-28) (PDB id:1BJC). The fragment 1-16 exists in the coil form. Different NMR conformations of this 1-16 fragment were selected for each monomer of the fibril. Two identical ligands were inserted at both ends of the fibril, one at each side, in a distance 2.5 Å on average.

Molecular dynamics simulations. The prepared Aβ42-ligand systems were initially subjected to 10 000 steps of energy minimization and then 100 ns MD equilibration with increasing temperature from 20 K to 298 K. All energy minimizations and molecular dynamics (MD) simulations were performed in NAMD program version 2.10 using all-atom force field CHARMM22 in implicit solvent. The MD simulations were conducted using Langevin (stochastic) dynamics which is default in the NAMD program. The molecules in the system interact with a stochastic heat bath via random forces and dissipative forces. The friction coefficient of 50 ps⁻¹ was used and temperature was set to 298 K. Non-bonded interactions were damped employing a switching function for van
der Waals and electrostatic interactions using cutoff of 14 Å. For each investigated system 600 ns MD simulation was performed with a time step of 2 fs. All bond lengths were constrained using SHAKE algorithm. All figures of molecular structures were created using VMD program (v.1.9.2).

**Details of ligand binding to helical form of Aβ₄₂.** For compound 2, one catechol moiety forms hydrogen bonds with side chains of E11, H14 and N27 while the second one with side chains of N23 and S26. The central ring does not form any bond, and spermine tail of compound 2 forms hydrogen bonds with Y10 (sc – side chain), H13 (mc – main chain), Q15 (sc), N27 (mc) and A30 (mc). The hydrogen bonds with main chains were created at the ends of α-helices. In complex of compound 8 there are much less hydrogen bonds than for 1: one catechol moiety forms a hydrogen bond with Q15 (sc) of α-helix and A30 (mc) of a loop, while the second catechol moiety also forms a hydrogen bond with Q15 (sc) of π-helix of another monomer. The charged nitrogen of the central ring of compound 8 forms a hydrogen bond with L17 (mc). Helices are mostly unfolded (they were converted into β-turns) and there is small number of π-helices (two helix turns). Spermine, which is highly positively charged as compound 2, forms some hydrogen bonds to Q15 (sc), F19 (mc), E22 (mc), and N27 (sc). With D23 (sc) it forms a distant salt bridge.

**Details of ligand binding to fibril form of Aβ₄₂.** There are many interactions between compound 2 and beta-amyloid, mostly hydrogen bonds, involving catechol rings: R5 (mc) and F20 (mc) for one ring, and G33 (mc) with M35 (mc) for the second ring; and also spermine tail: A21 (mc), E22 (sc), D1 (sc), N27 (mc), V24 (mc), N27 (ms). Both copies of compound 2 are perpendicular to β-sheet of amyloid fibril. Compound 8 forms much less of hydrogen bonds than compound 2 at both ends of the fibril: E11 (mc), V36 (mc), E22 (sc), and additionally there is some number of π-π interactions: H13 (sc), H14 (sc), F20 (sc). At one end of fibril the compound 8 is parallel to β-sheet while at the other end it is perpendicular. Spermine binds to the fibril in extended conformation and creates
many hydrogen bonds with main chain and also side chains of amino acids: D1 (sc), F20 (mc), E22 (sc), V24 (sc), S26 (mc), N27 (sc), G37 (mc).
Figure S1. Antioxidant activity of compounds 2 and 3 on oxidative stress induced by tert-Butyl hydroperoxide (TBHP). T67 cells were pretreated for 4 hours with 10µM of compounds 2 and 3. After this time, ROS were detected by following DCFDA oxidation after 30 minutes exposure to 100 µM TBHP.
Figure S2. Complexes of compound 8 (a) and spermine (b) and five helical monomers of Aβ42 after 600 ns MD simulation. Left panels – side view, right panels – top view. Colors used: α-helix – blue, π-helix – red, β-sheet and β-bridge – yellow, β-turn – green, coil – white. Compounds are shown as
fat sticks while amino acid residues in vicinity of compounds as thin sticks. Hydrogen bonds are shown as dashed lines.

(a)

(b)

**Figure S3.** Complexes of compound 8 (a) and spermine (b) and a fibril of Aβ_{42} after 600 ns MD simulation. Two copies of each compound were located at each side of the fibril.
Figure S4. Complexes of compound 2 with Aβ42 in helical and fibril conformation after 600 ns MD simulation showing closely located M35. The residue M35 is shown as medium size sticks.

Figure S5. Effects of Compound 2 and compound 8 on neuronal survival in mixed cortical neurons. Compound 2 and compound 8 were applied to neuronal cultures at increasing concentrations (250 nM-50 μM). The intrinsic toxicity of compounds 2 and 8 was assessed by cell counting after trypan blue staining. Cell counts was performed in three random microscopic fields/well.
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