Acetylcholinesterase and butyrylcholinesterase inhibitory activities of khellactone coumarin derivatives isolated from *Peucedanum japonicum* Thurnberg

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Cholinesterase (ChE) and monoamine oxidase (MAO) inhibitors have been attracted as candidate treatments for Alzheimer's disease (AD). Fifteen khellactone-type coumarins from the roots of *Peucedanum japonicum* Thunberg were tested for acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and MAO inhibitory activities. Compound 3'-angeloyl-4'-(2-methylbutyryl)khellactone (PJ13) most potently inhibited AChE (IC₅₀ = 9.28 µM), followed by 3'-isovaleryl-4'-(2-methylbutyroyl)khellactone (PJ15) (IC₅₀ = 10.0 µM). Compound senecioyl-4'-angeloyl-khellactone (PJ5) most potently inhibited BChE (IC₅₀ = 7.22 µM) and had the highest selectivity index (> 5.54), followed by 3'-senecioyl-4'- (2-methylbutyryl)khellactone (PJ10) and 3',4'-disenecioylkhellactone (PJ4) (IC₅₀ = 10.2 and 10.7 µM, respectively). Compounds PJ13, PJ15, and PJ5 showed reversible and mixed-types of inhibition with Kᵢ values of 5.98, 10.4 (for AChE), and 4.16 µM (for BChE), respectively. However, all 15 compounds weakly inhibited MAO-A and MAO-B. Molecular docking simulation revealed that PJ13 had a higher binding affinity (~9.3 kcal/mol) with AChE than PJ15 (~7.8 kcal/mol) or PJ5 (~5.4 kcal/mol), due to the formation of a hydrogen bond with Tyr121 (distance: 2.52 Å). On the other hand, the binding affinity of PJ5 (~10.0 kcal/mol) with BChE was higher than for PJ13 (~7.7 kcal/mol) or PJ15 (~8.1 kcal/mol), due to the formation of a hydrogen bond with Ser198 (distance: 2.05 Å). These results suggest that PJ13 and PJ5 are potential reversible selective inhibitors of AChE and BChE, respectively, for the treatment of AD.

Acetylcholinesterase (AChE) is a member of α/β hydrolase protein superfamily and breaks down an acetylcholine (ACh) into acetate and choline¹. Alzheimer's disease (AD) is an age-associated memory/cognitive disorder, and its mechanism has not been determined, and no curative therapy has been developed². Since cholinergic deficiency is present in AD, the relation between AChE and AD has been extensively studied¹³. AChE inhibitors (AChEIs) inhibit the hydrolysis of ACh (a neurotransmitter in the central nervous system), and as a result, increase ACh levels and ACh half-lives in autonomic ganglia and neuromuscular junctions, which are rich in ACh receptors⁴. AChEIs may be reversible or irreversible⁵. Commercially available AChEIs include piperidine-based (e.g., donepezil, Aricept)⁷, carbamate-based (rivastigmine, Exelon)⁸, phenanthrene-based (galantamine, Reminyl)⁹, and other inhibitors. The common potential side effects of AChEIs are diarrhea, headache, insomnia, nausea, and vomiting¹⁰. Butyrylcholinesterase (BChE) is mainly expressed in glial cells and white matter in the human brain, and as its name indicated, it breaks down butyrylcholine (BCh). BChE levels are significantly elevated...
in AD, and in BChE knockout AD mice, a reported reduction in fibrin Aβ plaque by up to 70% suggests that BChE inhibition has therapeutic value. Furthermore, AChE and BChE are known to be related to AD and to act independently of each other, which may lead to the diagnosis of disease and the development of potential drug targets.

Recently dual- or multi-targeting inhibitors of acetylcholinesterase (AChE) and monoamine oxidase (MAO) have attracted research attention as candidate treatments for AD. MAO catalyzes the oxidation of monoamines, and has two isoforms (MAO-A and MAO-B). MAO was discovered almost a century ago and has been the subject of many structural, pharmacological, and biochemical studies on neurotransmitters. MAO inhibitors (MAOIs) are currently used to treat depression and Parkinson's disease, and several studies have concluded that MAOIs reduce Aβ plaque, and thus, MAOIs are considered possible future treatments for AD.

