Electronic Supplementary Material (ESI) for RSC Advances.

Identification of 2-subsituted Benzothiazole Derivatives as Triple-functional agents with potential for AD Therapy

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Contents

1. Physicochemical properties prediction

To evaluate the drug-like properties of target compounds, their physicochemical properties were calculated using Chemoffice software, including molecular weight (MW), calculated logarithm of octanol–water partition coefficient (clogP), number of hydrogen-bond acceptors (HBA), number of hydrogenbond donors (HBD), and topological polar surface area (PSA). Log BB was calcultaed using Clark's equation[1]. log BB = -0.0148TPSA + 0.152clogP+ 0.139

2. Molecular docking Study

Molecular docking Study

The docking studies of compounds **HL_{12}** and **9a** with $A\beta_{1-42}$ monomer model (PDB ID: 1IYT) [2] were performed by using the FlexiDock program in SYBYL-X 1.3 software package (Tripos, Inc.). The 3D-sturcture of ligands were generated and optimized under a Tripos forcefield using the Powell method. The receptor was prepared checking the atom types, adding hydrogens, and Kollman charges were assigned. The binding pocket was defined to cover all the amino acid residues within 30 Å radius sphere. Docking experiments were carried out taking into account the flexibility of both ligand and receptor. The structural optimization was performed for 100,000 generations using a TRIPOS forcefield and genetic algorithm. The 10 best scoring complexes were kept for further analyses and the conformation with the lowest energy was selected. Docked models of the ligand-receptor complex were visualized using PyMOL.

3. Amyloid-β Peptide Experiments

ThT assay

$A\beta_{1-42}$ was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) by brief sonication for 10min, and kept overnight at ambient temperature. The HFIP was removed by evaporation under vacuum and the residue was redissolved in DMSO and diluted with buffer solution to get a stock solution (~2 mM) [3,4].

For the inhibition of Aβ self-aggregation experiment, a mixture of $A\beta_{1-42}$ peptide (20 μL, 25 μM) with or without the tested compound (20μL, 20 μM) was incubated at 37°C for 24 h. For the disaggregation experiment, the peptide was incubated at 37°C for 24 h before the addition of tested compounds, then the mixture was incubated for another 24h. The sample was diluted to a

final volume of 100 μL with 5mM glycine−NaOH buffer (pH 8.5) containing thioflavin T (5 μM) in a 96-well plate. The fluorescence intensities were recorded five minutes later (λ_{ex} = 450 nm, λ_{em} = 485 nm). The percent inhibition of aggregation was calculated by the expression $(1 - IF_i/IF_c) \times 100$, in which IF_i and IF_c are the fluorescence intensities obtained for $A\beta_{1-42}$ in the presence and absence of inhibitors after subtracting the background, respectively.

TEM Assay

For the self-mediated Aβ aggregation, the peptide was incubated at 37°C for 24 h before the addition of tested compounds, the mixture was incubated for another 24h. the Aβ stock solution was diluted with 10 mM phosphate buffer (pH 7.4) to 50 μM before use. The solutions of samples were contained Aβ (25 μM), ± compounds (20 μM, 1% final DMSO concentration) and incubated for 24 h at 37 °C with constant agitation. For the Cu^{2+}-induced Aβ aggregation study, Aβ (25 μM) solutions, ± $CuCl_2$ (25 μM), ± compounds (50 μM) were incubated for 24 h at 37 °C with constant agitation. For the disaggregation of copper-induced $A\beta_{1-42}$ fibrils experiment, the peptide was incubated with copper at 37°C for 24 h before the addition of tested compounds, then the mixture was incubated for another 24h. The buffered solution (20 μM HEPES, pH 6.6, 150 μM NaCl) was used for this study. Aliquots (5 μL) of the samples were placed on a carbon-coated copper/rhodium grid for 2 min. Each grid was stained with uranyl acetate (1%, 5 μL) for 2 min. After draining off the excess staining solution, the specimen was transferred for imaging by transmission electron microscopy (JEOL JEM-1230, Japan).

