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# 7-(4-Hydroxy-3-methoxyphenyl)-1-phenyl-4*E*-hepten-3-one alleviates $A\beta_{1-42}$ induced cytotoxicity through PI3K-mTOR pathways

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ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disease in the elderly. Increasing evidence has shown that β-amyloid protein (Aβ) production is the key pathological cause of AD. 7-(4-Hydroxy-3-methoxyphenyl)-1-phenyl-4*E*-hepten-3-one (AO-2), a natural diarylheptanoid, is previously found to have activities in neuronal differentiation and neurite outgrowth, and its analogue shows protective effects against Aβ. In this study, we further investigated the function of AO-2 toward Aβ-induced injuries in PC12 cells and hippocampal neurons. Pretreatment of PC12 cells with AO-2 restored cell viability in a concentration-dependent manner against Aβ-induced neurotoxicity. Moreover, the Aβ stimulated apoptosis and caspase-3 activation were markedly inhibited by AO-2. We found that AO-2 prevented the downregulation of PI3K-Akt-mTOR signaling after Aβ damage, and blockade of either PI3K or mTOR activity led to the failure of AO-2 on caspase-3 inhibition. We further showed that AO-2 was protective against two devastating effects of Aβ, increased reactive oxygen species (ROS) production and dendrite injury, and this protection was also dependent on PI3K and mTOR activities. Taken together, this study showed that AO-2 acts against Aβ-induced damages in PC12 cells and hippocampal neurons through PI3K-mTOR pathways, thus providing a new neuroprotective compound which may shed light on drug development of AD.



## 1. Introduction

Alzheimer's disease (AD), the most common cause of dementia [1,2], is characterized by amyloid β (Aβ) deposits and neurofibrillary tangles [3–5]. $A\beta_{1-42}$, which consists of 42 amino acids of the peptide, is the most found toxic forms in the pathogenesis of AD [6,7]. $A\beta_{1-42}$ causes oxidative stress which leads to DNA damage and caspase-dependent neuronal apoptosis, and this process plays an important role in neurodegeneration [8,9]. Moreover, there is cumulative evidence that dystrophic neurites and dendritic simplification are associated with AD [10,11]. Therefore, treatments that alleviate $A\beta_{1-42}$ induced caspase-dependent apoptosis and impairments in dendrites may be a therapeutic approach for AD [12,13].

An increasing number of natural products have been served as the source for drug development of AD, such as curcumin and resveratrol [14,15]. Our previous study showed that 7-(4-hydroxyphenyl)-1-phenyl-4*E*-hepten-3-one (AO-1) and 7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-4*E*-hepten-3-one (AO-2; Fig. 1A), two closely related, curcumin-like diarylheptanoids, promote differentiation and neurite outgrowth of neuroblastoma cells and hippocampal neurons [16]. AO-1 also shows a neuroprotective effect against $A\beta_{1-42}$ induced toxicity [17]. However, whether AO-2 is another beneficial compound against $A\beta_{1-42}$ is still unknown.

In the present study, we first examined the protective effects of AO-2 in PC12 cells, a well-established neuronal cell line for neuroprotective studies. Cytotoxicity, apoptosis, and the reactive oxygen species (ROS) levels were measured to evaluate the effects of AO-2 in the presence of $A\beta_{1-42}$. Moreover, we used primary hippocampal neurons to study whether neuronal death and dendritic injury induced by $A\beta_{1-42}$ were attenuated or rescued by AO-2. We also explored the molecular action of AO-2 by examining cleaved caspase-3 production and several signaling pathways. Our study demonstrated that AO-2 exhibited potent neuroprotective effects against neuronal apoptosis, ROS production, and dendritic damages induced by $A\beta_{1-42}$, which was dependent on PI3K-mTOR pathway.

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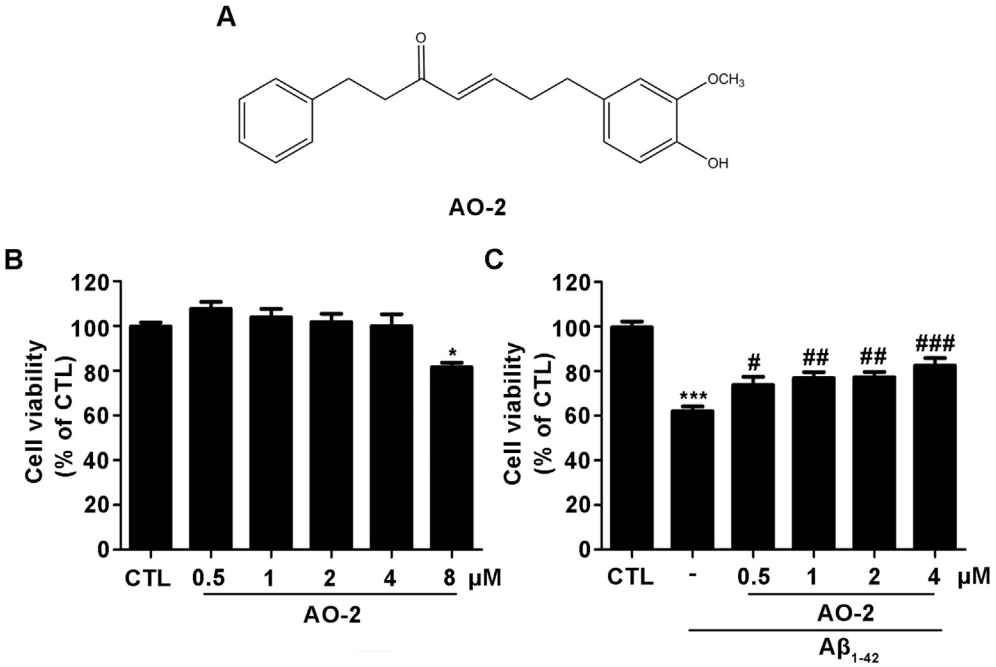


