INHIBITION OF AMYLOID-β AGGREGATION BY p-TERPHENYLS FROM THE MUSHROOM POLYOZELLUS MULTIPLEX AND THEIR NEUROPROTECTIVE EFFECTS

Shoko Nakabayashi, a Ayaka Ishikura, a Koji Fujihara, a Shuntaro Hirabayashi, a Shin Koike, b Hiroaki Sasaki, a Yuki Ogasawara, b Kiyotaka Koyama, a and Kaoru Kinoshita**

aDepartment of Pharmacognosy and Phytochemistry, Meiji Pharmaceutical University, Noshio 2-522-1, Kiyose-shi, Tokyo 204-8588, Japan. bDepartment of Analytical Biochemistry, Meiji Pharmaceutical University, Noshio 2-522-1, Kiyose-shi, Tokyo 204-8588, Japan. E-mail: kaoru@my-pharm.ac.jp

Abstract – The main pathogenesis of Alzheimer’s disease (AD) is related to the accumulation of amyloid-β (Aβ) peptides in the brain that leads to neuronal cell death. In this study, we identified compounds in a methanol extract of the fruiting body of Polyozellus multiplex that inhibited Aβ aggregation and are neuroprotective. Seven compounds against Aβ40 aggregation were obtained through bioactivity-guided fractionation of the extract, including polyozellin (1), kynapcin-12 (2), NSC617425 (3), cycloleucomelone (4), Bl-V (5), succinic acid (6), and protocatechuic acid (7). Compounds 1–5 inhibited Aβ40 aggregation in a dose-dependent manner. Moreover, compounds 2–5 protected SH-SY5Y cells from Aβ toxicity. Therefore, these compounds are potential agents in AD treatment.

Dementia is a neurodegenerative disease caused by acquired organic brain damage. The disease is characterized by a persistent loss of cognitive function that interferes with daily and social life. Alzheimer’s disease (AD) is the most frequent cause of dementia1; its pathological features include neuronal loss (mainly in the cerebral cortex), neurofibrillary tangles, and the occurrence of senile plaques in the brain parenchyma. Senile plaques are composed of amyloid-β (Aβ) peptides.2 Normally, Aβ peptides are degraded and/or eliminated. However, excessive accumulation of Aβ forms senile plaques, eventually leading to AD, which is known as the amyloid cascade hypothesis.3 Aβ is a partial fragment of amyloid precursor protein (APP), which is produced by the cleavage of β-secretase 1 (BACE1) and γ-secretase and is subsequently secreted into the extracellular milieu.4,6 Aβ undergoes degradation by various extracellular pathways. Therefore,
regulation of BACE1 and γ-secretase activity and activation of the Aβ degradation pathway may be an important strategy for the treatment of AD. In 2021, the US Food & Drug Administration announced the approval of a new drug, aducanumab (brand name Aduhelm™), to treat AD. Aducanumab, an antibody that reduces Aβ deposition, has proven to be clinically useful for the amyloid cascade hypothesis. However, further research is necessary on Aβ inhibitors, as well as BACE1 inhibitors, which have not been clinically used to date. In our laboratory, we screened 110 extracts of various mushrooms for their inhibitory effects on Aβ aggregation. We have already reported on meroterpenoids isolated from the fruiting bodies of Boletinus asiaticus and Albatrellus yasudae that inhibit Aβ aggregation and/or BACE1. In addition to these mushrooms, a methanol extract from the fruiting body of Polyozellus multiplex showed inhibitory activity against Aβ aggregation. The edible black mushroom P. multiplex belongs to the Thelephoraceae family; it is often harvested in Japan, Korea, and North America. p-Terphenyl compounds isolated from P. multiplex have been reported to have anti-inflammatory, anti-tumorigenic, and anti-prolylendopeptidase effects. The four p-terphenyls identified from the P. multiplex extracts, polyozellin, polyozellinic acid, thelephoric acid, and kynapcin-12, have been reported to have BACE1 inhibitory activity and neuroprotective effects and are expected to have anti-AD activity. However, no reports evaluating the Aβ aggregation inhibitory activity of P. multiplex are known. Recently, Aβ42 aggregation inhibitory activity was reported for p-terphenyl compounds isolated from Boletopsis leucomelas of the same family as P. multiplex. Based on this, it would be meaningful to evaluate the Aβ aggregation inhibitory activity of P. multiplex to explore their potential anti-AD activity. Here, seven compounds were isolated from this extract through bioactivity-guided separation and evaluated for their Aβ aggregation inhibitory activity, BACE1 inhibitory activity, and neuroprotective effects. Furthermore, their structure-activity relationships (SARs) were examined.