4. Trolox-equivalent antioxidant capacity (TEAC) assay

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical cation scavenging activity assay was used to determine the antioxidant ability according to the previously reported method.[5,6,7]

5. Metal-chelating study

The metal chelating activity of targeted compounds were studied using UV-vis spectroscopy. A solution of the tested compounds (50 μM, 10mM stock solutions in methanol) in double-distilled water was incubated with or without 25 μM metal ions ($CuCl_2$, $ZnCl_2$, $FeSO_4$ or $FeCl_3$) at 25 ºC.

The spectra of each compound alone or in the presence of metal ions were recorded after 5 min incubation with wavelength ranging from 200 to 700 nm.

The stoichiometry of the **9c**-Cu^{2+} and **9i**- Cu^{2+}complex was determined by Job's method[3,8]. Separate solutions of compounds and $CuCl_2$ were used to prepare solutions in which the sum of the concentrations of both species (50 μM) was constant in all samples, but the proportions of either component varied between 0% and 100%. The absorbance differences at 341 nm for **9c** and 370 nm for **9i** were plotted versus the mole fraction. The minimum revealed the stoichiometry of the compound-Cu^{2+} complex.

6. Cell viability studies

The cytotoxic activity of compounds **9c** and **9i** in U251 cells was measured using the SRB method. U251 cells were grown in culture dishes (10cm) and maintained under 5% CO_2 atmosphere at 37 °C. DMEM and 10% FBS was used as growth medium and changed on alternate days. The cells were harvested by a 0.25% w/v trypsin–EDTA solution (Sigma, USA) once 90% confluency was achieved and subcultured in 96-well plates at a density of 8000 cells in100 μL medium per well. Once the cells reached confluency, cells were treated with different concentrations of compound. Following 24h drug exposure, culture medium was removed, the 60μL of cold trichloroacetic acid (TCA) (10%) were added in 96-well plates. The cell culture plates were incubated for 60 min at 4°C and then washed three times with distilled water. Once plates were air dried, 60μL of 0.4% SRB stain containing 1% acetic acid was added to each well. The plates were then incubated for 20 min at room temperature and washed with 1% acetic acid. Once the stained plates were air dried, 60μL of 10 mM Tris Base was added to each well and plates were gently agitated for 5 min. The optical density was then read using a microplate reader at 490 nm. The background signal from media-alone controls was subtracted and data were normalized to untreated cells.