**Peucedanum japonicum** Thunberg is a herb found on the cliffs of islands in Korea, Japan, and the Philippines, and has traditionally been used to treat coughs, cramps, pain, rheumatism, asthma, and angina. Furthermore, it has been shown to have anti-diabetic and anti-obesity, anti-nociceptive, anti-osteoporotic, and anti-allergic lung inflammatory effects. In traditional medicine, *P. japonicum* Thunberg is also believed to prevent stroke and vascular disease. On the other hand, an extract of *P. japonicum* Thunberg was found to contain a norisorenoid glucoside, 4-O-cafeoylquinic acid, 5-O-cafeoylquinic acid, ci nidioside A, preraoside II, preraoside III, apterin, esculin, (R)-peucedanol, and (R)-peucedanol 7-O-ß-D-glucopyranoside were identified. In addition, a 4-O-cafeoylquinic acid, 5-O-cafeoylquinic acid, citridioside A, preraoside II, preraoside III, apterin, esculin, (R)-peucedanol, and (R)-peucedanol 7-O-ß-D-glucopyranoside were identified.

In a study, 80% EtOH was found to contain peucedanol 5-O-ß-D-glucopyranoside and myo-inositol. Recently, khellactone coumarins were isolated from subfractions of *P. japonicum* roots by recycling HPLC, and reported to reduce NO levels in LPS-stimulated RAW264.7 cells and to inhibit anti-inflammatory response.

However, little information is available about the anticholinergic actions of khellactone coumarins. Accordingly, we investigated the inhibitory effects of khellactone coumarins from *P. japonicum* Thunberg on AChE, BChE, and MAOs. In addition, we investigated the bindings and kinetics of the potent inhibitors senecioyl-4'-angeloyl-khellactone (PJ15), 3'-angeloyl-4'- (2-methylbutyryl)k hellactone (PJ12), and 3'-isovaleryl-4'- (2-methylbutyryl)k hellactone (PJ15), and performed molecular docking simulations of these three compounds with AChE and BChE.

**Materials and methods**

**Compounds.** Fifteen khellactone-type compounds were isolated from *P. japonicum* Thunberg (voucher specimen: PBC-484), and the structures were determined, as described previously. Briefly, the dried roots of *P. japonicum* (5.0 kg) were extracted with 80% ethanol (EtOH) at room temperature three times to obtain 1.62 kg of solid extract. The 80% EtOH extract was further partitioned between n-hexane (114.2 g) and H2O (1.50 kg), and the solid extract was subjected to preparative reverse phase chromatography (Xbridge Prep C18, 5 µm, Waters Corporation, Milford, MA, USA) using methanol (MeOH) and H2O (0–52.0 min, 66–88% MeOH; 52.0–53.0 min, 100% MeOH). The fractions (Frs. 1–8) were collected and concentrated on a rotary evaporator under reduced pressure. Purification was conducted by recycling preparative HPLC. The yield of the khellactone-type coumarins obtained was ~1.5% from 80% EtOH extract determined. The reference reversible inhibitors of AChE and BChE, MAO-A, and MAO-B used were tacrine, and lazabemide was purchased from Sigma-Aldrich (St. Louis, MO, USA). Clorgyline and pargyline (irreversible reference inhibitors of MAO-A and MAO-B, respectively) were from BioAssay Systems (Hayward, CA, USA).

**Chemicals and enzymes.** AChE (Type VI-S; from *Electrophorus electricus*), recombinant human MAO-A, MAO-B, and BChE (from equine serum), acetylthiocholine iodide (ATCI), kynuramine, benzylamine, S-butyrylthiocholine iodide (BTCI), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), tacrine, donepezil, tolazamide, and lazabemide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Clorgyline and pargyline (irreversible reference inhibitors of MAO-A and MAO-B, respectively) were from BioAssay Systems (Hayward, CA, USA).

**Enzyme assays.** AChE assays were performed as described by Ellman et al. with slight modifications. In brief, assays were performed using ~0.2 U/mL of AChE in the presence of 0.5 mM DTNB and 0.5 mM ACTI in 0.5 mL reaction mixtures, and continuously monitored for 10 min at 412 nm. DTNB was used for color development, caused by reaction between it and thiocholine (a product of AChE). For inhibitory assays, compounds were preincubated with AChE for 15 min prior to ATCI and DTNB addition. BChE activity was assayed using the same method, but BTCI was used instead of ATCI. MAO-A activity was continuously assayed using kynuramine (a substrate) at 316 nm for 20 min, and MAO-B activity was assayed using benzylamine at 250 nm for 30 min, as described previously.

