Electronic Supplementary Material (ESI) for Inorganic Chemistry Frontiers.

Supporting information for

Enantioselective modulation of amyloid burden and memory deficits by chiral polyoxometalates for Alzheimer's disease treatment

Shuyang Hu[a], Xinlu Ning[a], Jie Lv[a,b], Yuxin Wei[a], Huiran Zhang*[a] and Meng Li*[a]

[a]College of Pharmacy, Key Laboratory of Innovative Drug Development and Evaluation, Hebei Medical University, Shijiazhuang, 050017, China.
[b]Postdoctoral Mobile Station of Basic Medicine, Hebei Medical University, Shijiazhuang 050017, China.

Table of content

1. Supplementary methods

1.1 Measurements

The UV-Vis absorption spectra were monitored by a Shimadzu UV-2700 spectrophotometer (Japan). Fluorescence spectra were recorded on a fluorescence spectrophotometer (F-380, Gangdong Technology, China). Circular dichroism (CD) spectra were recorded by a J-1500 CD spectrophotometer (JASCO). Fourier translation infrared (FT-IR) spectra and attenuated total reflectance FT-IR spectra (ATR-FTIR) were obtained on a SHIMADZU FT-IR-8400S. Transmission electron microscope (TEM) images were recorded using a Hitachi 7650B microscope operating at 200 kV. NMR spectra were monitored on a Bruker 600-MHz AVANCE NMR spectrometer equipped with a triple channel cryoprobe.

1.2 Preparation of $K_6[\alpha\text{-}P_2W_{18}O_{62}]\cdot 14H_2O$

$K_6[\alpha\text{-}P_2W_{18}O_{62}]\cdot 14H_2O$ was synthesized according to the previously published method.[1] 210 mL of 85% phosphoric acid was added into 500 mL of deionized water containing 250 g of $Na_2WO_4\cdot 2H_2O$ (0.76 mol), and the solution was heated to reflux for 4 h. 100 g NH_4Cl was then added when the solution was cooled down to room temperature. After stirring for 10 min, the generated pale yellow salt was isolated by suction filtration and redissolved in 250 mL of deionized water at 45 °C. After cooling, 40 g of KCl was added. The resulting potassium salt was redissolved in 250 mL of deionized water at 80 °C. The solution was cooled to about 15 °C and filtrated to remove the precipitated white needle crystals. The filtrate was heated under reflux for 6 h to obtain a pure α-isomer solution of $K_6[\alpha\text{-}P_2W_{18}O_{62}]\cdot 14H_2O$. 25 g of KCl was added to the solution and the precipitate was air-dried.

1.3 Preparation of $Na_{12}[\alpha\text{-}P_2W_{15}O_{56}]\cdot 24H_2O$

$K_6[\alpha\text{-}P_2W_{18}O_{62}]\cdot 14H_2O$ was prepared according to the previous literature.[1] 38.5 g of $K_6[\alpha\text{-}P_2W_{18}O_{62}]\cdot 14H_2O$ was dissolved in 125 mL of deionized water, and then 35 g of $NaClO_4\cdot H_2O$ was added into the above solution. The reaction solution was stirred for 20 min and then cooled in an ice bath for 3 h. After that, the sodium perchlorate was removed by filtration. 100 mL of 10.6% Na_2CO_3 aqueous solution was added into the filtrate and the product $Na_{12}[\alpha\text{-}P_2W_{15}O_{56}]\cdot 24H_2O$ was collected by suction filtration. After washing with 16% NaCl solution (25 mL) and ethanol, the product was finally air dried.

