

## Supporting Information

for

### A novel hybrid of 6-chlorotacrine and metal-amyloid- $\beta$ modulator for inhibition of acetylcholinesterase and metal-induced amyloid- $\beta$ aggregation

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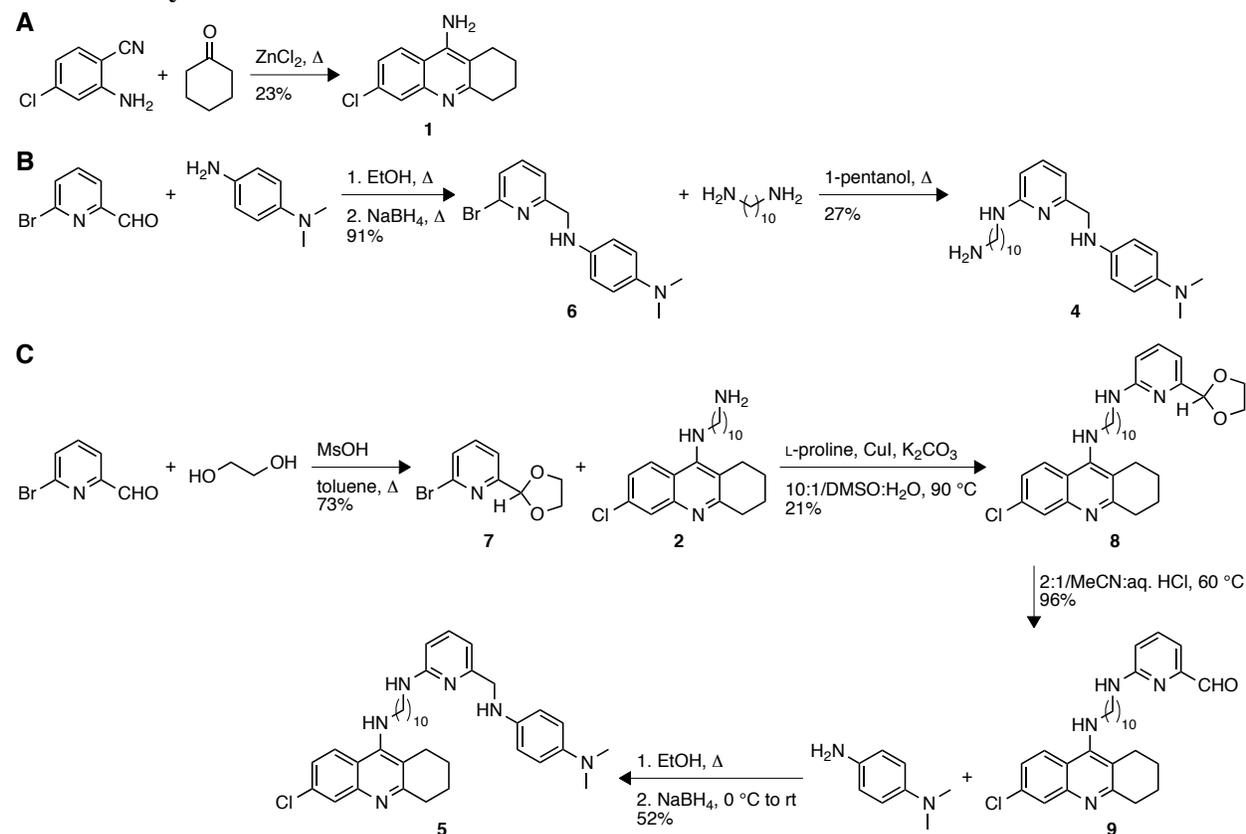
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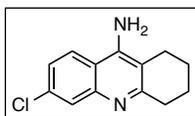
**Fig. S8.** Disaggregation studies using **1**, **2**, **3**, **4**, and **5** with and without AChE. **A.** Scheme of the disaggregation experiment. **B.** Visualization of various-sized  $\text{A}\beta$  species in the absence (left) and presence (right) of AChE by gel electrophoresis with Western blot (anti- $\text{A}\beta$  antibody, 6E10). Conditions: [ $\text{A}\beta$ ] = 25  $\mu\text{M}$ ; [ $\text{CuCl}_2$  or  $\text{ZnCl}_2$ ] = 25  $\mu\text{M}$ ; [AChE] = 25  $\mu\text{M}$  [compound] = 50  $\mu\text{M}$ ; pH 6.6 (for  $\text{Cu}^{2+}$  samples) or 7.4 (for metal-free and  $\text{Zn}^{2+}$  samples); 24 h incubation; 37  $^\circ\text{C}$ ; constant agitation.

## 1. Materials and instrumentation.

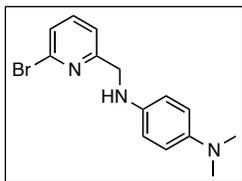
Compounds **2**<sup>1</sup> and **3**<sup>2</sup> were prepared as previously reported. A $\beta$ <sub>1-40</sub> (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVV) was purchased from Anaspec (Fremont, CA). All reagents for chemical synthesis were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Reactions were monitored by TLC (Merck, Silica gel 60 F<sub>254</sub>). Visualization was achieved using the following methods: UV absorption by fluorescence quenching or a ninhydrin stain (ninhydrin (1.5 g, *n*-butanol (100 mL), AcOH (3 mL)). Compounds were purified by SiO<sub>2</sub> flash chromatography (Dynamic Adsorbents Inc., Flash Silica Gel 32-63u). <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker Avance™ DPX 500 or Varian 400 MHz spectrometer. Liquid chromatography mass spectrometry (LCMS) was performed on a Shimadzu LCMS-2019EV equipped with a SPD-20AV UV-Vis detector and a LC-20AD liquid chromatograph. HRMS was performed on a Micromass AutoSpec Ultima Magnetic sector mass spectrometer. Optical spectra for metal binding studies were obtained by using an Agilent 8453 UV-Visible (UV-Vis) spectrophotometer. Analyses by UV-Vis assays (determination of IC<sub>50</sub> values and parallel artificial membrane permeability blood-brain barrier penetration (PAMPA-BBB) assay) were carried out on a multimode SpectraMax M5 plate reader (Molecular Devices, Sunnyvale, CA) using 96-well plates (Fisher Scientific). Transmission electron microscopy (TEM) images were recorded on a Philips CM-100 transmission electron microscope (Microscopy and Image Analysis Laboratory, University of Michigan, Ann Arbor, MI). Molecular modeling was performed using Sybyl-X and GOLD.<sup>3</sup>

