Regular Article

***N,N'*-1,10-Bis(Naringin) Triethylenetetraamine, Synthesis and as a Cu(II) Chelator for Alzheimer's Disease Therapy**Li-Xia Guo^{a,b} and Bin Sun^{*a,b}^aKey Laboratory of Natural Medicine Research of Chongqing Education Commission, Chongqing Technology and Business University; Chongqing 400067, P. R. China; and ^bCollege of Environment and Resources, Chongqing Technology and Business University; Chongqing 400067, P. R. China.

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The bis-Schiff base of *N,N'*-1,10-bis(naringin) triethylenetetraamine (**1**) was prepared, as a copper(II) ion chelator, compound **1** was used for Alzheimer's disease therapy *in vitro*. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay of compound **1** showed that this Schiff base could promote PC12 cells proliferation, and also, compound **1** could inhibit Cu²⁺-amyloid- β (A β)_{1–42} mediated cytotoxicity on PC12 cells. The thioflavine T (ThT) assay showed that **1** can effectively attenuate Cu²⁺-induced A β _{1–42} aggregation. In addition, compound **1** is determined to be potent antioxidants on the basis of *in vitro* antioxidant assay, it can effectively decrease the level of reactive oxygen species (ROS) in Cu²⁺-A β _{1–42}-treated PC12 cells and elevate the superoxide dismutase (SOD) activity in Cu²⁺-A β _{1–42}-treated PC12 cells. The results show that *N,N'*-1,10-bis(naringin) triethylenetetraamine is a potential agent for therapy of Alzheimer's disease.

Key words naringin; Schiff base; Cu²⁺ ion; amyloid-beta; antioxidant; Alzheimer's disease

INTRODUCTION

Alzheimer's disease (AD) is an age-related neurodegenerative progressive and fatal disorder.¹⁾ Although the development of cure for AD has been hindered by a lack of understanding of both the causes and mechanisms of this disease onset and progression, a series of hypothesis came forth to explain the cause of AD, among which amyloid cascade hypothesis, glycogen synthase kinase-3 (GSK-3) hypothesis, oxidative stress hypothesis, metal ion hypothesis and cholinergic hypothesis are of the central importance.^{2–6)} Among them, the aggregation of amyloid- β (A β) plays a major role in neuronal loss and cognitive impairment by forming senile plaques. This A β species form unbranched fibrils consisting of parallel and ordering β -sheet structures. A β , especially A β _{1–42}, is proven to form toxic aggregates, among them, soluble A β oligomers are the main toxic aggregates.^{7–11)} Therefore, preventing A β aggregation is a major strategy against AD.

Metals (such as Cu, Zn, Fe) play an important role in the pathogenesis of Alzheimer's disease,^{12–14)} particularly Cu and Zn, bind to A β peptides facilitating their aggregation,^{15–20)} moreover, dysregulated redox active metal ions, Cu(I/II) and Fe(II/III), both unbound and bound to A β peptides, are observed to promoted overproduction of reactive oxygen species (ROS) that damage biological molecules,²¹⁾ such as proteins, DNA and lipid.^{22–24)} Evidences show that metal chelators can prevent metal induced A β aggregation and ROS production,^{25–29)} and also can solubilize Cu(II) induced A β plaques, therefore, metal chelators are potential therapeutic agents of AD.^{30–34)}

Naringin is a dihydroflavone glycoside. It exhibits a variety of biological activities and pharmacological effects such as anti-inflammatory, anti-virus, anti-cancer, antioxidant and antihypertensive activities.³⁵⁾ Also, it can lower blood cholesterol, reduce the formation of blood clots, improve local microcirculation and nutrient supply reduce the formation of

blood clots.^{36–38)} Naringin can be used to produce drug for the prevention and treatment of cardiovascular and cerebrovascular diseases.³⁹⁾ In addition, naringin can attenuate neuronal apoptosis which induced by lipopolysaccharide (LPS), and improve the impairment of learning and memory in AD transgenic mouse model APPswe/PSDeltaE9.^{37,40)} Our group found that *N,N'*-bis(naringin)-1,3-propyldiamine (Fig. 1) expressed higher biological activities in inhibition of Cu²⁺ induced A β aggregation, attenuation of cytotoxicity of A β and anti-oxidation.⁴¹⁾ As a part of our continual interest in searching new medicines for curing AD, here we report the synthesis of *N,N'*-1,10-bis(naringin) triethylenetetraamine and *in vitro* studies of *N,N'*-1,10-bis(naringin) triethylenetetraamine as potential agent for Alzheimer's disease.