7. Supplementary Tables and Figures

Table S1. The physicochemical properties of compounds **9a-o**

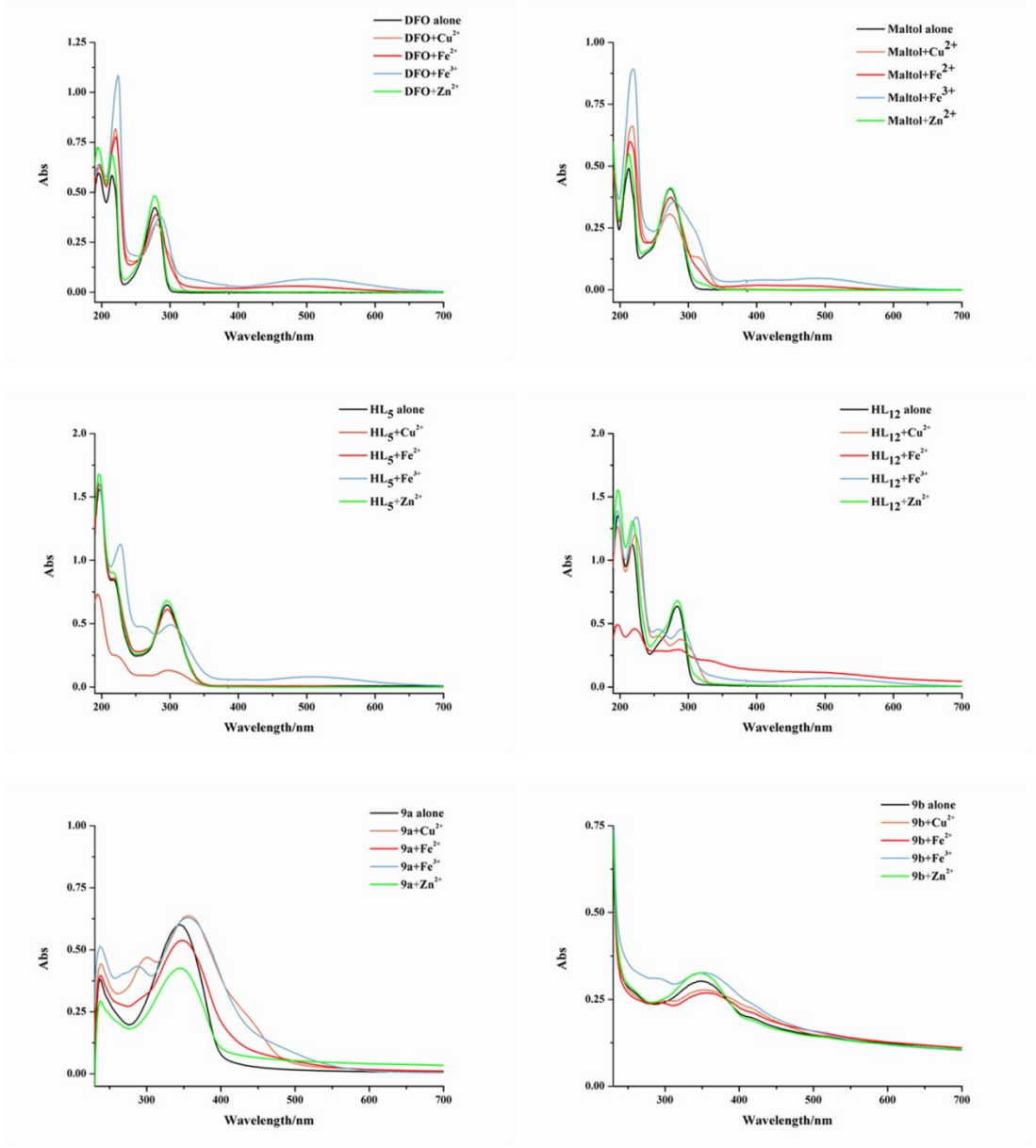
Compound	MW	clogP	PSA	HBA	HBD	logBB
9a	271.30	2.28	58.89	4	1	-0.39
9b	285.32	2.73	58.89	4	1	-0.32
9c	255.23	1.62	68.12	5	1	-0.62
9d	269.27	2.07	68.12	5	1	-0.56
9e	268.27	1.78	62.13	5	1	-0.51
9f	282.30	2.23	62.13	5	1	-0.44
9g	301.32	2.58	68.12	5	1	-0.48
9h	350.19	3.17	58.89	4	1	-0.25
9i	287.30	2.39	79.12	5	2	-0.67
9j	285.25	1.91	77.35	6	1	-0.72
9k	271.05	1.66	88.35	6	2	-0.91
9l	298.30	2.03	71.36	6	1	-0.61
9m	284.08	1.85	82.36	6	2	-0.80
9n	284.34	1.28	52.90	4	1	-0.45
9o	268.27	0.61	62.13	5	1	-0.69
Lipinski's rules	≤ 500	≤5	≤90	≤ 10	≤ 5	> -1.0

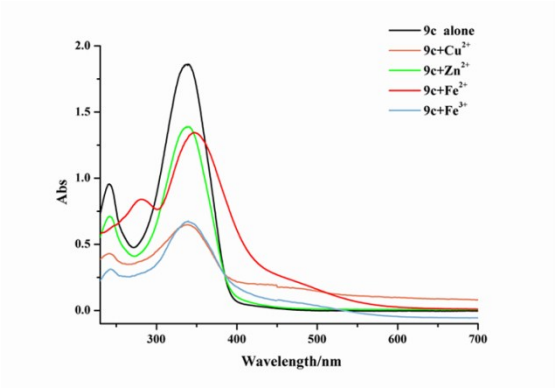
MW: molecular weight. clogP: calculated logarithm of the octanol-water partition coefficient.