## 2. Chemistry methods.

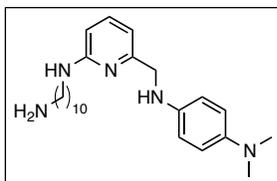




**2.1. Preparation of 6-chlorotacrine (1)** (Fig. S1A). The known compound **1** was prepared in a manner similar to those previously established in the literature.<sup>4,5</sup> Briefly, 2-amino-4-chlorobenzonitrile (382 mg, 2.5 mmol, 1 eq), cyclohexanone (1.26 mL, 12.2 mmol, 4.86 eq), and anhydrous ZnCl<sub>2</sub> (750 mg, 5.5 mmol, 2.2 eq) were combined and heated at 125 °C for 3 h. The reaction mixture was cooled to rt and treated with H<sub>2</sub>O (50 mL). The remaining solid was collected by vacuum filtration, treated with 1 M aq. NaOH (50 mL), and heated to reflux for 16 h. The reaction mixture was then cooled to rt and extracted with CHCl<sub>3</sub> (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure to afford a crude yellow solid, which was purified by flash column chromatography (SiO<sub>2</sub>, 9:1/EtOAc:MeOH, R<sub>f</sub> 0.15) to yield **1** (135 mg, 23%) as an off-white solid: <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>SO, 400 MHz) δ 8.18 (d, 1H, *J* = 9.0 Hz), 7.62 (d, 1H, *J* = 2.0 Hz), 7.28 (dd, 1H, *J*<sub>1</sub> = 9.0 Hz, *J*<sub>2</sub> = 2.0 Hz), 6.48 (s, 2H), 2.81 (t, 2H, *J* = 5.4 Hz), 2.53 (t, 2H, *J* = 5.5 Hz), 1.81 (m, 4H); <sup>13</sup>C NMR ((CD<sub>3</sub>)<sub>2</sub>SO, 100 MHz) δ 158.8, 148.3, 147.0, 132.4, 126.4, 124.2, 122.7, 115.6, 109.4, 33.5, 23.6, 22.5, 22.4; LRMS *m/z* calcd for C<sub>13</sub>H<sub>13</sub>ClN<sub>2</sub>: 232.08; found 233.00 [M+H]<sup>+</sup>.



**2.2. Preparation of N<sup>1</sup>-[(6-bromopyridin-2-yl)methyl]-N<sup>4</sup>,N<sup>4</sup>-dimethylbenzene-1,4-diamine (6)** (Fig. S1B). 6-Bromo-2-pyridinecarboxaldehyde (1 g, 5.38 mmol, 1 eq), *N,N*-dimethyl-*p*-phenylenediamine (733 mg, 5.38 mmol, 1 eq), and EtOH (40 mL) were combined and heated to reflux for 1 h. The reaction was cooled to rt, and NaBH<sub>4</sub> (1.1 g, 28.8 mmol, 5.4 eq) was added. The reaction was again heated to reflux for 1 h before being cooled to rt, quenched with H<sub>2</sub>O (100 mL), and extracted with Et<sub>2</sub>O (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure to afford a crude brown oil, which was purified by flash column chromatography (SiO<sub>2</sub>, 1:1/hexanes:EtOAc, R<sub>f</sub> 0.43) to yield **6** (1.5 g, 91%) as a brown solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 7.48 (t, 1H, *J* = 7.7 Hz), 7.35 (d, 1H, *J* = 7.8 Hz), 7.32 (d, 1H, *J* = 7.6 Hz), 6.76-6.58 (m, 4H), 4.41 (br s, 2H), 2.83 (br s, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ 161.5, 144.3, 141.6, 139.9, 139.1, 126.3, 120.3, 115.8, 114.5, 50.1, 42.2; LRMS *m/z* calcd for C<sub>14</sub>H<sub>16</sub>BrN<sub>3</sub>: 305.05; found 305.75 [M+H]<sup>+</sup>.



**2.3. Preparation of N<sup>1</sup>-[(6-((10-aminodecyl)amino)pyridin-2-yl)methyl]-N<sup>4</sup>,N<sup>4</sup>-dimethylbenzene-1,4-diamine (4)** (Fig. S1B). Compound **6** (136 mg, 0.443 mmol, 1 eq), 1,10-diaminodecane (305 mg, 1.77 mmol, 4 eq), and 1-pentanol (2 mL) were combined and heated to reflux for 14 h. The reaction was cooled to rt, diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL), and washed with 10% aq. KOH (2 x 50 mL), H<sub>2</sub>O (2 x 50 mL), and brine (50 mL). The organic layer was dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure to afford a crude yellow oil, which was purified by flash column chromatography (SiO<sub>2</sub>, 7:3/CH<sub>2</sub>Cl<sub>2</sub>:MeOH with NH<sub>4</sub>OH (7 mL/L of solvent), R<sub>f</sub> 0.76). Further purification by flash column chromatography (SiO<sub>2</sub>, 9:1/CH<sub>2</sub>Cl<sub>2</sub>:MeOH with NH<sub>4</sub>OH (7 mL/L of solvent), R<sub>f</sub> 0.23) removed remaining impurities and gave **4** (47 mg, 27%) as a dark yellow solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) (Fig. S2) δ 7.36 (t, 1H, *J* = 7.9 Hz), 6.73 (d, 2H, *J* = 8.8 Hz), 6.65 (d, 2H, *J* = 8.8 Hz), 6.58 (d, 1H, *J* = 7.3 Hz), 6.23 (d, 1H, *J* = 8.2 Hz), 4.56 (br t, 1H, *J* = 5.1 Hz), 4.19 (s, 2H), 3.22 (q, 2H, *J* = 6.6 Hz), 2.80 (s, 6H), 2.68 (t, 2H, *J* = 7.0 Hz), 1.61 (p, 2H, *J* = 7.2 Hz), 1.46-1.36 (m,

4H), 1.29 (s, 10H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz) (Fig. S3)  $\delta$  158.8, 157.4, 144.2, 141.0, 138.1, 116.0, 114.6, 110.4, 104.1, 50.4, 42.5, 42.4, 42.2, 33.5, 29.7, 29.63, 29.62, 29.53, 29.49, 27.2, 27.0; HRMS  $m/z$  calcd for  $\text{C}_{24}\text{H}_{39}\text{N}_5$ : 397.3205; found 398.3260  $[\text{M}+\text{H}]^+$ .

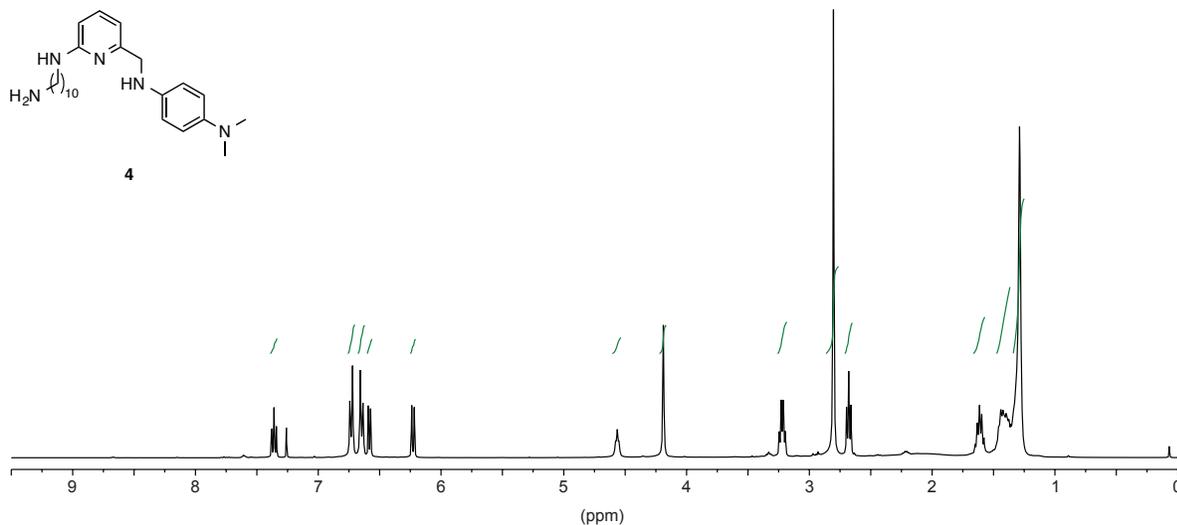


Fig. S2.  $^1\text{H}$  NMR spectrum for compound 4.