MATERIALS AND METHODS

Materials and Methods Chemicals used were reagent grade as supplied except where noted. Analytical TLC was performed using silica gel 60 F254 glass plates; Compound spots were visualized by UV light (254 nm) and by staining

Fig. 1. The Structure of *N,N'*-Bis(naringin)-1,3-propyldiamine

* To whom correspondence should be addressed. e-mail: sunbin@ctbu.edu.cn; binsunsh@yahoo.com

with a yellow solution containing $\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6$ (0.5 g) and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (24.0 g) in 6% H_2SO_4 (500 mL). Flash column chromatography was performed on silica gel 60 (200–400 Mesh). NMR spectra were referenced using Me_4Si (0 ppm). High-resolution mass spectra were recorded on a Q-TOF Ultima API LC-MS instrument with Waters 2795 Separation Module (Waters Corporation, Milford, MA, U.S.A.).

Dimethyl sulfoxide (DMSO), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), thioflavine T (ThT), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) kit and superoxide dismutase (SOD) kit were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). BCA protein assay kit was purchased from Thermo Fisher Scientific (Waltham, MA, U.S.A.). $\text{A}\beta_{1-42}$ (>98% purity) was purchased from ChinaPeptides (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from GIBCO (Grand Island, NY, U.S.A.).

Rat pheochromocytoma PC12 cell line was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Other chemicals were all of the highest purity available from local sources.

Synthesis

N,N'-1,10-Bis(naringin) Triethylenetetraamine

Naringin (5.81 g, 10 mmol) was dissolved in a mixture solvent of methanol and ethanol (v/v = 1:1) (100 mL). The reaction mixture was stirred and heated to 50 °C, a solution of triethylenetetraamine (0.74 mL, 5 mmol) in ethanol (10 mL) was added into the reaction mixture. After 30 min, a lot of yellow precipitate was formed. The reaction mixture was continued stirring for 1 h, and then the mixture was cooled down to room temperature. Filtered, the residue was washed with anhydrous ethanol (3 × 20 mL) 3 times and dried over a vacuum to obtain crude product (5.75 g). The crude product was purified by recrystallization with ethanol and water (3:1) to give product (5.50 g, 87%). $^1\text{H-NMR}$ (600 MHz, $\text{DMSO}-d_6$, ppm) δ : 12.02 (s, br, 2H), 9.58 (s, br, 2H), 7.30 (dd, J = 12.0, 4.8 Hz, 4H), 6.77 (d, J = 12.0 Hz, 4H), 6.09 (t, J = 3.0 Hz, 2H), 6.06 (t, J = 4.2 Hz, 2H), 5.52–5.42 (m, 2H), 5.27 (d, J = 6.3 Hz, 2H), 5.14–5.05 (m, 6H), 4.69 (d, J = 6.3 Hz, 2H), 4.63 (d, J = 6.0 Hz, 2H), 4.54 (t, J = 6.0 Hz, 2H), 4.44 (d, J = 2.4 Hz, 2H), 3.71–3.59 (m, 6H), 3.47–3.36 (m, 9H), 3.32–3.24 (m, 16H), 3.19–3.10 (m, 5H), 2.75–2.65 (m, 2H), 1.13 (d, J = 9.0 Hz, 6H). $^{13}\text{C-NMR}$ (150 MHz, D_2O , ppm) δ : 169.0, 164.1, 164.0, 163.5, 163.2, 158.0, 128.3, 127.9, 127.8, 127.7, 116.1, 116.0, 115.9, 103.8, 100.9, 97.4, 78.7, 77.5, 76.3, 75.6, 71.8, 70.0, 68.7, 60.3, 60.1, 57.3, 51.7, 48.9, 46.7, 66.5, 38.8, 38.4, 38.1, 16.7. HR-electrospray ionization (ESI)-MS: m/z : Calcd for $\text{C}_{60}\text{H}_{78}\text{N}_4\text{O}_{26}\text{Na}$: $[\text{M} + \text{Na}]^+$, 1293.4802. Found 1293.4864.