**Extraction, isolation, structural determination, and inhibitory activity of Aβ aggregation**

The methanol extract from the fruiting body of P. multiplex showed potent inhibitory activity against Aβ40 aggregation (an Aβ40-aggregation rate of 25.1%) at 10 µg/mL. Bioactivity-guided separation was performed using silica gel column chromatography (Si. C. C.) to yield seven fractions, A–G, and an insoluble fraction, H. The seven fractions A–G and the insoluble fraction H were evaluated for their inhibitory activity against Aβ40 aggregation at 10 µg/mL (A: 3.18 g, 49.0%; B: 5.80 g, 78.3%; C: 1.52 g, 59.3%; D: 0.31 g, 34.5%; E: 4.50 g, 30.3%; F: 33.50 g, 22.5%; G: 1.56 g, 14.7%; H: 23.91 g, 14.5%). Because of its relatively high Aβ40-aggregation inhibitory activity and large quantity, fraction F (22.5%) was further separated. The structures were elucidated as seven compounds 1–7 using mass spectrometry (MS) and comprehensive nuclear magnetic resonance (NMR) spectra, and were identified as polyozellin
(1),\textsuperscript{22,23} kynapcin-12 (2),\textsuperscript{19,24} NSC617425 (3),\textsuperscript{25} cycloleucomelone (4),\textsuperscript{26} Bl-V (5),\textsuperscript{27} succinic acid (6),\textsuperscript{28} and protocatechuic acid (7)\textsuperscript{28} (Figures, 1, S2, S3). This is the first report on the isolation of compounds 4–7 from \textit{P. multiplex}. Compounds 1–5 were classified as \textit{p}-terphenyl compounds.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{structures.png}
\caption{Structures of compounds 1–11}
\end{figure}
Based on thin layer chromatography (TLC), fraction D (34.5%) and E (30.3%) contained polyozellin (1), kynapcin-12 (2), and Bl-V (5). Fraction G (14.7%) showed a similar pattern with fraction F and contained more polyozellin (1). The insoluble fraction H (14.5%) contained mainly polyozellin (1) (Figure S15). The half-maximal inhibitory concentration (IC₅₀) values of compounds 1–7 were 0.615, 6.76, 6.45, 2.17, 3.61, >100, and >100 µM, respectively (Tables 1, S5). Compounds 1–5 inhibited Aβ₄₀ aggregation in a dose-dependent manner. The results of compound 5 supported the report of Kodori et al.²¹

<table>
<thead>
<tr>
<th></th>
<th>Aβ aggregation IC₅₀ (µM, n=3)</th>
<th>BACE1 IC₅₀ (µM, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.615</td>
<td>4.78d</td>
</tr>
<tr>
<td>2</td>
<td>6.76</td>
<td>15.79d</td>
</tr>
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<tr>
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<td>21.61</td>
<td>&gt;100</td>
</tr>
<tr>
<td>myricetina</td>
<td>9.9c</td>
<td>2.8e</td>
</tr>
<tr>
<td>inhibitor IVb</td>
<td>&gt;100c</td>
<td>0.015c</td>
</tr>
</tbody>
</table>

aMyricetin was used as a positive control for the inhibitory activity of Aβ aggregation.
bInhibitor IV was used as a positive control for BACE1 inhibitory activity.
c,d,e Data were referred to reference,¹³,²⁰,²⁹ respectively.