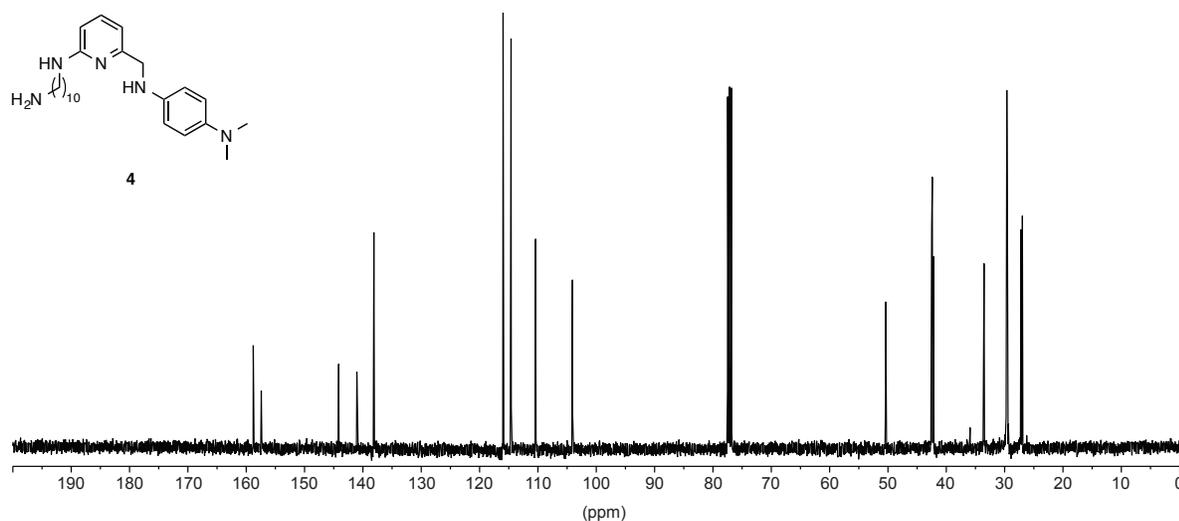
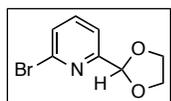
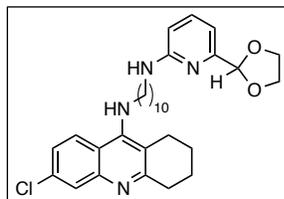


Fig. S3.  $^{13}\text{C}$  NMR spectrum for compound 4.

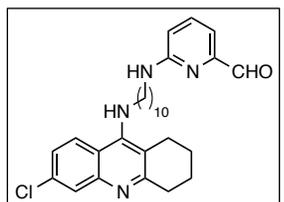


**2.4. Preparation of 2-bromo-6-(1,3-dioxolan-2-yl)pyridine (7)** (Fig. S1C). The known compound 7 was prepared by following a modified protocol of previously reported procedures.<sup>6,7</sup> A solution of 6-bromo-2-pyridinecarboxaldehyde (2.0 g, 10.8 mmol, 1 eq), ethylene glycol (1.2 mL, 21.5 mmol, 2 eq), and methanesulfonic acid (230  $\mu\text{L}$ , 3.55 mmol, 0.33 eq) in toluene (65 mL) was heated to reflux in a Dean-Stark apparatus for 24 h prior to being cooled to rt and neutralized with saturated aq.  $\text{NaHCO}_3$ . The organic layer was separated and the aqueous phase was extracted with  $\text{Et}_2\text{O}$  (3 x 50 mL). The combined organic layers were washed with  $\text{H}_2\text{O}$  (3 x 50 mL) and brine (50 mL), dried ( $\text{MgSO}_4$ ), filtered, and concentrated under reduced pressure to afford a crude yellow oil, which was purified by flash column chromatography ( $\text{SiO}_2$ , 1:1/hexanes: $\text{EtOAc}$ ,  $R_f$  0.66) to yield 7 (1.81 g, 73%) as a pale

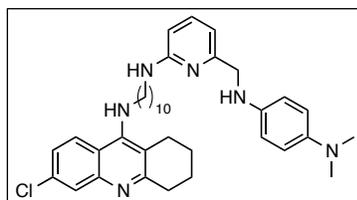
yellow oil:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.49 (t, 1H,  $J = 7.7$  Hz), 7.38 (d, 1H,  $J = 7.3$  Hz), 7.35 (d, 1H,  $J = 7.9$  Hz), 5.68 (s, 1H), 4.06-3.99 (m, 2H), 3.97-3.90 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  158.3, 141.3, 139.0, 128.2, 119.3, 102.5, 65.4.



**2.5. Preparation of  $N^1$ -[6-(1,3-dioxolan-2-yl)pyridin-2-yl]- $N^{10}$ -(6-chloro-tacrine)decane-1,10-diamine (8)** (Fig. S1C). Compound **7** (500 mg, 2.17 mmol, 1 eq), compound **2** (1.25 g, 3.23 mmol, 1.5 eq), CuI (21 mg, 0.109 mmol, 0.05 eq), L-proline (25 mg, 0.217 mmol, 0.10 eq),  $\text{K}_2\text{CO}_3$  (45 mg, 0.326 mmol, 0.15 eq), DMSO (8 mL), and  $\text{H}_2\text{O}$  (800  $\mu\text{L}$ ) were combined and stirred at 90  $^\circ\text{C}$  for 47 h. The reaction was cooled to rt, diluted with  $\text{H}_2\text{O}$  (150 mL), and extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 50 mL). The combined organic layers were washed with  $\text{H}_2\text{O}$  (3 x 50 mL) and brine (50 mL), dried ( $\text{MgSO}_4$ ), filtered, and concentrated under reduced pressure to afford a crude brown oil, which was purified by flash column chromatography ( $\text{SiO}_2$ , 49:1/ $\text{CH}_2\text{Cl}_2$ :MeOH with  $\text{NH}_4\text{OH}$  (7 mL/L of solvent) to 19:1/ $\text{CH}_2\text{Cl}_2$ :MeOH with  $\text{NH}_4\text{OH}$  (7 mL/L of solvent),  $R_f$  0.41 (9:1/ $\text{CH}_2\text{Cl}_2$ :MeOH with  $\text{NH}_4\text{OH}$  (7 mL/L of solvent))) to yield **8** (249 mg, 21%) as a yellow oil:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  7.84 (d, 1H,  $J = 9.0$  Hz), 7.83 (s, 1H), 7.39 (t, 1H,  $J = 7.8$  Hz), 7.20 (dd, 1H,  $J_1 = 9.0$  Hz,  $J_2 = 1.4$  Hz), 6.73 (d, 1H,  $J = 7.3$  Hz), 6.29 (d, 1H,  $J = 8.4$  Hz), 5.63 (s, 1H), 4.65 (br t, 1H,  $J = 5.0$  Hz), 4.13-4.05 (m, 2H), 4.02-3.96 (m, 2H), 3.94 (br s, 1H), 3.42 (br t, 2H), 3.15 (q, 2H,  $J = 6.6$  Hz), 2.97 (br t, 2H), 2.60 (br t, 2H), 1.85 (br p, 4H), 1.59 (p, 2H,  $J = 7.0$  Hz), 1.54 (p, 2H,  $J = 7.3$  Hz), 1.32-1.29 (m, 4H), 1.23 (br s, 8H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  159.4, 158.8, 155.4, 150.8, 148.1, 138.0, 133.8, 127.5, 124.6, 124.0, 118.4, 115.6, 108.9, 106.0, 103.6, 65.3, 49.6, 42.3, 34.0, 31.7, 29.42, 29.38, 29.27, 29.26, 27.0, 26.8, 24.5, 22.9, 22.6; LRMS  $m/z$  calcd for  $\text{C}_{31}\text{H}_{41}\text{ClN}_4\text{O}_2$ : 536.29; found 537.35 [ $\text{M}+\text{H}$ ] $^+$ .