Biological Assays

$\text{A}\beta_{1-42}$ Peptide Solution Preparation

In brief, $\text{A}\beta_{1-42}$ powder was firstly dissolved in HFIP at 1.0 mg/mL, sonicated for 10 min in ice bath to disrupt the pre-existing aggregates, and aliquoted into microcentrifuge tubes to obtain 0.1 mg stocks. The stocks were stored at room temperature and protected from light for 5–24 h. Thereafter, HFIP was removed by evaporation under N_2 . The thin transparent film of peptides at the internal surface of the tube was stored at –80 °C. In aggregation and inhibition experiments, $\text{A}\beta_{1-42}$ was dissolved in DMEM, and then diluted in PBS (pH 7.4) to a final concentration of 10 μM .

ThT Fluorescent Assay

$\text{A}\beta_{1-42}$ samples (10 μM) with CuCl_2 (5 μM) and different concentrations of *N,N'*-bis(naringin) triethylenetetraamine (dissolved in DMSO) were incubated at 37 °C. For ThT assays, 200 μL of ThT (ThT was dissolved in 0.1 M glycine buffer at pH 8.9) incubated samples were drawn carefully at 72 h. ThT fluorescence intensities (excitation wavelength 450 nm; emission wavelength 482 nm) were measured by a fluorescence spectrometer. The fluorescence intensity of the samples without $\text{A}\beta_{1-42}$ was subtracted as background from each read with $\text{A}\beta_{1-42}$. Each value reported is the average of three readings for every sample after subtraction of ThT fluorescence background. All ThT fluorescence experiments were performed in triplicate.

Cell Viability Assay

MTT assays were performed to assess the cytotoxicity of $\text{A}\beta_{1-42}$ aggregates using PC12 cells. The PC12 cells were cultured in DMEM supplemented with 10% FBS at 37 °C under 5% CO_2 . Prior to the MTT assays, PC12 cells were seeded onto 12-well plates at a density of 5×10^4 cells/well and cultured for 24 h. Then, the cells were treated with samples [mixtures of $\text{A}\beta_{1-42}$ (10 μM) with Cu^{2+} (5 μM), different concentrations of *N,N'*-bis(naringin) triethylenetetraamine], and incubated for additional 48 h. After that, 0.5 mg/mL MTT solutions (dissolved in water, sterile filtration) were added into each well. After 2–4 h incubation, the medium was replaced with DMSO to dissolve the formazan salt. Then, absorbance at 570 nm was measured by a multifunctional microplate reader.

Intracellular Determination of ROS

Intracellular ROS accumulation was monitored on a fluorescence spectrofluorometer (Beckman Coulter, Brea, CA, U.S.A.) with excitation and emission wavelengths of 485 and 530 nm, respectively using the fluorescent probe DCFH-DA. To do the test, cells were incubated with 20 $\mu\text{mol/L}$ of DCFH-DA (DCFH-DA was dissolved in DMSO and prepared into 10 mm storage solution) for 1 h at 37 °C in the dark after treatment with various concentrations of test agents. After incubation, the cells were washed twice with PBS and, finally, analyzed by a fluorescence spectrofluorometer. Protein concentration of samples was determined by BCA Protein assay kit with BSA used as a standard. Intracellular ROS accumulations of test compounds were assayed in triplicate.

SOD Activity Determination

SOD activity of the complexes was based on the inhibitory effect of SOD on the reduction of nitroblue tetrazolium (NBT) by the superoxide anion generated by the system xanthine/xanthine oxidase, measuring the absorption at 560 nm. PC12 cells were seeded onto 12-well plates at a density of 5×10^4 cells/well and cultured for 24 h. Then, the cells were treated with samples [mixtures of $\text{A}\beta_{1-42}$ (10 μM) with Cu^{2+} (5 μM), different concentrations of *N,N'*-1,10-bis(naringin) triethylenetetraamine, and incubated for additional 24 h. Then, the cells were washed two times with 0.1 M phosphate buffer saline (PBS, pH = 7.4).

The cell lysates were used to determine SOD activity using commercial kits according to the kit's instructions. Protein concentration of samples was determined by BCA Protein assay kit with BSA used as a standard. The SOD activities of test compounds were assayed in triplicate.