**Inhibitory activities and enzyme kinetics.** Inhibitions of the activities of AChE, BChE, MAO-A, and MAO-B by the 15 compounds were investigated at an inhibitor concentration of 10 µM. IC50 values were also determined. The reference reversible inhibitors of AChE and BChE, MAO-A, and MAO-B used were tacrine...
(or donepezil), tolloxatone and lazabemide, respectively, and the reference irreversible inhibitors of MAO-A and MAO-B used were clorgyline and pargyline, respectively. Kinetic parameters, inhibition types, and $K_i$ values of PJ5 (for BChE), PJ13 and PJ15 (for AChE) were determined as the methods previously described43. Enzyme kinetics were investigated at five different substrate concentrations, that is, at 0, ~1/2 × IC$_{50}$, IC$_{50}$, and 2 × IC$_{50}$ for each inhibitor. The inhibition types and $K_i$ values were determined using Lineweaver–Burk Plots and secondary plots.

Analysis of inhibitor reversibilities. Inhibitor reversibilities were examined using the dialysis method44, using with AChE or BChE, rather than MAO enzymes. In brief, the experiment was performed by preincubating an inhibitor at ~2 × IC$_{50}$ concentration with AChE or BChE for 30 min in 0.1 M sodium phosphate buffer (pH 7.2). Dialysis was conducted for 6 h with stirring and two buffer changes. Residual activities before ($A_U$) and after ($A_D$) dialysis were compared to those of non-treated controls, and reversibility types were determined by comparing $A_U$ and $A_D$ values.

Docking simulations of PJ5, PJ13, and PJ15 with AChE or BChE. To simulate the dockings of PJ5, PJ13, and PJ15 with AChE, we used Autodock Vina45, which has an automated docking facility. To define enzyme pockets, we used predefined active sites obtained from complexes between AChE and 3-[(1S)-1-(dimethylamino)ethyl]phenol (PDB ID: 1GQS) or donepezil (PDB ID: 6O4W), BChE and butyl-[(2-~{S})-1-(2-cycloheptylthylamino)-3-[(5~{H})-indol-3-yl]-1-oxidanylidenepropan-2-yl]azanium (PDB ID: 6QAA), MAO-A and 7-methoxy-1-methyl-9H-β-carboline complex (PDB ID: 2Z5X), and MAO-B and pioglitazone complex (PDB ID: 4A79). To prepare PJ5, PJ13, and PJ15 for docking simulation, ChemOffice program (http://www.cambridgesoft.com) was used to create the 2D structures of PJ5, PJ13, and PJ15, to convert them into 3D structures, and to perform energy minimizations. Docking simulations of the enzymes with PJ5, PJ13, and PJ15 were performed using Chimera46. Based on the results of docking simulations, we checked for possible hydrogen bonding using bonding relaxation constraints of 0.4 Å and 20.0° using Chimera47.

Analysis of pharmacokinetic properties using in silico method. Drug-like properties of the lead compounds of PJ5, PJ13, and PJ15 were analyzed using a web tool of SwissADME at http://www.swissadme.ch/48.
Inhibitory patterns. Modes of AChE inhibitions by PJ13 and PJ15 were investigated using Lineweaver–Burk plots. Plots of AChE inhibition by PJ13 were linear and lines intersected at a point, but not at the x- or y-axis (Fig. 3A). Secondary plots of the slopes of Lineweaver–Burk plots against inhibitor concentrations showed that the $K_I$ value of PJ13 for AChE inhibition was $5.99 \pm 0.21 \mu M$ (Fig. 3B). Plots of AChE inhibitions by PJ15 were also linear and did not intersect at the x- or y-axis (Fig. 3C), and the $K_I$ value of PJ15 for the AChE inhibition was $10.41 \pm 0.67 \mu M$ (Fig. 3D). These results show PJ13 and PJ15 acted as mixed-type inhibitors of AChE. In addition, plots of BChE inhibition by PJ5 were linear and intersected near the y-axis (Fig. 3E). Secondary plots showed the $K_I$ value of PJ5 for BChE inhibition was $4.16 \pm 0.72 \mu M$ (Fig. 3F), showing PJ5 acted as a mixed-type BChE inhibitor.