1.4 Preparation of L/D-POM

Briefly, 0.105 g of L-tartaric acid (L-TA) was added into 15 mL $ZrO(NO_3)_2\cdot 6H_2O$ aqueous solution (0.7 mM) with constantly stirring at room temperature, and the white slurry was obtained. Subsequently, the reaction was continued for 30 min after addition of $Na_{12}[\alpha\text{-}P_2W_{15}O_{56}]\cdot 24H_2O$ (1.03 g). Then, 0.4 g of dimethylamine hydrochloride was added into the above reaction system, and the reaction was conducted at 70 °C for 15 min. Finally, the solution was slowly evaporated at room temperature to obtain needle-like crystals $[(CH_3)_2NH_2]_{15}\{[\alpha\text{-}P_2W_{15}O_{55}(H_2O)]Zr_3(\mu_3\text{-}O)(H_2O)(L\text{-}tartH)[\alpha\text{-}P_2W_{16}O_{59}]\}$ (L-POM). The procedure of preparing D-POM was same as that of L-POM, in which L-TA was replaced by D-TA.

1.5 Atomic force microscopy (AFM) imaging

Aβ40 (100 μM) was treated with or without chiral POMs (20 μM) in aggregation buffer and incubated at 37 °C for 7 days. Subsequently, the samples were diluted with deionized water to a final concentration of 1 μM and then dropped onto a freshly cleaved mica surface with an additional 5 min incubation. After that, the substrate was washed twice with water. The AFM was operated in tapping mode.

1.6 Fluorescence titrations

The binding affinity of L-POM or D-POM to Aβ was determined by a tyrosine fluorescence quenching experiment. Typically, L/D-POM was gradually added into the Aβ40 solution (3 μM), and the inherent tyrosine fluorescence of Aβ40 after treating with different concentrations of chiral POMs was monitored (Ex: 278 nm, Em: 306 nm). And the binding constants of L/D-POM with Aβ40 were calculated according to the 1:1 binding stoichiometric equation. To get the proper fluorescence intensity, the internal filtration effect was rectified by the corresponding absorbance value.[2, 3] The fluorescence values were calculated in accordance with the following equation:

$F_{corr} = F_{obs} \log^{-1}[(A_{ex} + A_{em})/2]$

where F_{corr} and F_{obs} are the corrected and observed fluorescence intensity, respectively. A_{ex} is the absorbance value at the excitation wavelength and A_{em} is the absorbance value at the emission wavelength.

1.7 Cell culture

PC12 cells (rat pheochromocytoma, American Type Culture Collection) were maintained in Iscove modified Dulbecco medium (IMDM) (Gibco, BRL) supplemented with 5% fetal bovine serum and 10% heat-inactivated horse serum at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

1.8 Hemolysis experiment

The fresh blood was collected from SD rats with canthus blood collection method and then centrifuged at 3500 rpm for 10 min to collect red blood cells (RBCs). The RBCs was washed with PBS (10 mM, pH 7.4) for several times. Subsequently, the RBCs was diluted with PBS (10 mM, pH 7.4) at a ratio of 1:20. The L/D-POMs with different concentrations were added to dilute RBCs suspension. PBS (10 mM, pH 7.4) and deionized water diluted RBCs suspension were used as negative control group and positive control group, respectively. All samples were incubated at room temperature for 2 h. Finally, the RBCs suspension was centrifuged, and the absorbance of the supernatant was measured at 570 nm.

The hemolysis rate was calculated according to the following formula:

Hemolysis rate % = $(Abs_{(Sample)}\text{-}Abs_{(Negative\ control)})/(Abs_{(Positive\ sample)}\text{-}Abs_{(Negative\ control)}) \times 100$.

1.9 Morris water maze (MWM) test

Spatial memory was measured by MWM test.[4] The APP/PS1 mice were measured in a water maze with a diameter of 110 cm. During the first five days of training, the platform was installed 1 cm below the water surface in the targeted quadrant. The tracking system was used to record movement trajectories and escape latency of the mice. On the sixth day, spatial memory of mice was explored through a platform-free probe trial. The mice were released into the water at the

position opposite to the target quadrant and allowed to swim freely for 60 s. The escape latency and the time spent in the target quadrant were recorded.