**2.6. Preparation of 6-([10-((6-chloro-1,2,3,4-tacrine)amino)decyl]amino)picolinaldehyde (9)** (Fig. S1C). A solution of compound **8** (91 mg, 0.170 mmol) in MeCN (2.2 mL) and 2 M aq. HCl (1.1 mL) was stirred at 60  $^\circ\text{C}$  for 23 h. The reaction was cooled to rt, slowly quenched with saturated aq.  $\text{NaHCO}_3$ , and extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 25 mL). The combined organic layers were washed with brine (50 mL), dried ( $\text{MgSO}_4$ ), filtered, and concentrated under reduced pressure to yield **9** (81 mg, 96%) as a yellow oil, which was used without any further purification:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  9.82 (s, 1H), 7.85 (d, 1H,  $J = 9.1$  Hz), 7.84 (s, 1H), 7.48 (t, 1H,  $J = 7.8$  Hz), 7.20 (dd, 1H,  $J_1 = 9.1$  Hz,  $J_2 = 1.9$  Hz), 7.15 (d, 1H,  $J = 7.2$  Hz), 6.53 (d, 1H,  $J = 8.4$  Hz), 4.86 (br t, 1H), 4.03-3.97 (m, 1H), 3.43 (t, 2H,  $J = 7.2$  Hz), 3.27 (q, 2H,  $J = 6.7$  Hz), 2.98 (br t, 2H), 2.61 (br t, 2H), 1.85 (br p, 4H), 1.62 (p, 2H,  $J = 7.2$  Hz), 1.57 (p, 2H,  $J = 7.4$  Hz), 1.34-1.30 (m, 4H), 1.24 (br s, 8H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  193.8, 159.4, 159.1, 151.3, 150.9, 148.0, 137.8, 133.9, 127.4, 124.7, 124.1, 118.3, 115.6, 112.0, 111.5, 49.6, 42.1, 34.0, 31.8, 29.5, 29.4, 29.31, 29.28, 27.0, 26.9, 24.6, 22.9, 22.6;  $m/z$  calcd for  $\text{C}_{29}\text{H}_{37}\text{ClN}_4\text{O}$ : 492.27; found 493.20 [ $\text{M}+\text{H}$ ] $^+$ .



**2.7. Preparation of  $N^1$ -{[6-((10-((6-chloro-1,2,3,4-tetrahydroacridin-9-yl)amino)decyl)amino)pyridin-2-yl]methyl}- $N^4,N^4$ -dimethylbenzene-1,4-diamine (5)** (Fig. S1C). To a solution of compound **9** (64 mg, 0.129 mmol, 1 eq) in EtOH (2.5 mL) was added  $N,N$ -dimethyl- $p$ -phenylenediamine (18 mg,

0.129 mmol, 1 eq). The reaction mixture was heated to reflux for 1 h and cooled to 0 °C prior to addition of NaBH<sub>4</sub> (33 mg, 0.864 mmol, 6.7 eq). The reaction mixture was stirred at 0 °C for 5 min, warmed to rt, and stirred for an additional 30 min before quenching with H<sub>2</sub>O (10 mL) and extracting with Et<sub>2</sub>O (3 x 10 mL). The combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure to afford a crude yellow oil, which was purified by flash column chromatography (SiO<sub>2</sub>, 19:1/CH<sub>2</sub>Cl<sub>2</sub>:MeOH with NH<sub>4</sub>OH (7 mL/L of solvent), R<sub>f</sub> 0.20) to yield **5** (41 mg, 52%) as a yellow oil: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) (Fig. S4) δ 7.888 (d, 1H, *J* = 1.5 Hz), 7.886 (d, 1H, *J* = 8.8 Hz), 7.35 (t, 1H, *J* = 7.7 Hz), 7.25 (dd, 1H, *J*<sub>1</sub> = 8.8 Hz, *J*<sub>2</sub> = 1.5 Hz), 6.73 (d, 2H, *J* = 8.6 Hz), 6.64 (d, 2H, *J* = 8.6 Hz), 6.58 (d, 1H, *J* = 7.3 Hz), 6.22 (d, 1H, *J* = 8.3 Hz), 4.56 (br t, 1H, *J* = 4.9 Hz), 4.19 (s, 2H), 3.97 (br s, 1H), 3.47 (t, 2H, *J* = 7.0 Hz), 3.22 (q, 2H, *J* = 6.6 Hz), 3.02 (br t, 2H), 2.80 (s, 6H), 2.65 (br t, 2H), 1.90 (br p, 4H), 1.64 (p, 2H, *J* = 7.1 Hz), 1.61 (p, 2H, *J* = 7.4 Hz), 1.39-1.35 (m, 4H), 1.29 (s, 9H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) (Fig. S5) δ 159.5, 158.7, 157.4, 150.9, 148.2, 144.1, 141.0, 138.1, 134.0, 127.6, 124.7, 124.2, 118.5, 115.9, 115.7, 114.6, 110.4, 104.1, 50.3, 49.7, 42.44, 42.36, 34.1, 31.9, 29.7, 29.5, 29.44, 29.39, 27.2, 27.0, 24.6, 23.0, 22.7; HRMS *m/z* calcd for C<sub>37</sub>H<sub>49</sub>ClN<sub>6</sub>: 612.3707; found 613.3600 [M+H]<sup>+</sup>.

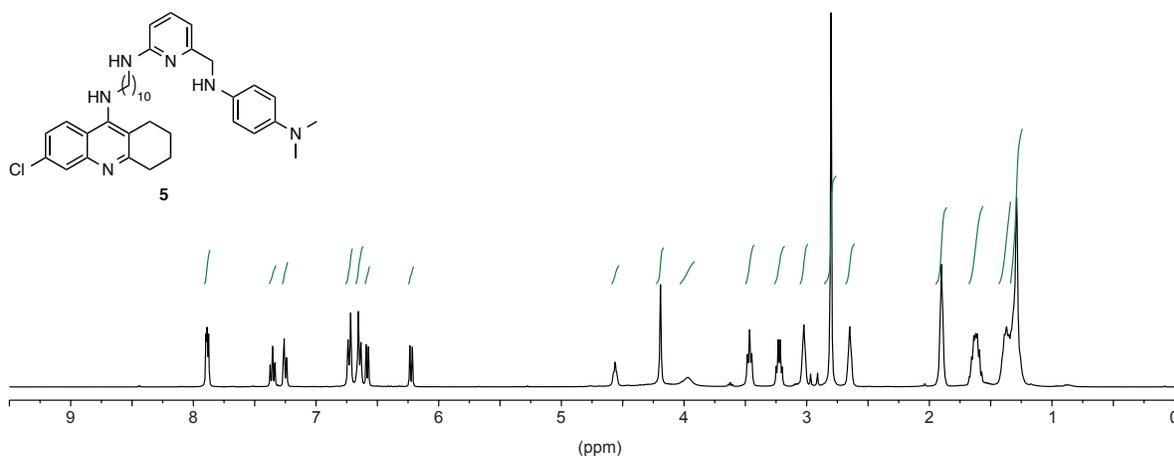


Fig. S4. <sup>1</sup>H NMR spectrum for compound **5**.