Structure-activity relationship (SAR)
To determine the SAR of inhibitory activity on Aβ₄₀ aggregation, p-terphenyl compounds 8–11, thelephoric acid (8), polyozellic acid (9), an acetone adduct from polyozellic acid (10), and 11,12-(methylenedioxy)polyozellic acid acetate (11), from our previous work were used (Figure 1).¹⁵ The IC₅₀ value of compounds 8–11 were 12.62, 1.29, 1.96, and 21.61 µM, respectively (Tables 1, S5). Compounds 8–10 exhibited inhibitory activity against Aβ₄₀ aggregation in a dose-dependent manner. Based on these
assay results, there are some consistent characteristics of compounds with inhibitory activity on Aβ40 aggregation. The first is the presence of catechol moieties. Compounds 1–5 and 8–10 contain one or two catechol moieties and tend to be relatively more active than compound 11, which has acetylated catechol moieties. Sato et al.30 reported a site-specific inhibitory mechanism against Aβ42 aggregation through a catechol-type flavonoid, (+)-taxifolin, which targets Lys16 and Lys28 after autoxidation. Furthermore, catechol-bearing compounds have been reported to have antioxidative and anti-inflammatory properties.31,32 However, compound 7, which contained a catechol moiety but no p-terphenyl structure, was active. This suggests that the p-terphenyl structure is important for Aβ40 aggregation inhibition. The second characteristic is the presence of furan rings. Compounds 1, 4, 5, and 8–11 contain one or two furan rings. Compounds 1, 9, and 10, with two furan rings in the same direction, tended to be more active than compound 8, with two furan rings in the opposite direction. Compound 11 was less active than compounds 1, 9, and 10, although two furan rings in the same direction are present. The presence of two acetyl groups in the central ring (compounds 1–3 and 5) does not appear to be important for Aβ40 aggregation inhibitory activity compared to the other compounds that showed activity.

**Inhibitory activity against BACE1**

In this study, compounds 3–7, 10, and 11 were evaluated for their inhibitory activities against BACE1. The IC$_{50}$ values of compounds 3–7, 10, and 11 were >100, 66.62, >100, >100, >100, >100, >100, >100, >100, >100, >100, 34.49, and >100 µM, respectively (Tables 1, S6). Compounds 4 and 10 showed weak BACE1 inhibitory activities in a dose-dependent manner. The BACE1 inhibitory activity of compounds 1, 2, 8, and 9 have reported by Chon et al.20

**Protective effect of compounds on Aβ42 toxicity in SH-SY5Y cells**

Since compounds 1–5 inhibited Aβ40 aggregation, their neuroprotective effects against Aβ42 toxicity were also evaluated using human neuroblastoma SH-SY5Y cells. Compounds 6 and 7, which did not inhibit Aβ40 aggregation, were evaluated as well. To avoid measuring a reduction in Aβ42 production due to the potential toxicity of these compounds, the effect of these compounds on SH-SY5Y cells was measured before the Aβ42 measurement and evaluated using doses below those observed to have no adverse effects for each compound (Figure S16). The results showed that SH-SY5Y cell viability after treatment with 10 µM Aβ42 was reduced to 31.7% of the control group, whereas the cell viabilities with co-incubation of compounds 1–7 and 10 µM Aβ42 were 29.0 ± 10.6% at 3.1 µM, 63.3 ± 2.60% at 25.0 µM, 74.7 ± 1.5% at 13.0 µM, 85.8 ± 6.8% at 25.0 µM, 51.6 ± 3.7% at 13.0 µM, 40.7 ± 2.6% at 13.0 µM, and 37.7 ± 7.9% at 13.0 µM, respectively (Figure 2). Cell viability was improved with the addition of compounds 2–5, and furthermore,
compounds 2–4 were dose-dependent. Compounds 2–5 also have catechol moieties, which might be important for neuroprotection as well as Aβ_{40} aggregation inhibitory activity. Although compound 1 has a catechol moiety, it showed no neuroprotective effects against Aβ_{42} toxicity. This is because the cytotoxicity of compound 1 was strong, so it is possible that there was no concentration that had activity but no toxicity.

![Figure 2](image)

**Figure 2.** Protective effects of seven compounds on SH-SY5Y cells against Aβ toxicity Cont. indicates the control group (cells in the absence of Aβ and compounds 1–7). Aβ indicates the group treated with 10 µM Aβ but no compound. 1–7 indicate the groups treated with each compound (1–7) and 10 µM Aβ. Statistical significance was analyzed using one-way ANOVA followed by Dunnett’s test. Values are expressed as mean ± SD, n=3, **p < 0.01, ***p < 0.001 as compared with Aβ.

