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Electronic Supplementary Material for

"Bis-8-hydroxyquinoline Ligands as Potential Anti-Alzheimer Agents" by
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Detailed Experimental

General remarks. UV-visible spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer. $CuCl_2$, $ZnCl_2$ or $FeCl_3$ (puriss p.a. grade) and L(+)-ascorbic acid sodium salt (Biochemica) were purchased from Fluka. Clioquinol was from Sigma. Ligand **1** has been prepared according to reference 1.

MilliQ water (Millipore) and buffers were treated before use with Chelex-100 resin (Biorad) (5.0 mg/mL) and filtered through a 0.2 µm filter (Whatman) to remove any metal ion or particulate matter. Stock solutions of ligand were prepared in DMSO.

$A\beta_{1-42}$ amyloid peptide was synthesized, purified (up to 95 % purity) and characterized by HPLC analysis and MALDI-TOF mass spectrometry. Peptide working solutions were prepared by dissolving 1.0 mg of lyophilized peptide in 500 µL water and 500 µL aqueous NaOH (pH 12) under stirring at 1400 rpm on a Thermomixer Comfort (Eppendorf). The peptide preparation was then centrifuged for 10 min at 5500 g and the supernatant was used. The Aβ concentration in this supernatant was immediately determined by MicroBCA Assay (Pierce). The quantification was done from a Bovine Serum Albumin standard curve. The Aβ solution was then aliquoted, fast frozen in liquid nitrogen and stored at - 20 °C.

All experiments involving the peptide were performed in duplicate when the discrepancy between experimental values was less than 5 %. In the other cases and for major results triplicates were done and standard deviations were calculated.

Stirrings were performed with a Thermomixer.

Log $D_{7.4}$ determination (adapted from reference 2). Ligand (2.0 mg) was dissolved in 2.00 mL of 1-octanol then 2.00 mL of 20 mM Tris·HCl buffer (pH 7.4), 150 mM NaCl was added. After a 3-min vortex at room temperature followed by centrifugation for 5 min at 5500 g, the

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concentration of ligand in each layer was determined by UV-vis spectrophotometry. Samples from 1-octanol layer were repartitioned until consistent partition coefficient values were obtained. The measurement was carried out in triplicate.

Inhibition of Aβ precipitation (adapted from reference 3; final concentrations were given). $A\beta_{1-42}$ (500 µL, 5.0 µM) in 20 mM Tris·HCl/150 mM NaCl buffer (pH 7.4) was incubated without metal ion or with $CuCl_2$, $ZnCl_2$ or $FeCl_3$ (12.5 or 20.0 µM) for 1 h at 37 °C under stirring at 1400 rpm. Ligand (50 µL; 200, 25 or 12.5 µM solution) or 50 µL of DMSO were added and the samples were incubated for 1 h at 37 °C under stirring. Samples were then centrifuged for 20 min at 5500 g and 500 µL of supernatant was removed. The test tube containing residual supernatant and pellet received 450 µL of the experimental buffer/DMSO (91/9, v/v). Then both supernatant and pellet were analyzed for the determination of protein concentration by MicroBCA Assay. This double quantification allowed to monitor the initial quantity of peptide and to confirm the validity of the measurements.

Hydrogen peroxide assay (adapted from reference 4). $A\beta_{1-42}$ (0.2 or 0.5 µM) and $CuCl_2$ (0.4 µM) were incubated for 1 h at 37 °C under stirring at 1400 rpm in 375 µL of sodium phosphate buffer (50 mM, pH 7.4). Then 2 µL of water or of stock solution of chelator (yielding a final concentration of 0.4 or 0.8 µM for 375 µL of initial volume) were added. After another 1 h of incubation, 2 µL of sodium ascorbate (10 µM final concentration for 375 µL) were added and the reaction mixture was incubated 5 min under stirring. Then one volume of AmplexRed/HRP mixture (Molecular Probes) in the same buffer was added. After one additional hour of incubation at 37 °C with moderate stirring (500 rpm), the amount of H_2O_2 produced was quantified at 563 nm from an analytical grade H_2O_2 standard curve.

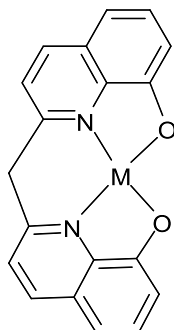

Figure S1: Metal complexes expected for the bis-8-hydroxyquinoline chelator **1** in the presence of Cu(II) or Zn(II). The structure for clioquinol in a L_2M form can be seen in reference 5.

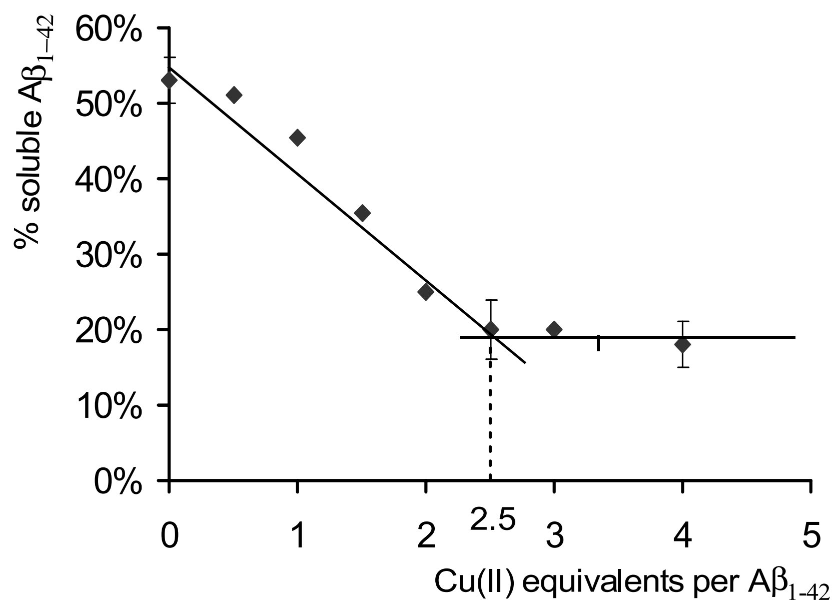

Figure S2: Variation of the percentage of soluble $A\beta_{1-42}$ peptide according to the $A\beta$/Cu(II) ratio.

$A\beta_{1-42}$ peptide (5 µM) was incubated for 2 hours at 37°C in 20 mM Tris·HCl buffer (pH 7.4), 150 mM NaCl in the presence of different ratios of Cu(II) then centrifuged. Percentage of peptide in the supernatant (soluble $A\beta$) and the pellet were quantified with MicroBCA Assay.

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