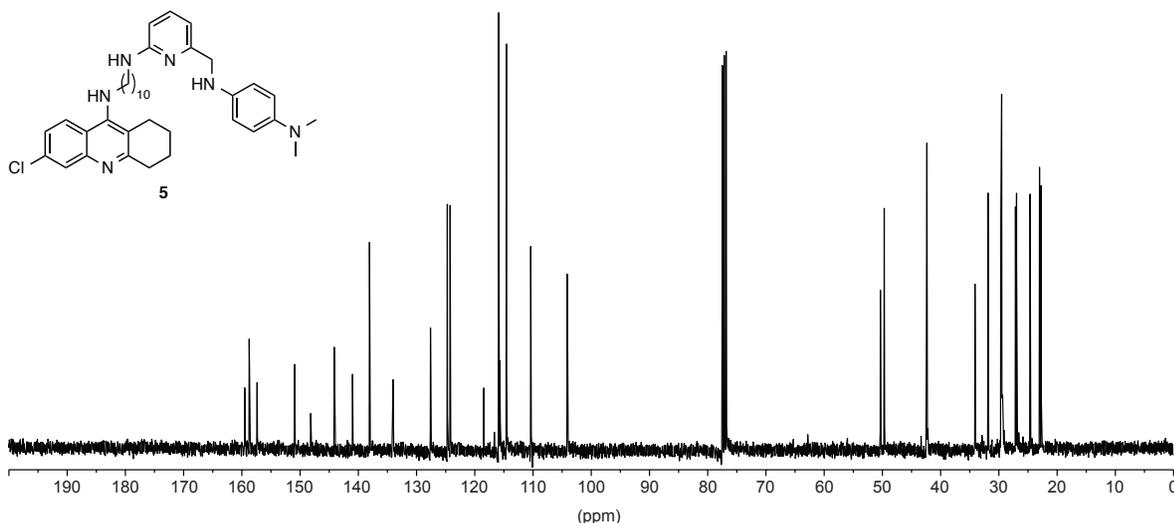


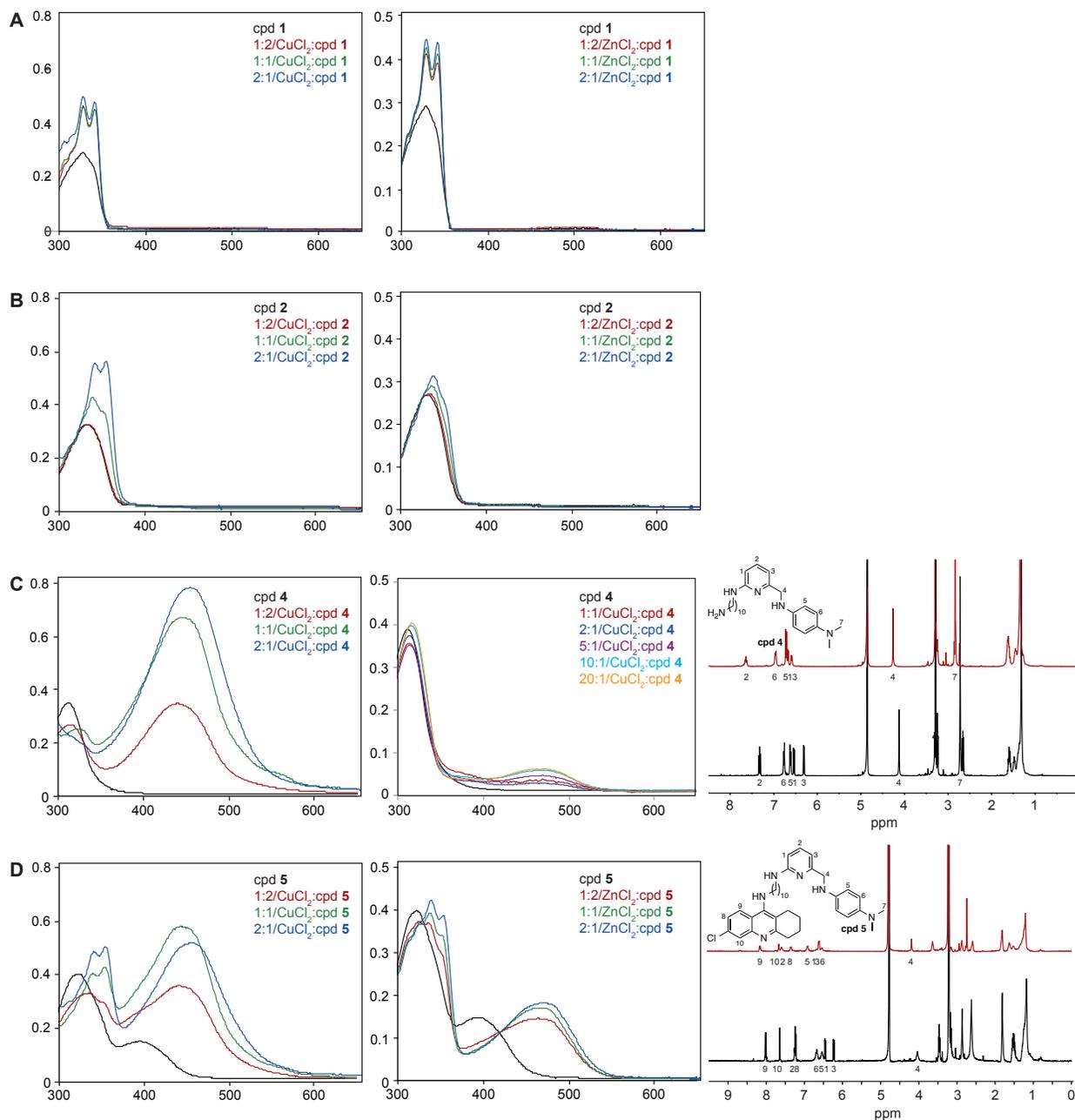
Fig. S5. <sup>13</sup>C NMR spectrum for compound **5**.