PSA: polar surface area. HBA: hydrogen-bond acceptor atoms. HBD: hydrogen-bond donor atoms.

LogBB = −0.0148×PSA + 0.152×clogP + 0.139.

Figure S1 UV spectra of compound **9a-9o**,**DFO**(deferiprone),Maltol,**HL_5**,**HL_{12}** (50μM) alone and in the presence of $CuCl_2$ (25μM), $ZnCl_2$(25μM) or $FeSO_4$(25μM) ,$FeCl_3$(25μM) in double-distilled water.

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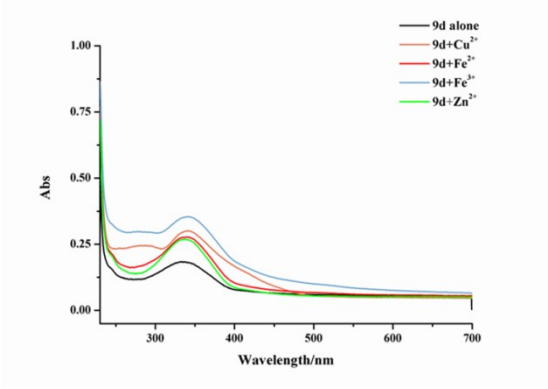
9c+Cu2+
9c+Zn2+
9c+Fe2+
9c+Fe3+
Abs
Wavelength/nm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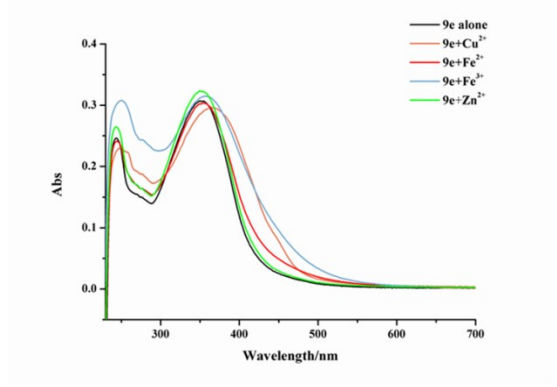
9d+Zn2+
Abs
Wavelength/nm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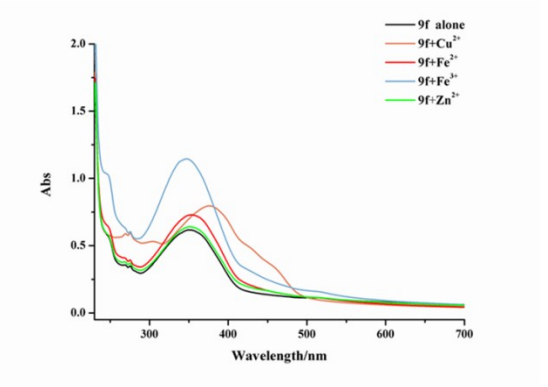
9e+Zn2+
Abs
Wavelength/nm


9f alone
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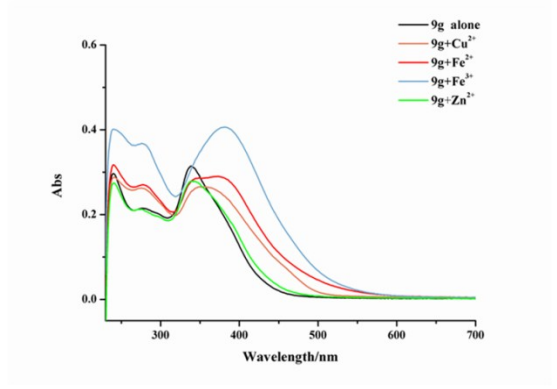
9f+Fe2+
9f+Fe3+
9f+Zn2+
Abs
Wavelength/nm