Based on SAR, the presence of the p-terphenyl structure and the C4 or C4’-linked hydroxyl group tended to enhance the protective effect against Aβ_{42} toxicity in SH-SY5Y cells, since no neuroprotective effect was observed with compounds 1, 6, and 7. Hanaki et al. reported on the inhibition of Aβ_{42} aggregation by flavonoids with non-catechol structures, and kaempferol with a C4’-linked hydroxy group may prevent the elongation phase in the aggregation stage of Aβ_{42}. We isolated five candidate compounds, 1–5, for the treatment of AD from the methanol extract of the fruiting body of *P. multiplex* and these compounds exhibited inhibitory activities against Aβ_{40} aggregation. Compounds 2–5 protected against Aβ_{42} toxicity in SH-SY5Y cells. These results suggest that compounds 2–5 might be developed into therapeutic agents that can prevent AD symptoms by protecting neurons after the deposition of Aβ_{42} in the brain. Furthermore, because compounds 2–5 also inhibited Aβ_{40} aggregation, they might be expected to be prophylactic agents against AD and mild cognitive impairment. Compound 1 exhibited inhibitory activity against both BACE1 and Aβ_{40} aggregation. According to previous studies based on the amyloid cascade hypothesis, most compounds reported to have the potential for AD therapy have been limited to either inhibitors of Aβ aggregation or BACE1. In general, no relationship has been found between the inhibition of Aβ aggregation and BACE1. However, there has been a finding similar to the present study for *A. yasudae*.

Therefore,
compounds that inhibit both activities, such as compound 1, may be useful as new prophylactic agents for the treatment of AD. Aβ peptides aggregate and form oligomers, protofibrils, and mature fibrils with various structures. It has been reported that some toxic aggregates are non-toxic conformations to neurons. 34-37 It is necessary for prophylactic and therapeutic drugs to suppress the aggregation of neurotoxic Aβ. One limitation of this study is that the thioflavin-T (Th-T) assay cannot distinguish the difference between toxic and non-toxic conformations due to the characteristics of the assay. In addition, compound 1 might need the addition of modifying groups to reduce its cytotoxicity.

CONCLUSION

In conclusion, in this study, we demonstrated that p-terphenyl compounds from a methanol extract of the fruiting body of P. multiplex, particularly compounds 1–5, have potential as prophylactic and therapeutic agents for the treatment of AD. In addition to the p-terphenyl compounds mentioned above, meroterpenoids isolated from the fruiting bodies of B. asiaticus and A. yasudae inhibit Aβ aggregation and/or BACE1. Therefore, mushrooms may be valuable natural resources for the treatment of AD.

EXPERIMENTAL

General experimental procedures

Column chromatography was performed using silica gel 60N (63–210 µm; Kanto Chemical, Tokyo, Japan), ODS silica gel YMC-GEL ODS-A (75 µm; YMC Co., Ltd., Kyoto, Japan), Chromatorex COOH MB100-75/200 (100 µm; Fuji Silysia Chemical Ltd., Aichi, Japan), and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden). Preparative high-performance liquid chromatography (HPLC) was performed on a Jasco PU-2080 Plus equipped with a Jasco UV-2075 Plus UV detector at 254 nm (Jasco Co., Tokyo, Japan). The HPLC columns were Inertsustain C18 column (10φ × 250 mm; GL Sciences Inc., Tokyo, Japan), Senshu Pak PEGASIL Silica SP100 (10φ × 250 mm; Senshu Scientific Co., Ltd.), and Inertsustain phenyl 5 µm column (10φ × 250 mm; GL Sciences Inc., Tokyo, Japan).

Fungal material

P. multiplex was collected on Mt. Mizugaki in Yamanashi Prefecture, Japan. A voucher specimen (PM-2018) was deposited in Sep. 2018. Species identification was confirmed by one of the authors (K. Koyama).

Extraction and isolation

Dried and fractured fruiting body of P. multiplex (734.0 g) was extracted three times each with CHCl3 and MeOH at room temperature. The MeOH extracts were evaluated for their inhibitory activity against Aβ aggregation with an Aβ aggregation rate of 25.1% at 10 µg/mL. The MeOH extract (75.0 g, 25.1%) was
Further fractionated by Si. C. C. using a stepwise gradient (CHCl$_3$-MeOH; 100:1, 75:1, 50:1, 20:1, 10:1) to yield seven fractions (A–G) and an insoluble fraction (H). Fractions A–H were evaluated for their inhibitory activity against Aβ aggregation at 10 µg/mL (A: 3.18 g, 49.0%; B: 5.80 g, 78.3%; C: 1.52 g, 59.3%; D: 0.31 g, 34.5%; E: 4.50 g, 30.3%; F: 33.50 g, 22.5%; G: 1.56 g, 14.7%; H: 23.91 g, 14.5%).

Fraction F (33.50 g, 22.5%) was further fractionated by Si. C. C. using a stepwise gradient (CHCl$_3$-MeOH; 100:1, 75:1, 50:1, 20:1, 10:1) to yield five fractions (F1–F5). Fractions F1–F5 were evaluated for their inhibitory activity against Aβ aggregation at 10 µg/mL (F1: 0.12 g, 25.3%; F2: 0.17 g, 32.5%; F3: 2.20 g, 14.2%; F4: 3.07 g, 9.8%; F5: 18.70 g, 13.9%).