### 3. Biochemical, biophysical, and computational methods.

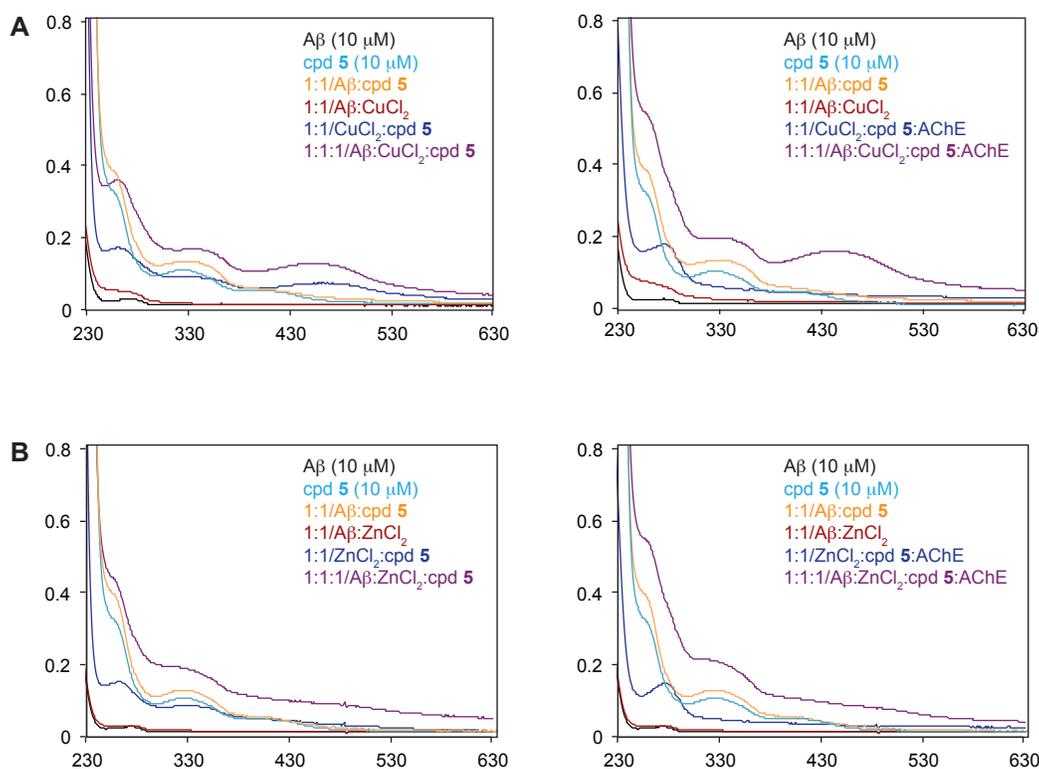
**3.1. *In vitro* acetyl- and butyrylcholinesterase (AChE and BChE) assay.** Compounds of interest were dissolved in sodium phosphate (dibasic) buffer ((100  $\mu$ L), 0.1 M, pH 8.0 adjusted at rt). AChE was added to the solution of inhibitors (50  $\mu$ L, containing 0.08 U/mL (~0.29 nM) AChE (final concentration) (Sigma-Aldrich cat #C2888 from eel) in sodium phosphate (dibasic) buffer (100 mM, pH 8.0 adjusted at rt)). The mixture of inhibitor and enzyme was incubated for 10 min before initiation with a DTNB:acetylthiocholine (ATC) (0.25 mM:0.5 mM final concentration, respectively) mixture (50  $\mu$ L) in phosphate buffer (100 mM, pH 8.0 adjusted at rt). The reaction was monitored at 412 nm taking measurements every 30 sec for 20 min on a SpectraMax M5 plate reader. Data was corrected with the negative control (no ATC) and normalized to the positive control (no inhibitor) using the initial rates (first 2-5 min). All assays were performed in triplicate. The data was fitted to a Hill-plot and  $IC_{50}$  values calculated using KaleidaGraph 4.1.1. All  $IC_{50}$  values are reported in Table 1. All butyrylcholinesterase (BChE) experiments were performed using identical conditions substituting butyrylthiocholine (BTC) for ATC.

**3.2. *In vitro* AChE and BChE inactivation assay in a ROS environment.** In the wells of a 96-well plate, horseradish peroxidase (0.25  $\mu$ M),  $H_2O_2$  (100  $\mu$ M), AChE (0.08 U/mL, ~0.29 nM), inhibitors (25  $\mu$ M to 13 pM), and DETAPAC (100  $\mu$ M) were dissolved in sodium acetate buffer (50 mM, pH 6.0 adjusted at rt) and incubated at 37 °C for 30 min. Note: all concentrations are reported as final concentrations. A DTNB:acetylthiocholine (ATC) (0.25 mM:0.5 mM final concentration, respectively) mixture in sodium phosphate (dibasic) buffer (50 mM, pH 7.4 adjusted at rt) was added to the AChE/inhibitor solution to initiate the reactions. The crude data was processed to obtain  $IC_{50}$  values as described in section 3.1. All  $IC_{50}$  values are reported in Table 1. BChE experiments were done in an identical manner substituting BTC for ATC.

**3.3. Metal binding studies by UV-Vis and NMR spectroscopy.** The interaction of compounds **1-5** with 0.5-2 eq of  $Cu^{2+}$  or  $Zn^{2+}$  in EtOH was monitored by UV-Vis ([compound] = 40  $\mu$ M (1% v/v final DMSO concentration; incubation for 30 min (4 h for  $Zn^{2+}$  binding for cpd **4**); rt) (Fig. S6). Metal binding properties of compound **5** in the presence of  $A\beta$  and/or AChE were also studied by UV-Vis (Fig. S7).  $A\beta$  (10  $\mu$ M) was treated for 2 min with  $CuCl_2$  or  $ZnCl_2$  (10  $\mu$ M) in HEPES (20 mM, pH 6.6 (for  $Cu^{2+}$ ) or pH 7.4 (for  $Zn^{2+}$ ) and NaCl (150 mM). AChE (10  $\mu$ M) was added to the solution containing  $A\beta$  and  $Cu^{2+}$  or  $Zn^{2+}$ . The resulting sample was incubated for 5 min at rt and treated with compound **5** (10  $\mu$ M, 5% v/v final DMSO concentration) followed by 5 min incubation. For comparison, optical spectra of the samples generated from incubation of compound **5** (10  $\mu$ M) with  $CuCl_2$  or  $ZnCl_2$  (10  $\mu$ M) for 5 min were measured. The interaction of compounds **4** and **5** with  $ZnCl_2$  was observed by  $^1H$  NMR (Fig. S6C,D). Compound **4** or **5** (2 mM) was dissolved in  $CD_3OD$  and treated with 1 eq of  $ZnCl_2$ . The resulting solution was incubated for 30 min prior to NMR measurement. Sequentially, 0.2 eq of  $ZnCl_2$  was added to this solution until no further change in the NMR spectrum or precipitation was observed.