Table 1. Inhibitions of AChE, BChE, MAO-A, and MAO-B by khellactone coumarins from Peucedanum japonicum Thunberg roots. The values above are the means ± SEs of duplicate or triplicate experiments. Values for AChE and BChE were determined after preincubation of the enzymes with each compound for 15 min. $^a$ SI = IC$_{50}$ of AChE/ IC$_{50}$ of BChE, $^b$ IC$_{50}$ value.
Molecular docking simulation. AutoDock Vina docking simulations showed that PJ5, PJ13, and PJ15 located well at the binding sites of 3-[(1S)-(1-dimethylamino)ethyl]phenol complexed with AChE and at that of butyl-[(2-[(S)]=1-(2-cycloheptylethylamino)-3-(1-[H]-indol-3-yl)-1-oxidanylidenepropan-2-yl]azanium complexed with BChE. The results of the docking simulation for AChE showed that PJ13 interacted by forming a hydrogen bond with Tyr121 (distance: 2.52 Å). However, no hydrogen bond interaction was predicted for PJ5 and PJ15 (Fig. 4A–C). Docking simulation of PJ5 with BChE implied that a hydrogen bonding interaction was established with Ser198 (distance: 2.05 Å) of BChE, whereas no hydrogen bond was proposed for PJ13 and PJ15 (Fig. 4D–F). The binding affinity of PJ13 (−9.3 kcal/mol) for AChE was higher than that of PJ15 (−7.8 kcal/mol) or PJ5 (−5.4 kcal/mol) (Table 2). In addition, PJ5 had higher binding affinity for BChE (−10.0 kcal/mol) than PJ13 (−7.7 kcal/mol) or PJ15 (−8.1 kcal/mol). The binding affinities of PJ5, PJ13, and PJ15 with MAO-A or MAO-B were predicted to be weaker than those with AChE or BChE (Table 2). Docking simulations were provided in Supplementary Figure S17 (A–F). The binding score (−4.8 kcal/mol) of PJ13 for MAO-B was relatively higher than those of PJ5 and PJ15 in accordance with the residual activities at 10 µM.

When the crystal structure of AChE complexed with donepezil (PDB ID: 6O4W) and the binding pockets for BChE, MAO-A, and MAO-B defined with donepezil were used for docking simulations, the binding scores of PJ compounds were similar to the values obtained with their complexed ligands (Tables 2 and Supplementary Table S3). From docking simulations with the AChE/donepezil complex (PDB ID: 6O4W), it was predicted that PJ13 and PJ15 formed one hydrogen bond with Tyr124 (distances = 2.602 and 2.994 Å, respectively), but PJ5 did not form the bond. On the contrary, PJ5 could form a hydrogen bond with Thr120 of BChE (distance = 3.354 Å), but PJ13 and PJ15 did not form (Supplementary Fig. S18).
Figure 3. Lineweaver–Burk plots for the inhibitions of AChE by PJ13 (A) and PJ15 (C), and of BChE by PJ5 (E), and respective secondary plots (B, D, F) of slopes against inhibitor concentration. Substrates were used at five different concentrations (0.05–1.0 mM). Experiments were carried out at three inhibitor concentrations at around their respective IC\textsubscript{50} values. Initial reaction rates are expressed as increases in absorbance per min. \( K_m \) values of AChE and BChE were 0.1 and 0.18 mM, respectively.
Pharmacokinetic properties using in silico method. From the SwissADME analysis, it was predicted that the lead compounds of PJ5, PJ13, and PJ15 had high gastrointestinal adsorption abilities and cytochrome P450 inhibitory activities for 2C19, 2C9, and 3A4, however, they did not have blood–brain barrier (BBB) permeabilities (Table 3).
| Compounds | GI absorption | BBB permeant | P-gp substrate | CYP1A2 inhibitor | CYP2C19 inhibitor | CYP2C9 inhibitor | CYP2D6 inhibitor | CYP3A4 inhibitor | Log \( K_p \) (Skin permeation) (cm/s) |
|-----------|---------------|--------------|----------------|-----------------|-----------------|-----------------|-----------------|----------------|-------------------------------|
| PJ5       | High          | No           | No             | No              | Yes             | Yes             | No              | Yes            | 5.64                          |
| PJ13      | High          | No           | No             | No              | Yes             | Yes             | No              | Yes            | 5.68                          |
| PJ15      | High          | No           | No             | No              | Yes             | Yes             | No              | Yes            | 5.65                          |

Table 3. Predicted pharmacokinetic properties of PJ5, PJ13, and PJ15. GI, gastrointestinal absorption; BBB, blood–brain barrier; P-gp, P-glycoprotein; CYP, cytochrome P450.