Fraction F3 (2.20 g, 14.2%) was further fractionated by a Sephadex LH-20 with CHCl$_3$-MeOH-acetone (1:1:1) to yield six fractions (F3a–F3f) and an insoluble fraction (fraction F3g). Fractions F3a–F3g were evaluated for their inhibitory activity against Aβ aggregation at 10 µg/mL (F3a: 72.2 mg, 28.5%; F3b: 324.2 mg, 25.9%; F3c: 1.42 g, 5.1%; F3d: 262.7 mg, 18.9%; F3e: 18.4 mg, 23.2%; F3f: 15.8 mg, 5.6%; F3g: 35.8 mg, 5.6%). Fraction F3f was obtained as polyozellin (1, 15.8 mg).

Fraction F3c (1.42 g, 5.1%) was subjected to four chromatography steps: (1) ODS C. C. with MeOH-H$_2$O (50:0, 100:0), (2) Si-COOH. C. C. with CHCl$_3$-MeOH (50:1, 20:1, 10:1, 5:1, 2:1, 0:100), (3) preparative HPLC by ODS C. C. eluted with MeOH-H$_2$O (45:55) to obtain NSC617425 (3) (5.6 mg, $t_R$ 8.0 min) and kynapcin-12 (2) (33.0 mg, $t_R$ 11.2 min), (4) preparative HPLC by ODS C. C. eluted with MeOH-H$_2$O (5:5) to obtain Bl-V (5) (9.1 mg, $t_R$ 11.2 min), and (5) preparative HPLC by ODS C. C. eluted with MeOH-H$_2$O (2:8) to obtain succinic acid (6) (13.4 mg, $t_R$ 8.0 min).

Fraction F3d (262.7 mg, 18.9%) was subjected to three chromatography steps: (1) Sephadex LH-20 with CHCl$_3$-MeOH-acetone (1:1:1), (2) Si-COOH. C. C. with CHCl$_3$-MeOH (50:1, 0:100), and (3) ODS C. C. with MeOH-H$_2$O (80:20, 100:0) to obtain protocatechuic acid (7, 3.5 mg).

Fraction F4 (3.07 g, 9.8%) was fractionated by Sephadex LH-20 with CHCl$_3$-MeOH-acetone (1:1:1) to yield eleven fractions (F4a–F4k) and an insoluble fraction (F4l). Fractions F4a–F4l were evaluated for their inhibitory activity against Aβ aggregation at 10 µg/mL (F4a: 499.3 mg, 52.2%; F4b: 779.6 mg, 43.0%; F4c: 305.0 mg, 43.2%; F4d: 120.2 mg, 39.1%; F4e: 590.1 mg, 35.3%; F4f: 158.9 mg, 25.7%; F4g: 227.6 mg, 11.7%; F4h: 73.3 mg, 9.1%; F4i: 36.2 mg, 5.4%; F4j: 19.3 mg, 13.7%; F4k: 18.1 mg, 6.1%; F4l: 546.8 mg, 12.8%). Fractions F4g and F4h (300.9 mg) were fractionated by Sephadex LH-20 with CHCl$_3$-MeOH-acetone (1:1:1) to obtain cycloleucomelone (4, 12 mg).