9g alone
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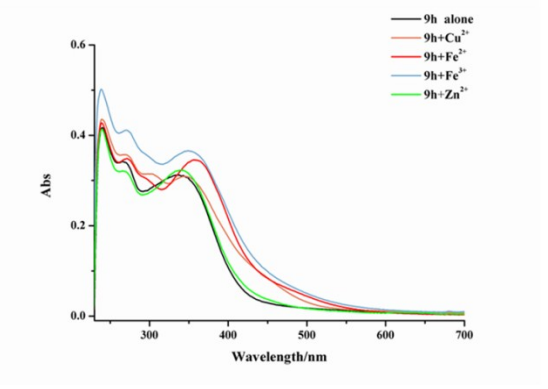
9g+Fe2+
9g+Fe3+
9g+Zn2+
Abs
Wavelength/nm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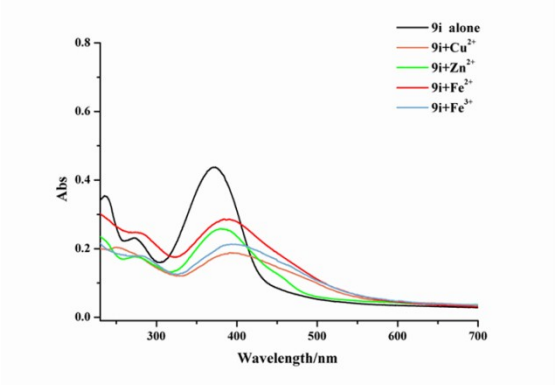
9h+Fe2+
9h+Fe3+
9h+Zn2+
Abs
Wavelength/nm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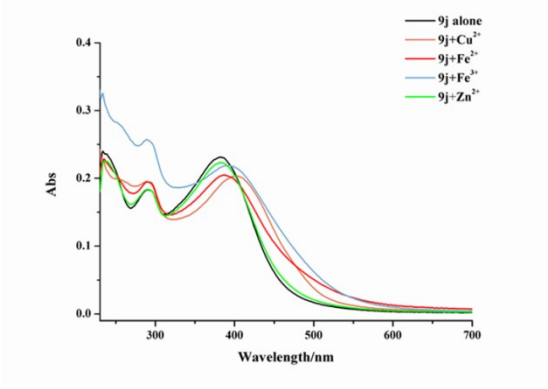
9i+Zn2+
9i+Fe2+
9i+Fe3+
Abs
Wavelength/nm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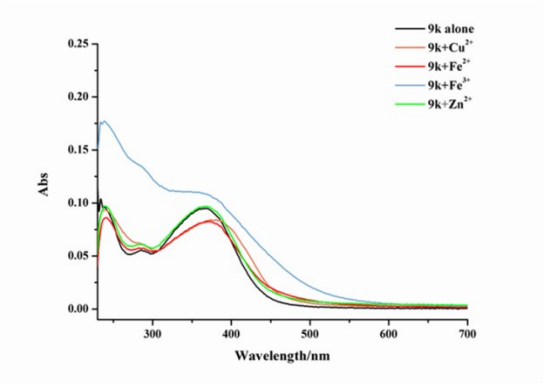
9j+Cu2+
9j+Fe2+
9j+Fe3+
9j+Zn2+
Abs
Wavelength/nm