Discussion

In this study, fifteen khellactone coumarin compounds from *P. japonicum* were analyzed for their abilities to inhibit AChE, BChE, MAO-A, and MAO-B. Compound PJ13 (IC\(_{50}\) = 9.28 \(\mu\)M) most potently inhibited AChE, followed by PJ15 and PJ7 (10.0 and 17.9 \(\mu\)M, respectively), which indicated all three are highly potent natural AChE inhibitors, based on the IC\(_{50}\) values of < 20 \(\mu\)M. The IC\(_{50}\) values of PJ13 and PJ15 were lower than those of the \(\text{C-}\)-glucosylflavone, isovitexin-7-\(\text{O}\)-methyl ether (swertisin) (32.09 \(\mu\)g/mL, i.e., 71.9 \(\mu\)M) from *Anthocleista vogelii*, the flavonoids tiloside (23.5 \(\mu\)M) and quercetin (19.8 \(\mu\)M) from *Agrimonia pilosa*, and the verbascosides decaffeoylverbascoside (16.1 \(\mu\)M) and acteoside (19.9 \(\mu\)M) from *Harpagophytum procumbens*, but higher than those of sargachromanol I (SCI, 0.79 \(\mu\)M) from the brown alga *Sargassum silphastrum* and dihydroberbine (1.18 \(\mu\)M) from *Captis chinensis*. Compared to other coumarin derivatives, the values of PJ13 and PJ15 were lower than those of scopoletin (52 \(\mu\)M) from *Vaccinium oldhami* Miquel, a dihydropyranocoumarin decursinol (28 \(\mu\)M) from *Angelica gigas Nakai*, masoonone E (23.5 \(\mu\)M) from *Mansonia gagei*, daphnetin (11.57 \(\mu\)M) from *Artemisia capillaris*, and a furanocoumarin 4′-hydroxy Pd–C–III (1.09 \(\mu\)M) from *Angelica decussiva* and a 4-phenyl coumarin mesuagenin B (0.7 \(\mu\)M) from *Mesua elegans*.

Regarding BChE inhibition, PJ5 (IC\(_{50}\) = 7.22 \(\mu\)M) was the most potent inhibitor, followed by PJ10 and PJ4 (IC\(_{50}\) = 10.16 and 16.66 \(\mu\)M, respectively). The IC\(_{50}\) value of PJ5 in this study was lower than those of broussonin A (7.50 \(\mu\)M) from *Anemarrhena asphodeloides*, isoaecoside (29.7 \(\mu\)M) from *H. procumbens*, corenone B (10.9 \(\mu\)g/mL, i.e., 49.5 \(\mu\)M) from *Niphogoton dissecta*, and kaempferol (62.5 \(\mu\)M) from *Cleistsocalyx operculata*, but higher than that of 4′-hydroxy Pd–C–III (5.78 \(\mu\)M) from *A. decussiva*. Compared to other coumarins, the IC\(_{50}\) value of PJ5 for BChE inhibition was lower than those of hyuganin C (38.86 \(\mu\)M) from *Mutellina purpurea*, a coumarin pteryxin (12.96 \(\mu\)g/mL, i.e., 33.5 \(\mu\)M) from *Coptis chinensis*, and the daphnetin (8.66 \(\mu\)M), and it might be concluded that PJ5 is the most potent BChE inhibitor in natural coumarins reported.

These results show that PJ5 is potent and selective inhibitor of BChE, while PJ13 and PJ15 are selective inhibitors of AChE. It might be suggested that combination of compounds effectively inhibit ChE. The possibility of dual inhibition of AChE and MAO enzymes was investigated for dual- or multi-targeting therapeutic purposes in AD and PD. However, in the present study, no tested khellactone coumarin showed dual inhibitory activity.