Statistical Analysis All data are presented as means $\pm$ standard deviation (S.D.). Statistical analyses were

conducted using Origin version 8.0 software (OriginLab Corporation, MA, U.S.A.). Data were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test, for differences among treatments. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Chemistry

Synthesis of Ligand (Chart 1)

In order to avoid naringin in product, the reactant should be completely dissolved in solvent. Therefore, a solvent having a moderate solubility for naringin is required, which can completely dissolve naringin, and also, the product is easy to precipitate from the solvent after the reaction finished. The solubility of naringin in methanol is good, however, there is no product precipitate from solvent when the solution of naringin in methanol react with triethylenetetraamine. The solubility of naringin in ethanol is much worse than that in methanol, the proper amount of ethanol cannot completely dissolve the naringin. After repeated attempts, the mixture solvent of methanol and ethanol with 1:1 ratio can obtain product precipitates with 96% yield.

Biological Activities

Inhibition of Cu^{2+} -Induced $\text{A}\beta_{1-42}$ Aggregation by N,N' -1,10-Bis(naringin) Triethylenetetraamine

Cu^{2+} and Zn^{2+} are known to promote the rapid aggregation of $\text{A}\beta$ peptides in solution and this has been implicated in several neurodegenerative diseases such as Alzheimer and Parkinson disease.^{18,20} It has been shown previously that metal chelators can attenuate these effects.²⁴⁻²⁸ However, treatment of Tg2576 mice with traditional Cu chelator triethylenetetraamine (TETA), did not inhibit amyloid deposition.³³ In the ThT assay, we found that TETA also did not effectively pre-

vent the Cu^{2+} -induced $\text{A}\beta_{1-42}$ accumulation. TETA ($100\mu\text{M}$) was incubated with $\text{A}\beta_{1-42}$ and Cu^{2+} for 72 h, the inhibition rate was only 3% (Fig. S3). The activity of naringin for preventing of Cu^{2+} -induced $\text{A}\beta_{1-42}$ aggregation is better than that of TETA, naringin ($100\mu\text{M}$) was incubated with $\text{A}\beta_{1-42}$ and Cu^{2+} for 72 h, the inhibition rate was 13% (Fig. 2B). Comparing TETA and naringin, N,N' -1,10-bis(naringin) triethylenetetraamine was shown high activity to reduce Cu^{2+} -induced $\text{A}\beta_{1-42}$ aggregation (Fig. 2A), this bis-Schiff base ($10\mu\text{M}$) was incubated with $\text{A}\beta_{1-42}$ and Cu^{2+} for 72 h, the inhibition rate was 33%, when its concentration increased to $100\mu\text{M}$, the inhibition rate increased to 55%, these results were obviously better than that of bis(naringin)-1,3-propyldiamine (when its concentration is separately 10 and $100\mu\text{M}$, the inhibition rate is separately 3 and 26%, Fig. S4), and the attenuation of this Schiff base was increased with the prolongation of action time (24–72 h). These results indicated that compound **1** has much better ability to inhibit Cu^{2+} -induced $\text{A}\beta$ aggregation than TETA, naringin and N,N' -bis(naringin)-1,3-propylenediamine.

Protection Effects of N,N' -1,10-Bis(naringin) Triethylenetetraamine on $\text{A}\beta_{1-42}$ - Cu^{2+} -Induced Cytotoxicity of PC12 Cells

To study the influence of N,N' -1,10-bis(naringin) triethylenetetraamine itself on the cell viability, the PC12 cell line was chosen, as it is a neuronal cell line, and the brain is the target organ of the compound. The compound was added to PC12 cells at increasing concentrations (10 – $200\mu\text{M}$) and incubated for 48 h or 72 h. The cell cytotoxicity was assessed with the MTT assay. Figure S5 illustrates cell viability in relation to concentrations of compound; as can be seen, the compound had not a significant effect on cytotoxicity, and also, it can promote PC12 cell proliferation, when the concentration of compound **1** exceeded $25\mu\text{M}$, it incubated with PC12 cells for 72 h, the cell viability was more than 100% (in the same condition, the cell viability of control is 100%).