1.10 Immunohistochemistry

The mice were euthanized after MWM test. And the whole brain was collected for staining and imaging. Aβ staining was used to study the deposition of Aβ in the hippocampus. The inflammatory factors were detected by TNF-α staining, and Nissl staining sections were used to detect Nissl bodies in the cytoplasm of mouse hippocampal neurons.

2. Supplementary figures

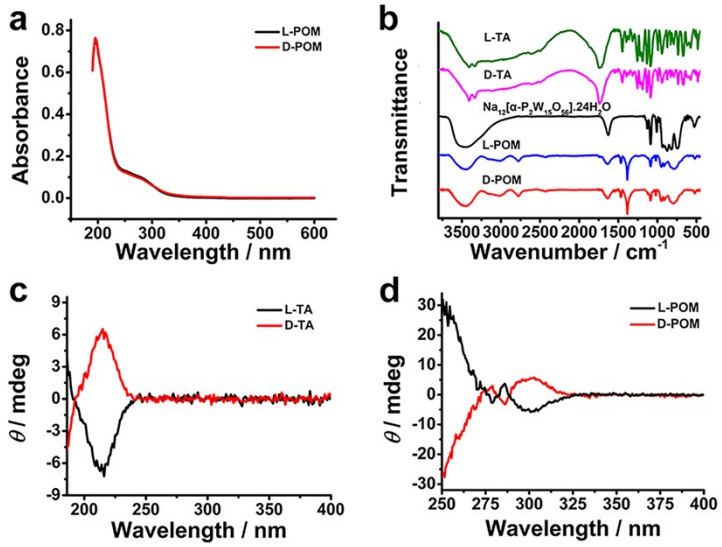

Fig. S1 Characterization of L/D-POM. (a) UV–Vis spectra of L/D-POM. (b) FT-IR spectra of L/D-TA, $Na_{12}[\alpha\text{-}P_2W_{15}O_{56}]\cdot 24H_2O$ and L/D-POM. CD spectra of (c) L/D-TA and (d) L/D-POM.

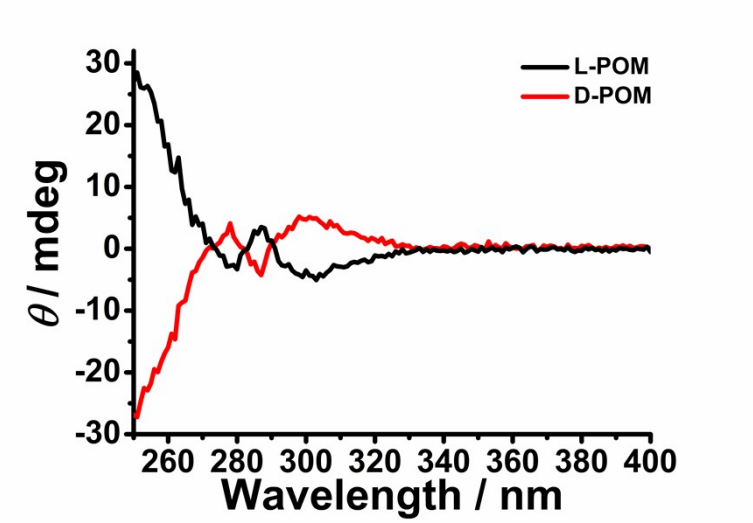

Fig. S2 CD spectra of L/D-POM after 3 months of storage in aqueous solution.