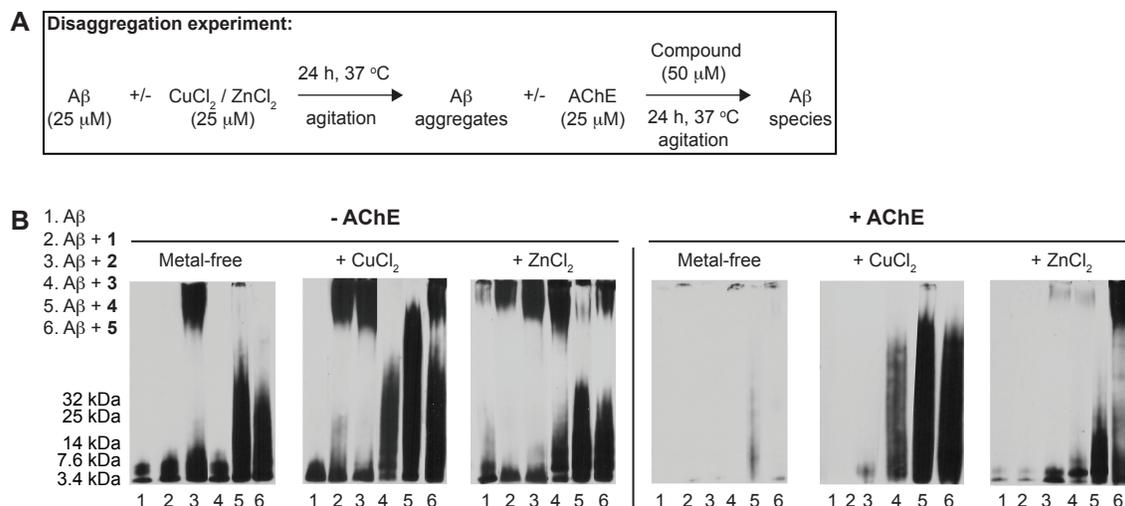


**Fig. S6.** Metal binding studies of **A.** cpd 1, **B.** cpd 2, **C.** cpd 4, and **D.** cpd 5 with  $\text{CuCl}_2$  (left) or  $\text{ZnCl}_2$  (middle) by UV-Vis. Conditions: [compound] = 40  $\mu\text{M}$ ; [ $\text{CuCl}_2$  or  $\text{ZnCl}_2$ ] = 20-800  $\mu\text{M}$ ; 30 min incubation (4 h for  $\text{Zn}^{2+}$  binding for cpd 4); rt. **C** and **D.** right panel =  $\text{Zn}^{2+}$  binding of 4 or 5 by  $^1\text{H}$  NMR. NMR spectra of 4 or 5 (black, 2.0 mM) with  $\text{ZnCl}_2$  (red, 3.2 mM) in  $\text{CD}_3\text{OD}$  at rt.



**Fig. S7.** Metal binding studies of **5** with **A.**  $\text{CuCl}_2$  or **B.**  $\text{ZnCl}_2$  in the absence and presence of  $\text{A}\beta$  and/or AChE at pH 6.6 ( $\text{Cu}^{2+}$ ) and 7.4 ( $\text{Zn}^{2+}$ ), monitored by UV-Vis. Spectra of  $\text{A}\beta$ , **5**, [ $\text{A}\beta$  + **5**], [ $\text{A}\beta$  +  $\text{MCl}_2$ ], [ $\text{MCl}_2$  + **5**], and [ $\text{A}\beta$  +  $\text{MCl}_2$  + **5**  $\pm$  AChE] are depicted in black, light blue, yellow, red, green, and blue, respectively. Conditions: [ $\text{A}\beta$ ] = 10  $\mu\text{M}$ ; [ $\text{CuCl}_2$  or  $\text{ZnCl}_2$ ] = 10  $\mu\text{M}$ ; [AChE] = 10  $\mu\text{M}$ ; [**5**] = 10  $\mu\text{M}$  (5% v/v DMSO); 20 mM HEPES, pH 6.6 ( $\text{Cu}^{2+}$ ) and 7.4 ( $\text{Zn}^{2+}$ ), 150 mM NaCl; rt.

**3.4. Amyloid- $\beta$  ( $\text{A}\beta$ ) peptide experiments.** To aliquot the  $\text{A}\beta_{1-40}$  (1 mg), it was completely dissolved with the  $\text{NH}_4\text{OH}$  provided by the supplier, split into 5 aliquots, lyophilized, and stored at  $-80^\circ\text{C}$ . For assays,  $\text{A}\beta_{1-40}$  solutions were prepared by addition of  $\text{NH}_4\text{OH}$  (10  $\mu\text{L}$ , 1% v/v, aq) to the above aliquots and diluted with dd $\text{H}_2\text{O}$  to obtain *ca.* 200  $\mu\text{M}$  as determined by UV-Vis (280 nm, rt). For both inhibition and disaggregation experiments (Fig. 2A in the main text for inhibition and Fig. S8A for disaggregation), the buffer solution (20  $\mu\text{M}$  HEPES, pH 6.6 (for  $\text{Cu}^{2+}$ ) or pH 7.4 (for metal-free and  $\text{Zn}^{2+}$ ), 150  $\mu\text{M}$  NaCl) was used. For the inhibition experiment,  $\text{A}\beta$  (25  $\mu\text{M}$ ) was first treated with either  $\text{CuCl}_2$  or  $\text{ZnCl}_2$  (25  $\mu\text{M}$ ) for 2 min at rt followed by addition and incubation of AChE (25  $\mu\text{M}$ ) for 5 min (only for AChE-indicated samples). The resulting samples were then incubated with compounds **1-5** (50  $\mu\text{M}$ , 1% v/v final DMSO concentration) at 37  $^\circ\text{C}$  for 24 h with constant agitation. For the disaggregation experiment,  $\text{A}\beta$  (25  $\mu\text{M}$ ) was first incubated with  $\text{CuCl}_2$  or  $\text{ZnCl}_2$  (25  $\mu\text{M}$ ) at 37  $^\circ\text{C}$  for 24 h with continuous agitation. The samples were then treated sequentially with AChE (25  $\mu\text{M}$ , only for AChE-indicated samples) for 5 min followed by addition of compounds **1-5** (50  $\mu\text{M}$ ; 1% v/v final DMSO concentration). These resulting solutions were incubated for an additional 24 h at 37  $^\circ\text{C}$  with constant agitation.



**Fig. S8.** Disaggregation studies using **1**, **2**, **3**, **4**, and **5** with and without AChE. **A.** Scheme of the disaggregation experiment. **B.** Visualization of various-sized A $\beta_{1-40}$  species in the absence (left) and presence (right) of AChE by gel electrophoresis with Western blot (anti-A $\beta$  antibody, 6E10). Conditions: [A $\beta$ ] = 25  $\mu$ M; [CuCl<sub>2</sub> or ZnCl<sub>2</sub>] = 25  $\mu$ M; [AChE] = 25  $\mu$ M [compound] = 50  $\mu$ M; pH 6.6 (for Cu<sup>2+</sup> samples) or 7.4 (for metal-free and Zn<sup>2+</sup> samples); 24 h incubation; 37 °C; constant agitation.

**3.5. Gel electrophoresis with Western blotting.** The A $\beta$  peptide experiments (described in section 3.4) were analyzed by gel electrophoresis with Western blotting using anti-A $\beta$  antibody (6E10) (Fig. 2B and Fig. S8B).<sup>2, 8</sup> Various A $\beta$  species generated by both inhibition and disaggregation experiments were separated by a 10-20% Tris-tricine gel (Invitrogen). The gel was transferred to a nitrocellulose membrane and blocked for 2 h with BSA (Sigma, 3% w/v) dissolved in Tris-buffered saline (TBS, Fisher) containing 0.1% Tween-20 (TBS-T, Sigma). The membrane was treated with 6E10 (1:2,000; 2% BSA in TBS-T, Covance, Princeton, NJ) overnight at 4 °C with gentle agitation and probed with a horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:5,000; Cayman Chemical, Ann Arbor, MI) in 2% BSA in TBS-T solution for 1 h at rt. The protein bands were visualized by using the ThermoScientific Supersignal West Pico Chemiluminescent Substrate (Fisher).