**Thioflavin-T (Th-T assay)**

Similar data were previously obtained for Aβ$_{40}$ and Aβ$_{42}$ using both test conditions, and thus Aβ$_{40}$ was used in this study.$^{38}$ Th-T assays of extracts, fractions, and isolated compounds were performed using thioflavine-T (FUJIFILM Wako Pure Chemical Co., Ltd., Japan) and Aβ$_{40}$ (Peptide Institute, Japan). The
aggregation of Aβ was evaluated using a slight modification of the Th-T method developed by Naiki and co-workers. In brief, 80 μL of 50 mM sodium phosphate buffer containing 100 mM NaCl at pH 7.4 was aliquoted into a 0.5 mL tube, followed by the addition of 10 μL of each test sample that was dissolved with DMSO. Then, 10 μL of Aβ 40 (250 μM in 0.02% NH₄OH) was added to the tube so that the final concentration was Aβ 40 25 μM, respectively. All procedures were performed on ice. The resultant solution was incubated at 37 °C for 24 h, and then mixed with 300 μL of 5.0 μM Th-T in 5.0 mM Gly-NaOH buffer at pH 8.5. The mixture was transferred to black 96-well plates at 100 μL per well and then gently vortexed for 30 min. Fluorescence intensity was measured at 440 nm excitation and 485 nm emission using a Synergy HTX Multi-Mode Reader (BioTek, USA). The Aβ aggregation rate was calculated by the following equation: Aβ aggregation rate (%) = (S – B)/(C – B) × 100, where C is the fluorescence intensity of a DMSO control (assay buffer, 25 μM Aβ 40, and DMSO containing no test sample) after 24 h of incubation, B is the fluorescence intensity of a blank (assay buffer, 25 μM Aβ 40, and DMSO containing no test sample) without incubation, S is the fluorescence intensity of the tested samples (assay buffer, 25 μM Aβ 40, and DMSO containing test sample) after 24 h of incubation. Myricetin (Tokyo Chemical Industry Co., Ltd., Japan) was used as a positive control. The IC₅₀ values were calculated by GraphPad Prism version 5.02 (GraphPad Software, USA).

BACE1 FRET assay

BACE1 assays were performed using the BACE1 FRET assay kit, Red (Thermo Fisher Scientific, USA). 9 μL of test sample, 9 μL of 722 nM BACE1 substrate (Rh-EVNNLDAEFK-Quencher in 50 mM ammonium bicarbonate), and 9 μL of BACE1 enzyme (1 U/mL) were mixed on black 384-well plates and incubated for 3 h at room temperature. Fluorescence intensity was measured at 540 nm excitation and 590 nm emission using a Synergy HTX Multi-Mode Reader (BioTek, USA). The BACE1 inhibition rate was calculated by the following equation: BACE1 inhibition rate (%) = [1 – {(S – S₀) – (B – B₀)}/(C – C₀) – (B – B₀)] × 100, where C is the fluorescence of a DMSO control (enzyme, substrate, and assay buffer with DMSO) after 3 h of incubation, C₀ is the fluorescence of the DMSO control at 1 h after incubation, B is the fluorescence of a no-enzyme control (substrate and assay buffer with DMSO) after 3 h of incubation, B₀ is the fluorescence of the non-enzyme control at 1 h after incubation, S is the fluorescence of the tested samples (enzyme, sample solution, and substrate) after 3 h of incubation, and S₀ is the fluorescence of the tested samples at 1 h after incubation. β-secretase inhibitor IV (Merck, Germany) was used as a positive control. The IC₅₀ values were calculated by GraphPad Prism version 5.02 (GraphPad Software, USA).
Cell culture

Human neuroblastoma SH-SY5Y cells were purchased from ATCC and cultured in Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium (DMEM/F12) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin and 15 mM HEPES. The cells were cultured and maintained in T75 flasks at 37 °C in 5% CO₂.

Protective effect on SH-SY5Y against Aβ toxicity assay

Each compound was dissolved in DMSO. The final DMSO concentration in the culture medium was 0.1%. The assay was conducted in Type I collagen-coated 96-well cell culture plates (Nippi Inc., Tokyo, Japan), and 9.6 × 10³ cells/well were seeded. After incubation for 72 h, the medium was removed. Each test sample and 250 µM Aβ42 in 0.02% NH₄OH (final concentration of Aβ42: 10 µM) was added in DMEM/F12 medium containing 2% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin. After incubation for 24 h at 37 °C in 5% CO₂, cell viability was determined using the Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). The plate was incubated for 3 h at 37 °C in 5% CO₂, then the absorbance at 450 nm was measured using the Synergy HTX Multi-Mode Reader while blank wells containing no-cells, no-Aβ42 peptides, and test samples were also prepared and measured at 450 nm in the same manner. The relative cell viability was calculated by the following equation: relative cell viability (%) = (S–B₅)/(C–B₇) × 100, where C is the absorbance at 450 nm of DMSO control (9.6 × 10³ cells/well, 10 µM Aβ42, and DMSO containing no test samples), S is the absorbance at 450 nm of DMSO control (9.6 × 10³ cells/well, 10 µM Aβ42, and DMSO containing test sample), B₅ is the absorbance at 450 nm of DMSO control (no cells, no Aβ42 peptide, and DMSO containing no test samples), B₇ is the absorbance at 450 nm of DMSO control (no cells, no Aβ42 peptide, and DMSO containing test samples), each absorbance was measured in the same procedure.

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REFERENCES AND NOTES