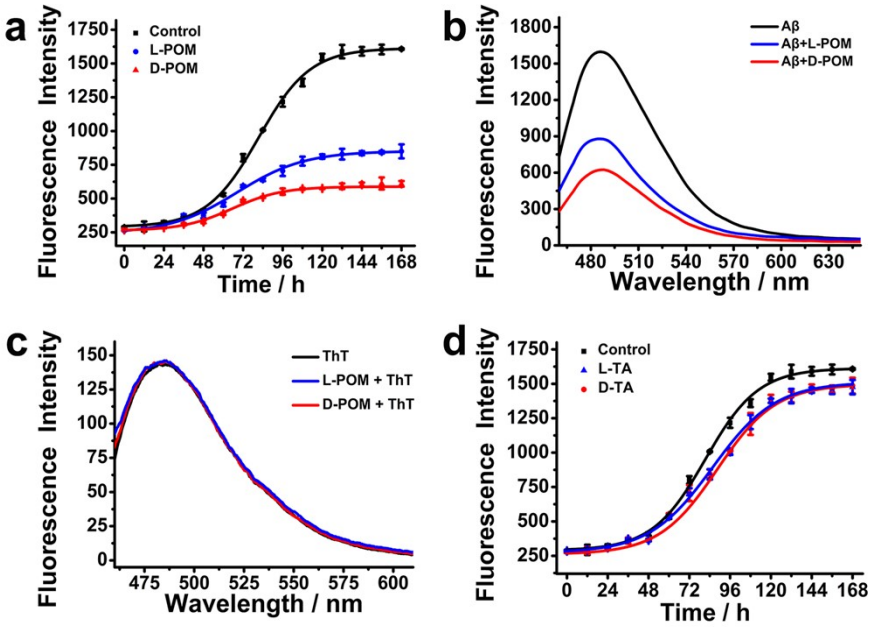

Fig. S3 (a) Fibrillation kinetics of Aβ40 were monitored by the development of ThT binding in the absence or presence of L/D-POM. (b) ThT analysis of amyloid samples after incubation for 168 h. (c) The influence of L/D-POM on ThT fluorescence. (d) Fibrillation kinetics of Aβ40 were monitored by the development of ThT binding in the absence or presence of L/D-TA.

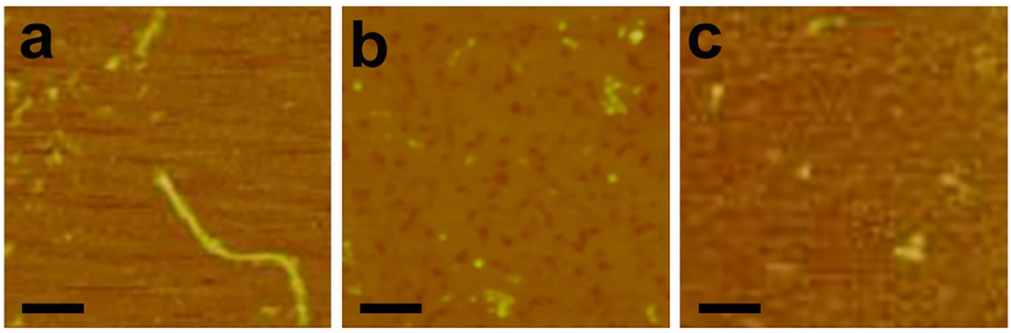

Fig. S4 The morphology of Aβ40 aggregates was analyzed by AFM images: (a) 100 μM Aβ40, (b) 100 μM Aβ40 in the presence of 20 μM L-POM, (c) 100 μM Aβ40 in the presence of 20 μM D-POM. Scale bar=200 nm.