9k alone
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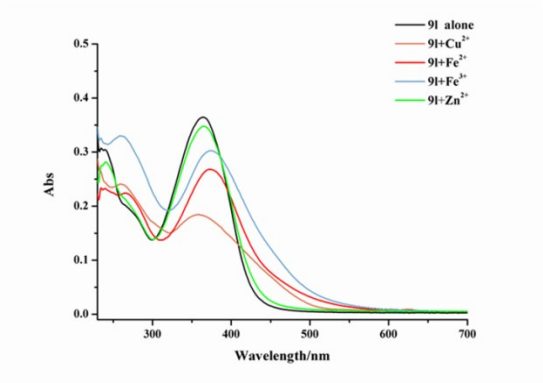
9k+Fe2+
9k+Fe3+
9k+Zn2+
Abs
Wavelength/nm


9l alone
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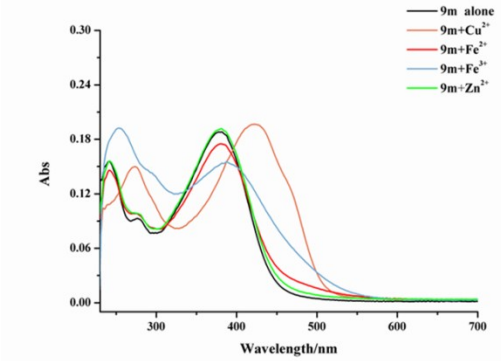
9l+Fe2+
9l+Fe3+
9l+Zn2+
Abs
Wavelength/nm


9m alone
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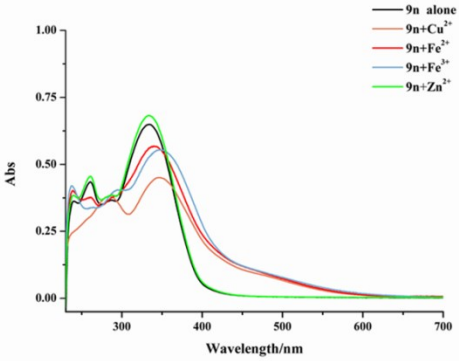
9m+Fe2+
9m+Fe3+
9m+Zn2+
Abs
Wavelength/nm


9n alone
9n+Cu2+
9n+Fe2+
9n+Fe3+
9n+Zn2+
Abs
Wavelength/nm

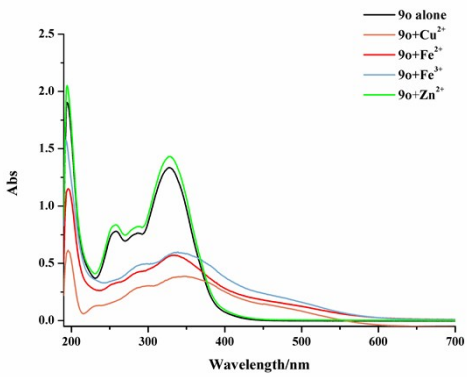
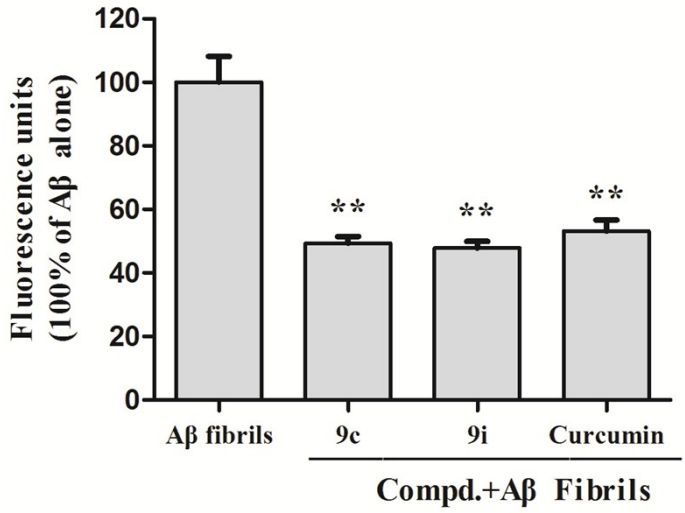
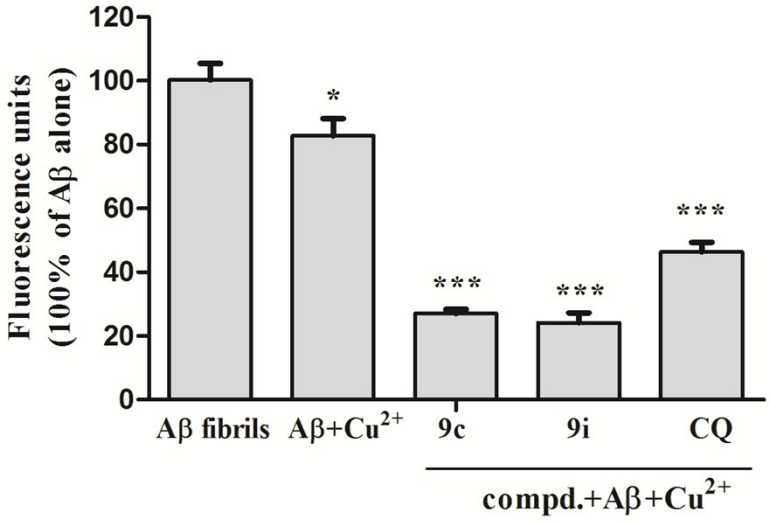