Chart 1. The Synthesis of N,N' -Bis(naringin) Triethylenetetraamine

Fig. 2. ThT Assay on the Cu^{2+} -Induced Aggregation of $\text{A}\beta_{1-42}$ Peptide in the Absence or Presence of N,N' -Bis(naringin) Triethylenetetraamine (A) and Naringin (B) at Indicated Concentrations

Fig. 3. Protection Effects of 1,10-*N,N'*-Bis(naringin) Triethylenetetraamine (A) and Naringin (B) on $A\beta_{1-42}$ - Cu^{2+} -Induced Cytotoxicity of PC 12 Cells

##, $p < 0.01$ vs. control and *, $p < 0.05$ vs. the group of $A\beta_{1-42}$ - Cu^{2+} alone.

The ability of *N,N'*-1,10-bis(naringin) triethylenetetraamine to inhibit Cu^{2+} - $A\beta_{1-42}$ assembly suggested that it might be helpful in blocking $A\beta$ -mediated cellular toxicity. As shown in Fig. 3(A), treatment of the PC12 cells with $5 \mu M$ $A\beta$ - Cu^{2+} for 48 h the cell viability reduced to 50.2%. When the PC12 cells with $5 \mu M$ $A\beta$ - Cu^{2+} and $10 \mu M$ naringin Schiff base were incubated for 48 h, the survival of the cells increased to about 60.2%. These results showed that the compound was not only effective as a Cu^{2+} -induced $A\beta$ assembly inhibitor, but also could decrease $A\beta$ -mediated cellular toxicity in PC12 cell lines. The same assays were done for naringin (Fig. 3B) and *N,N'*-bis(naringin)-1,3-propylenediamine (Fig. S6), the results showed that naringin did not obviously increase the cell viability of PC12 ($p > 0.05$), and when the concentration of *N,N'*-bis(naringin)-1,3-propylenediamine is $10 \mu M$, the cell viability of PC12 cell is not obvious increasing yet. In addition, When the PC12 cells with $5 \mu M$ $A\beta$ - Cu^{2+} and $10 \mu M$ triethylenetetraamine (TETA) were incubated for 48 h, the cell viability reduced to 45.2% (Fig. S7). These results indicated that *N,N'*-1,10-bis(naringin) triethylenetetraamine had more ability to attenuate $A\beta$ -mediated cellular toxicity in PC12 cell lines than that of naringin, TETA and *N,N'*-bis(naringin)-1,3-propylenediamine.

N,N'-1,10-Bis(naringin) Triethylenetetraamine Attenuates the Production of ROS in $A\beta_{1-42}$ - Cu^{2+} -Treated PC12 Cells

The interaction of the $A\beta$ peptides with redox-active metal ions such as Cu^{2+} has been proposed to lead to formation of ROS and oxidative stress associated with $A\beta$ neurotoxicity.⁴²⁾ The oxidative stress which associated with high levels of the bio-metals copper (or iron) in the neurodegenerative AD naturally has triggered the potential use of chelating agents to connected with this metal-induced brain damage.^{42,43)} To evaluate the effect of *N,N'*-1,10-bis(naringin) triethylenetetraamine on cell viability at the molecular level, we detected the reduction of the amount of ROS by this naringin bis-Schiff bases with the fluorescent probe DCFH in PC12 cells in the presence of $A\beta_{1-42}$ - Cu^{2+} . The results demonstrated that incubation with $5 \mu M$ $A\beta_{1-42}$ - Cu^{2+} increased ROS levels compared to control, and by contrast, *N,N'*-1,10-bis(naringin) triethylenetetraamine dose-dependently decreased the level of ROS in $A\beta_{1-42}$ - Cu^{2+} treated PC12 cells, when the concentration of this bis-Schiff base was $25 \mu M$, the ROS level was 74 (Fig. 4). The same assay was carried out for *N,N'*-bis(naringin)-1,3-propylenediamine, when its concentration is $25 \mu M$, the ROS level was 115 (Fig. S8). These results showed that the decrease-

Fig. 4. *N,N'*-Bis(naringin) Triethylenetetraamine Decreases the Production of ROS in $A\beta_{1-42}$ - Cu^{2+} -Treated PC12 Cells

##, $p < 0.01$ vs. control, and *, $p < 0.05$, **, $p < 0.01$ vs. the group of $A\beta_{1-42}$ - Cu^{2+} alone.

ing of the level of ROS was not simply caused by hydroxyl group on naringin. It maybe that compound **1** can chelate Cu^{2+} from $A\beta$ - Cu complex, so as to relieve the oxidative stress which caused by $A\beta$ - Cu complex.