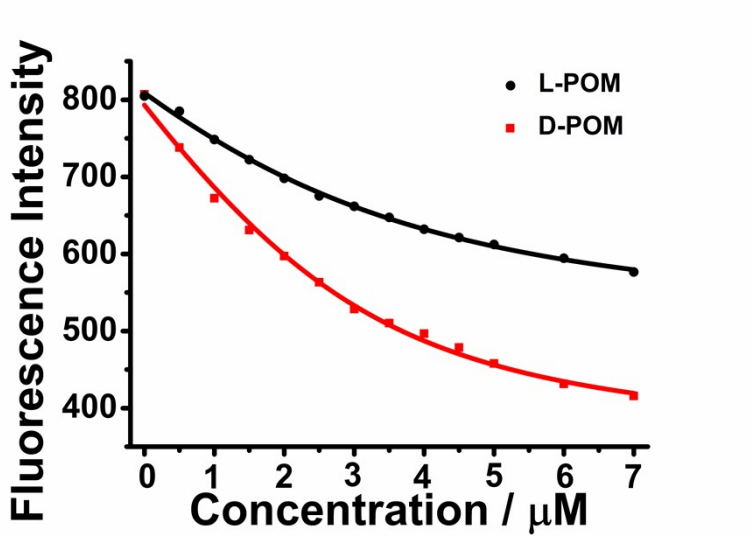

Fig. S5 Fluorescence titration of Aβ (3 μM) with various concentrations of L/D-POM in Tris-NaCl buffer. The excitation wavelength was 278 nm and the emission intensity at 305 nm was used for analysis.

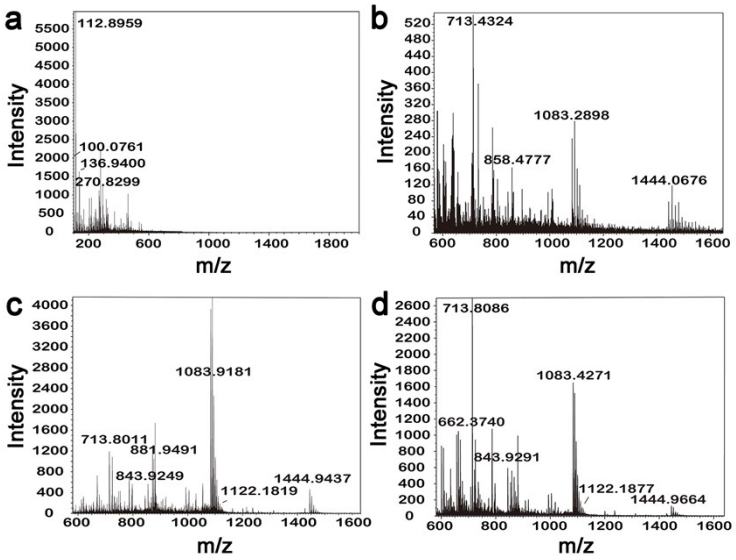

Fig. S6 ESI-MS spectra of: (a) L-POM, (b) Aβ40 peptide, (c) Aβ40 incubated in the presence of D-POM and (d) Aβ40 incubated in the presence of L-POM.

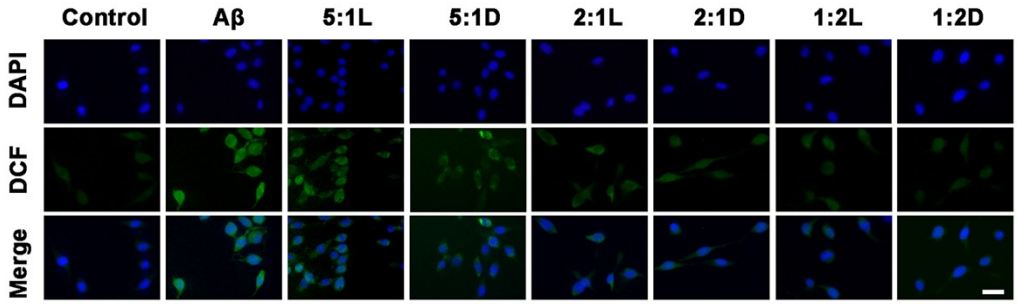

Fig. S7 Fluorescence microscope images of PC12 cells stained by DAPI and DCFH-DA. Scale bar=25 μm.