**Fig. 1.** Protective effects of AO-2 against $A\beta_{1\text{-}42}$-induced cytotoxicity in PC12 cells. A. The chemical structure of AO-2 is shown. B. PC12 cells were exposed to indicated concentrations of AO-2 and cell viability was determined at 24 h later by MTT assay. $^{*}P < 0.05$, AO-2 (8 μM) vs. CTL (control). C. AO-2 prevents $A\beta_{1\text{-}42}$ induced decrease of cell viability. PC12 cells were pretreated with different concentrations of AO-2 (0.5, 1, 2, 4 μM) for 2 h followed by exposure to 1 μM $A\beta_{1\text{-}42}$ for 24 h. Cell viability was determined using MTT assay. $^{***}P < 0.001$, $A\beta_{1\text{-}42}$ vs. CTL. $\#P < 0.05$, $\#\#P < 0.01$ and $\#\#\#P < 0.001$, AO-2 + $A\beta_{1\text{-}42}$ vs. $A\beta_{1\text{-}42}$ alone. For B and C, Data are expressed as percentages of values of that in untreated control cells, and are expressed as mean ± SEM of three independent experiments.

## 2. Materials and methods

### 2.1. Chemicals, reagents and materials

AO-2 (7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-4*E*-hepten-3-one) was extracted from the rhizomes of *A. officinarum* Hance as previously reported [16]. Stock AO-2 was dissolved in DMSO at a concentration of 10 mM. Different concentrations of AO-2 (0.5–8 μM) was used to study its protective effects, and DMSO with the volume equal to the highest concentration of AO-2 was added as the vehicle control in each experiment.

Rabbit monoclonal antibodies against caspase-3, p38, Akt and S6K were purchased from Cell Signaling Technology; α-tubulin was from Sigma-Aldrich; GAPDH was from Abcam; Anti-Microtubule-Associated Protein 2 (MAP2) antibody and LY294002 were from Millipore; Torin1 was from Tocris; 2′, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethyl-sulfoxide (DMSO) and curcumin were from Sigma-Aldrich.

### 2.2. Preparation of oligomerized $A\beta_{1\text{–}42}$

$A\beta_{1\text{-}42}$ was purchased from rPeptide and was dissolved in phosphate-buffered saline (PBS) at a concentration of 1 mM. Prior to the treatment, peptides were preincubated at 37 °C for 7 days to promote aggregation and then diluted in medium to desired concentrations as described previously [17]. Soluble oligomerized $A\beta_{1\text{-}42}$ peptides (equivalent to 1 μM peptides) were added to cells to induce damaging effects.

### 2.3. Cell culture

PC12 cells were obtained from the American Type Culture Collection (ATCC) and were cultured in Dulbecco's modified Eagle's medium supplemented with 6% FBS, 6% HS and 1% penicillin/streptomycin under a humidified atmosphere of 5% $CO_2$ and 95% air at 37 °C. Cells were plated at $1 \times 10^4$ cells per well in 96 well plates for cytotoxicity study, $3 \times 10^5$/35 mm dish for flow cytometry and western blot analysis.

Primary hippocampal neurons were prepared from E18 Sprague Dawley (SD) rat embryos as previously described [18]. Briefly, Rat hippocampal neurons ($1 \times 10^5$ per coverslip) were plated on 18 mm coverslips coated with poly-D-lysine (1 mg/ml; Sigma) and fed with 0.2% B27 (Invitrogen), 1 mM L-glutamine (Life Technologies) and 1% penicillin/streptomycin. All experimental procedures involving the use of animals were approved by the Ethics Committee on Animal Experiments at Jinan University, China, and were strictly performed according to the guidelines of the Care and Use of Laboratory Animals.

### 2.4. Measurement of cell viability

PC12 cells were preincubated with AO-2 for 2 h and exposed to 1 μM $A\beta_{1\text{-}42}$ for 24 h, and cell viability was measured by MTT assay as previously reported [16]. Briefly, MTT was added to each well for 4 h, and then the formazan was dissolved in DMSO. The optical density (OD) values were detected at 595 nm in a multimode detector (Beckman coulter). Cell viability was expressed as a percentage of the value against the control using the following formula: Cell viability (%) = (absorption of sample - absorption of background)/(absorption of control - absorption of background) × 100%.

### 2.5. Detection of apoptosis by flow cytometry

The protective effect of AO-2 against cell apoptosis was evaluated using the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Detection Kit (Biouniquer Tech) according to the manufacturer's instructions. Briefly, cells were

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