9o alone
9o+Cu2+
9o+Fe2+
9o+Fe3+
9o+Zn2+
Abs
Wavelength/nm

Fig S2 The ThT binding assay results of the self-mediated Aβ disaggregation experiment.



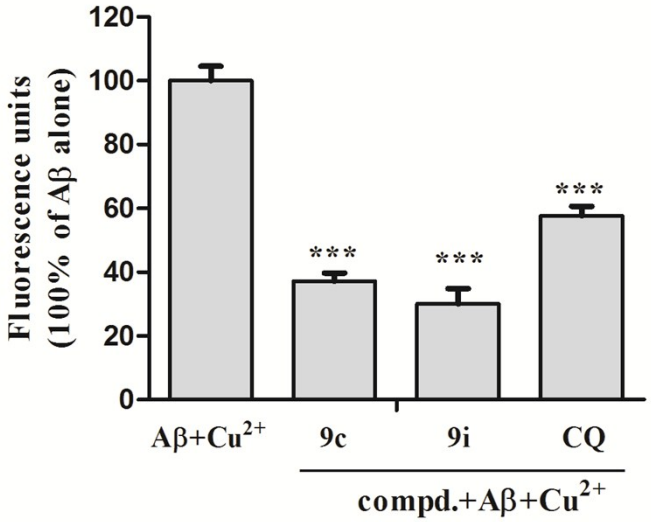
ThT fluorescence of disaggregation of self-induced Aβ fibrillization, measured upon incubation at 37℃ for 24h (PBS, [Aβ] = 25 μM; [compound] = 20 μM). Statistical significance was analyzed by ANOVA: (∗∗),p< 0.01 versus control.

Figure S3 The ThT binding assay results of the Cu^{2+}- mediated Aβ aggregation experiment.



ThT fluorescence of inhibition of Aβ-Cu^{2+} fibrillization, measured upon incubation at 37℃ for 24h. (HEPERS, [Aβ] = 25 μM; [Cu^{2+}] = 25 μM; [compound] = 50 μM). Statistical significance was analyzed by ANOVA: (∗∗∗)p< 0.001, (∗),p< 0.05 versus control.

Figure S4 The ThT binding assay results of Cu^{2+}-mediated Aβ disaggregation experiment.



ThT fluorescence of disaggregation of Aβ-Cu^{2+} fibrillization, measured upon incubation at 37℃ for 24h (HEPERS, [Aβ] = 25 μM; [Cu^{2+}] = 25 μM; [compound] = 50 μM). Statistical significance was analyzed by ANOVA: (***)p< 0.001, versus control.

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