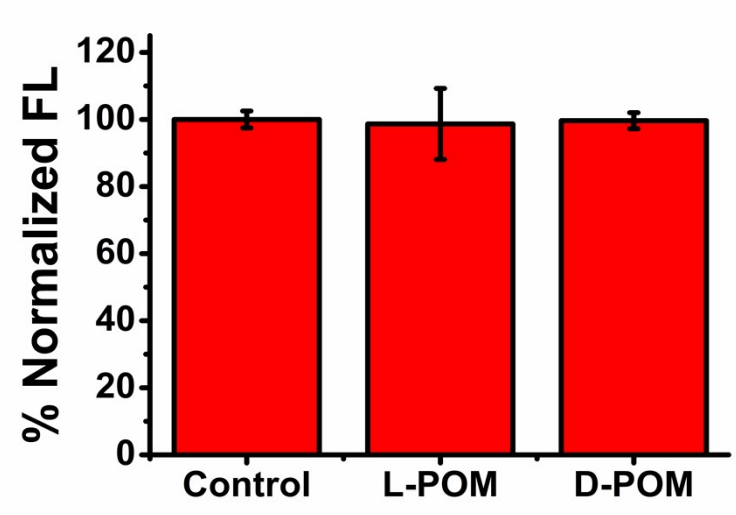

Fig. S8 Effect of L/D-POM (20 μM) on intracellular ROS formation in normal PC12 cells.

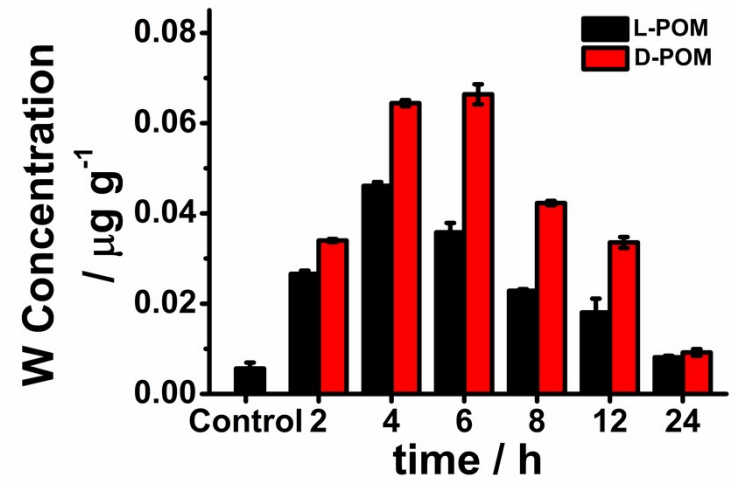

Fig. S9 The biodistribution of L/D-POM in the brain of healthy mice at different time based on ICP-MS analysis.

2. Supplementary tables

Table S1 IC_{50} values of L/D-POM for inhibition of fibril formation.

	L-POM	D-POM
IC_{50} values (μM)	17.38	2.63

Table S2 Binding constants of L/D-POM with Aβ40.

	L-POM	D-POM
Binding constants (M^{-1})	5.40×10^5	1.07×10^6

Table S3 The quenching constants of the chiral POM-Aβ system.

Chiral POM	Aβ	Quenching constants (M^{-1})
L-POM	Aβ1-40	1.80×10^6
D-POM	Aβ1-40	3.54×10^6
L-POM	Aβ12-28	2.05×10^6
D-POM	Aβ12-28	3.70×10^6

Table S4 Binding energies predicted for chiral L/D-POM with Aβ monomers.

Chiral POM	Binding energy (kcal mol^{-1})
L-POM	-476.00
D-POM	-556.98

4. References

1 R. Contant, Potassium octadecatungstodiphosphates(V) and related lacunary compounds, *Inorg. Synth.*, 1990, **27**, 106.

2 D. E. Schlamadinger, D. I. Kats and J. E. Kim, Quenching of tryptophan fluorescence in unfolded cytochrome c: A biophysics experiment for physical chemistry students, *J. Chem. Educ.*, 2010, **87**, 961-964.

3 M. M. Puchalski, M. J. Morra and R. von Wandruszka, Assessment of inner filter effect corrections in fluorimetry, *Fresenius J. Anal. Chem.*, 1991, **340**, 341-344.

4 M. Amini and Z. Abdolmaleki, The effect of cannabidiol coated by nano-chitosan on learning and memory, hippocampal CB1 and CB2 levels, and amyloid plaques in an Alzheimer's disease rat model, *Neuropsychobiology*, 2022, **81**, 171-183.