Structurally, PJ5, PJ13, and PJ15 contain a coumarin ring system, and the coumarins are known to have a variety of biological functions, which include anti-inflammatory, anticancer, antiviral, antioxidant, and anti-depressant effects, and some have been shown to inhibit AChE and BChE. PJ13 and PJ15 differ structurally as different substituents are bound to the 3C ester. PJ13 [(9R,10R)-8,8-dimethyl-10-((2-methylbutanoyloxy)-2-oxo-9,10-dihydro-2H,8H-pyran][2,3-|f|chromen-9-yl(1-2-methylbut-2-en-2-yl) with a double bond between 1 and 2C in the sec-butyl structure, whereas PJ15 [(9R,10R)-8,8-dimethyl-10-((2-methylbutanoyloxy)-2-oxo-9,10-dihydro-2H,8H-pyran][2,3-|f|chromen-9-yl-3-methylbutanoate] has an isobutyl group in this position. The AChE inhibitory activity of PJ15 was slightly higher than that of PJ13, which contains a (Z)-but-2-en-2-yl group. PJ4, PJ5, and PJ10 share a common 3-methylbut-2-en-2-enoate structure, and showed relatively higher BChE activities than other compounds. The higher BChE inhibitory activity of PJ9 than PJ8 appeared to be due to the different position of the double bond.

AChE or BChE inhibitors have been reported to exhibit competitive, noncompetitive, and mixed-type inhibitory patterns. In the present study, potent inhibitions of AChE by PJ13 and PJ15 and of BChE by PJ5 were found to be reversible and to exhibit mixed-type inhibition, with \( K_s \) values of 5.98, 10.4, and 4.16 \(\mu\)M, respectively. These results suggest that PJ13, PJ15, and PJ5 bind to the allosteric site or the substrate-binding site of AChE.
Docking simulation analysis with AChE revealed that the PJ13 interacted with the phenolic hydroxyl group of Tyr121 to form a hydrogen bond, while no hydrogen-bond was predicted for PJ5 and PJ15. In addition, the oxygen of the carbonyl group of PJ5 formed a hydrogen bond with Ser198 of BChE, whereas no hydrogen bonding was suggested for PJ13 and PJ15. These results imply that the existence of the hydrogen bond in the complex has major effects on binding energies. Furthermore, the results concur with the Kᵣ values and binding affinities of AChE or BChE for PJ5, PJ13, or PJ15.

To explain the reason PJ15 inhibits AChE more selectively than PJ5, Van der Waals (VDW) distances and interactions were examined at C16, C17, C18, and C19 (for PJ15) or C21 (for PJ5) atoms in the docked ligands, according to the difference between PJ15 and PJ5, i.e., the 2-methyl-butane and the 2-methyl-buten group, respectively (Figs. 1 and Supplementary Fig. S16). It was predicted that thirteen and five VDW interactions were formed with PJ15 and PJ5, respectively, within a distance of 4 Å (Supplementary Table S1 and S2). The VDW interactions of PJ15 could inhibit AChE more selectively than PJ5.

In molecular dynamics analysis, average root mean square deviation (RMSD) values of PJ5, PJ13, and PJ15 for AChE were estimated to be 0.767, 0.684, and 0.752 Å, respectively, and those for BChE were 0.738, 0.823, 0.757 Å, respectively (Supplementary Figure S19). The results supported well the experimental data and the docking simulations in this study.

In a previous study, it was observed that PJ5, PJ13, and PJ15 were non-toxic up to 10 µg/µL (i.e., ~25 mM) and exhibited potent for anti-inflammatory effects at 10 µg/µL in previous study, which suggests PJ5, PJ13, and PJ15 be considered candidates for the treatment of AD as ChE inhibitors with anti-inflammatory activities.

**Conclusion**

Among the fifteen khellactone coumarin compounds isolated from *P. japonicum* Thunberg, PJ5 and PJ13 were found to potently and effectively inhibited BChE and AChE, respectively. Furthermore, these inhibitors were reversible and caused by mixed inhibition. Molecular docking simulations showed that PJ13 had the highest binding affinity for AChE at −9.3 kcal/mol, and that PJ5 had the highest binding affinity for BChE at −10.0 kcal/mol. These results supported the notion that PJ13 and PJ5 should be considered novel, potent, and selective inhibitors of AChE and BChE, respectively. In addition, our findings suggest that PJ5, PJ13, and PJ15 are non-toxic, reversible AChE and BChE inhibitors and candidates for the treatment of AD.
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Author contributions
J.H.H., B.H.E., and J.E.P. tested biological activities of the compounds and wrote primarily the main manuscript text; H.W.R., D.-Y.K., J.-H.K., and S.-R.O. isolated and wrote the part; M.-G.K. and D.P. analyzed docking data and wrote the part; H.K. reviewed and finalized the manuscript.

Competing interests
The authors declare no competing interests.

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