**3.6. Transmission electron microscopy (TEM).** TEM samples were prepared following a previously reported method.<sup>2, 8</sup> Glow-discharged grids (Formar/Carbon 300-mesh, Electron Microscopy Sciences, Hatfield, PA) were treated with the samples (5  $\mu$ L) from either the inhibition or disaggregation experiment for 2 min at rt. Excess sample was removed with filter paper. The grids were washed 5 times with ddH<sub>2</sub>O, stained with uranyl acetate (1% w/v, ddH<sub>2</sub>O, 5  $\mu$ L) for 1 min, and dried for 15 min at rt. A Philips CM-100 transmission electron microscope (80 kV, 25,000x magnification) was used for obtaining TEM images of the samples (Fig. 2C).

**3.7. Effect of metals and A $\beta$  peptide on AChE and BChE inhibition by compound 5.** The AChE inhibitor **5** (10  $\mu$ M to 5 pM) was dissolved in phosphate buffer (100  $\mu$ L, 100 mM final concentration, pH 8.0 adjusted at rt) and one of three conditions was followed. Conditions (a), (d), and (g): AChE (25  $\mu$ L, 0.08 U/mL final concentration) was added to the inhibitor solutions (100  $\mu$ L) and incubated for 10 min prior to addition of CuCl<sub>2</sub> or ZnCl<sub>2</sub> or A $\beta$  peptide (25  $\mu$ L, 10  $\mu$ M final concentration). After 10 min, the reactions were initialized with a DTNB:ATC (0.25

mM:0.5 mM final concentration, respectively) mixture (50  $\mu$ L) in phosphate buffer (100 mM final concentration, pH 8.0 adjusted at rt). Conditions (b), (e), and (h):  $\text{CuCl}_2$  or  $\text{ZnCl}_2$  or  $\text{A}\beta$  peptide (25  $\mu$ L, 10  $\mu$ M final concentration) were added to the inhibitor solutions (100  $\mu$ L) and incubated for 10 min prior to addition AChE (0.08 U/mL final concentration). After 10 min, the reactions were initialized with a DTNB:ATC (0.25 mM:0.5 mM final concentration, respectively) mixture (50  $\mu$ L) in phosphate buffer (100 mM final concentration, pH 8.0 adjusted at rt). Conditions (c), (f), and (i): A mixture (50  $\mu$ L) of AChE (0.08 U/mL final concentration) and  $\text{CuCl}_2$  or  $\text{ZnCl}_2$  or  $\text{A}\beta$  peptide (10  $\mu$ M final concentration) (50  $\mu$ L total) was added to the inhibitor solutions (100  $\mu$ L). After 10 min, the reactions were initialized with a DTNB:ATC (0.25 mM:0.5 mM final concentration, respectively) mixture (50  $\mu$ L) in phosphate buffer (100 mM final concentration, pH 8.0 adjusted at rt).  $\text{IC}_{50}$  values were determined as described in section 3.1 and are reported in Table 1. Outside of using BTC in lieu of ATC, all BChE experiments were performed as AChE experiments.

**3.8. Molecular modeling.** Compound **5** was built using the Sybyl-X software and minimized to 0.01 kcal/mol by the Powell method, using Gasteiger-Hückel charges and the Tripos force fields. The coordinates of acetylcholinesterase (*TcAChE*) and amyloid-beta ( $\text{A}\beta_{1-40}$ ) were downloaded from the Protein Data Bank website (PDB codes: 1ACJ and 2LFM, respectively). The  $\text{H}_2\text{O}$  molecules and all other ligands were removed from the two proteins. Hydrogen atoms were added and the energy of both proteins was minimized separately using the Amber force fields with Amber charges. Then, the energy-optimized ligand (compound **5**) was docked into the tacrine-binding site in the energy minimized tacrine-free *TcAChE* using GOLD.<sup>3</sup> The parameters were set as the default values for GOLD. The maximum distance between hydrogen bond donors and acceptors for hydrogen bonding was set to 3.5 Å. After docking, the first-ranked conformation of compound **5** was merged into the corresponding tacrine-free *TcAChE* (to determine the proper binding area for  $\text{A}\beta_{1-40}$  binding).

The energy-optimized  $\text{A}\beta$  was then docked close to the surface of *TcAChE* using GOLD. A distance constraint was applied between the His6 residue of  $\text{A}\beta_{1-40}$  and the Gln74 of *TcAChE* (that was chosen due its vicinity to the metal-chelator portion of compound **5** in the compound **5**-*TcAChE* complex). Here again, the parameters were set as the default values for GOLD. The maximum distance between hydrogen bond donors and acceptors for hydrogen bonding was set to 3.5 Å. After docking, the first-ranked conformation of  $\text{A}\beta_{1-40}$  was merged into the corresponding tacrine-free *TcAChE*. The new  $\text{A}\beta_{1-40}$ -*TcAChE* complex was subsequently subjected to energy minimization using the Amber force fields with Amber charges. During the energy minimization, the structure of  $\text{A}\beta_{1-40}$  and residues within a 7-Å radius were allowed to move. The remaining residues were kept frozen in order to save calculation time. The energy minimization was performed using the Powell method with a 0.05 kcal/mol energy gradient convergence criterion and a distance dependent dielectric function.

Compound **5** was then docked into the tacrine-binding site in the obtained energy optimized  $\text{A}\beta_{1-40}$ -*TcAChE* complex as described above. The first-ranked conformation of compound **5** was merged into the tacrine-free  $\text{A}\beta_{1-40}$ -*TcAChE* complex (Fig. 3).

**3.9. Parallel artificial membrane permeability assay adapted for blood-brain barrier (PAMPA-BBB).** Previously reported protocols with modification using the PAMPA Explorer kit (Pion, Inc.) were applied to our PAMPA-BBB experiment.<sup>2, 9-11</sup> Each stock solution of the

compounds was diluted to a final concentration of 10  $\mu\text{M}$  (1% v/v final DMSO concentration) with pH 7.4 Prisma HT buffer (Pion). The resulting solution (200  $\mu\text{L}$ ) was added to each of the wells of the donor plate (number of replicates per sample = 12). The BBB-1 lipid (Pion formulation, 5  $\mu\text{L}$ ) was used to coat the polyvinylidene fluoride (PDVF, 0.45  $\mu\text{M}$ ) filter membrane on the acceptor plate. The acceptor plate was placed on the top of the donor plate generating a “sandwich” and each well of the acceptor plate was filled with the brain sink buffer (200  $\mu\text{L}$ , Pion). The sandwich was incubated at rt for 4 h without stirring. A microplate reader was used to obtain the optical spectra (250-500 nm) of the solutions in the reference, acceptor, and donor plates. The  $-\log P_e$  for each compound was calculated using the PAMPA Explorer software c. 3.5 (Pion). CNS+/- assignment was determined in comparison to compounds identified previously.<sup>9-11</sup> Compounds categorized as CNS+ have the ability to permeate through the BBB and target the CNS. Compounds assigned as CNS- have poor permeability through the BBB and therefore, their bioavailability into the CNS is considered to be minimal. All values (MW,  $c\log P$ , HBA, HBD, PSA,  $\log\text{BB}$ , and  $-\log P_e$ ) for compounds **1-5** are reported in Table 2.

#### 4. Abbreviations.

AChE, acetylcholinesterase; ATC, acetylthiocholine; BSA, bovine serum albumin; BTC, butyrylthiocholine; DETAPAC, diethylenetriaminepentaacetic acid; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; ROS, reactive oxygen species; TLC, thin layer chromatography.

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