N,N'-1,10-bis(naringin) Triethylenetetraamine Elevates the SOD Activities in $A\beta_{1-42}$ - Cu^{2+} -Treated PC12 Cells (Fig. 5)

Some research suggests that the possible mechanisms for neurotoxicity in AD are an imbalance in the production and detoxification of free radicals both an increased production of ROS and insufficient antioxidant defense.^{44,45)} The immediate evidence is the alterations in enzymatic antioxidant metabolism in postmortem AD brains.⁴⁶⁾ SOD multigene family play an important role in the clearance of ROS and imbalance in oxidative stress. Mn-SOD deficiency has been shown to accelerate the onset of a number of behavioral deficits in hAPP mice.⁴⁷⁾ And impaired Cu/Zn-SOD activity contributes to increased oxidative damage in amyloid precursor protein (APP) transgenic mice.^{48,49)} Instead, overexpression of Mn-SOD reduces hippocampal superoxide and $A\beta$ levels, improves the axonal transport deficits and mitochondrial respiratory function in the Tg2576 Alzheimer model mice.^{50,51)} Considering the fact that the active centers of SOD are metal complexes, we speculate that *N,N'*-1,10-bis(naringin) triethylenetetraamine, as a chelator, could capture Cu from $A\beta_{1-42}$ - Cu^{2+} complex, and chelator-Cu complex may form a dinuclear Cu complex with naringin Schiff bases (Fig. 6, the complex structure was certificated by ESI-HRMS, FTIR, UV and the results of elemental analysis, in supporting information), and this di-

Fig. 5. the Activity of SOD in $A\beta_{1-42}$ - Cu^{2+} -Treated PC12 Cells in the Absence or Presence of N,N' -Bis(naringin) Triethylenetetraamine at Indicated Concentrations

##, $p < 0.01$ vs. control, and **, $p < 0.01$ vs. the group of $A\beta_{1-42}$ - Cu^{2+} alone.

Fig. 6. The Structure of Copper Complex with N,N' -1,10-Bis(naringin) Triethylenetetraamine Bis-Schiff Bases

nuclear Cu complex could mimic SOD. The SOD activity was quantified using a modified NBT assay system. The results indicated that $A\beta_{1-42}$ - Cu^{2+} complex as expected showed little SOD activity compared to control (Fig. 5), and by contrast, N,N' -1,10-bis(naringin) triethylenetetraamine dose-dependently increased the activity of SOD in $A\beta_{1-42}$ - Cu^{2+} treated PC12 cells, when the concentration of compound **1** is separately 0, 10, 20, 50 and $100 \mu M$, the activity of SOD is separately 22, 25, 43, 50, and 82. The same assay was done for N,N' -bis(naringin)-1,3-propylenediamine, when its concentration is separately 0, 10, 20, 50 and $100 \mu M$, the activity of SOD is separately 22, 18, 28, 36, and 50 (Fig. S9). These results indicated that the copper(II) complex with N,N' -1,10-bis(naringin) triethylenetetraamine can more stimulate the action of SOD than the copper(II) complex with N,N' -bis(naringin)-1,3-propylenediamine.

In conclusion, in this study, we synthesized N,N' -1,10-bis(naringin) triethylenetetraamine bis-Schiff bases, and studied the bioactivities of this compound *in vitro*. This compound is designed to attenuate the $A\beta$ aggregation caused by Cu^{2+} , inhibit cellular toxicity mediated by the accumulation of $A\beta$ and prevent the production of reactive oxygen species. The results of biological assays show that this compound can effectively prevent Cu^{2+} -induced $A\beta_{1-42}$ aggregation, attenuation of $A\beta_{1-42}$ -mediated cytotoxicity in PC12 cell lines, decrease the level of ROS in $A\beta_{1-42}$ - Cu^{2+} treated PC12 cells, and increase the activity of SOD in $A\beta_{1-42}$ - Cu^{2+} treated PC12 cells. These studies show that N,N' -1,10-bis(naringin)triethylenetetraamine will be a potential drugs for AD therapy.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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