Tryptamine–Triazole Hybrid Compounds for Selective Butyrylcholinesterase Inhibition

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Received February 23, 2019, Accepted March 29, 2019, Published online April 23, 2019

Tryptamine–triazole hybrid compounds (11–18) were synthesized via click reaction between tryptamine azide and propargylated natural compounds. Their cholinesterase inhibitory activity was evaluated. Among the eight compounds, compound 11 showed the most potent inhibitory activity [IC50 = 0.42 ± 0.29 μM for equine butyrylcholinesterase (BuChE) and 1.96 ± 0.15 μM for human BuChE]. From the molecular modeling study, compound 11 was bound to the catalytic anionic site, anionic subsite, peripheral anionic subsite, acyl-binding pocket, and oxyanion hole of human or horse BuChE by forming a hairpin or U-shaped structure. The Lineweaver-Burk plot of compound 11 against BuChE suggests a mixed type of inhibition which corresponds well with the molecular modeling study.

Keywords: Tryptamine–triazole hybrid compounds, Cholinesterase inhibitory activity, Alzheimer’s disease, Molecular docking calculation, Molecular dynamics simulation

Introduction

For the last 10 years, new drugs have not been introduced to the market for Alzheimer’s disease (AD) treatment. Therefore, strategies are needed to develop drug candidates for AD. There are many targets for AD drugs such as beta-amyloid, tau-protein, cholinesterase, γ- and β-secretase, neurotransmitter systems (serotonergic 5-HT6 and histaminergic H3), etc. Among them, cholinesterase inhibitors based on the cholinergic hypothesis are still the most valuable targets for novel AD drugs. Currently commercially available AD drugs such as donepezil, rivastigmine, and galantamine are targeted primarily to acetylcholinesterase (AChE). However, they are not a complete remedy for AD and have many side effects that require a new type of target for cholinergic hypothesis. The activity of AChE in AD patients is decreases but that of BuChE is increased to 40–90%. When a selective inhibitor of BuChE, such as a cymserine analogue, is applied to rodents, the concentration of acetylcholine (ACh) in the brain increases. Thus, selective inhibition of BuChE may increase ACh levels in AD patients with superior drug candidates.

Synthesis of derivatives of natural or secondary metabolites with physiological activities such as antioxidant, anti-inflammatory, or cytoprotective and verifying that they meet cholinergic hypothesis can be a good strategy for developing AD therapeutic compounds. In this study, we have synthesized derivatives of natural compounds such as lipoic acid, polyphenols, or paeonol with various physiological activities and studied whether they can act as selective BuChE inhibitors.

α-Lipoic acid (ALA) and its reduced form, dihydrolipoic acid, show various physiological activities. ALA is often referred to as an “antioxidant of antioxidants” because of its ability to act as a multifunctional antioxidant against a variety of reactive oxygen species (ROS) and to promote new synthesis of endogenous antioxidants such as glutathione, α-tocopherol, and vitamin C. ALA conjugated compounds such as ALA-tacrin conjugate (Lipocrine; IC50 = 6.96 ± 0.45 nM for AChE) and ALA-triazole-coumarin conjugate (IC50 = 7.8 ± 3.6 μM for BuChE) showed cholinesterase inhibitory activity.

Natural polyphenols (PPs) are known as natural antioxidants by reducing ROS levels in vivo. PP also reduces the inflammation effect on coronary artery disease and slows down the process of wrinkling the skin. Paeonol is a natural compound isolated from Paeonia suffruticosa. Paeonol and its derivatives have many beneficial anticancer, antimicrobial, antimutagenic, anti-inflammatory, and analgesic properties.

Previously, we have reported that a benzoisoxazole-trypamine hybrid compound (1) showed good BuChE...
In this study, compound 1 derivatives were synthesized by converting the benzoisoxazole moiety to biologically active natural compounds. The click reaction between tryptamine azide and propargylated lipoic acid, polyphenols, and paeonol was carried out. We also evaluated the effect of compound 1-derivatives on BuChE inhibition in vitro. Molecular modeling studies using compound 11 in human or horse BuChE have been performed to understand the binding mode.

**Experimental**

**General Methods.** $^1$H NMR and $^{13}$C NMR spectra were recorded on a Varian Mercury 400 (400 MHz) spectrometer (Varian, Palo Alto, California, USA) and mass spectra were taken using an Agilent G1956B mass spectrometer (Agilent, Santa Clara, California, USA). Flash column chromatography was performed using E. Merck silica gel (60, particle size 0.040–0.063 mm) and analytical thin layer chromatography (TLC) was performed using pre-coated TLC plates with silica gel 60 F254 (Merck, Darmstadt, Germany). The following is a general reaction procedure for the synthesis of propargylated derivatives (3–10). EDC (1.5 equiv) was added to the mixture of 2,4-dihydroxyacetophenone or paeonol (1.1 equiv), and Cs$_2$CO$_3$ (1.1 equiv) in acetone at room temperature. The reaction mixture was refluxed for 24 h and water was added. The solution was extracted three times with DCM and dried over anhydrous MgSO$_4$. The solvent was removed in vacuo and the residues were purified by silica gel column chromatography (DCM; EA: Hex = 4:1) to give the propargylated compound (3–10).

**General Procedure (B).** The following is a general reaction procedure for the synthesis of propargylated derivatives (5 and 6). Propargyl bromide (1.5 equiv) was added to the mixture of 2,4-dihydroxyacetophenone or paeonol (1.1 equiv), and Cs$_2$CO$_3$ (1.1 equiv) in acetone at room temperature. The reaction mixture was refluxed for 24 h and water was added. The solution was extracted three times with DCM and dried over anhydrous MgSO$_4$. The solvent was removed in vacuo and the residues were purified by silica gel column chromatography (DCM; EA: Hex = 1:6) to give propargylated compounds (5 and 6), respectively.

**General Procedure (C).** The following is a general reaction procedure for the synthesis of compound (1)-derivatives by the click reaction using a microwave reactor. Tryptamine azide (2, 1.1 equiv) was prepared by treating tryptamine with imidazole sulfonyl azide, which was added to a solution of propargylated compounds (3–10, 1.0 equiv) in acetone in the presence of Cu(PPh$_3$)$_3$Br (0.1 equiv). The reaction mixture was reacted in a microwave reactor (Model: Initiator Exp Eu, Sweden). Unless otherwise noted, the reagents used were reagent grade microwave reactor (Model: Initiator Exp Eu, Sweden).

**General Synthetic Procedure**

**General Procedure (A).** The following is a general reaction procedure for the synthesis of propargylated derivatives (3–10). EDC (1.5 equiv), hydroxybenzotriazole (HOBT, 0.1 equiv), and propargylamine (1.1 equiv) were added at room temperature to a dichloromethane (DCM) solution of the carboxylic acids. The reaction mixture is stirred at room temperature for 4 h and then water is added. The solution was extracted three times with DCM and dried over anhydrous MgSO$_4$. The solvent was removed in vacuo and the residue was purified by silica gel column chromatography (DCM; EA = 4:1) to give the propargylated compound (3–10).

**General Procedure (B).** The following is a general reaction procedure for the synthesis of propargylated derivatives (5 and 6). Propargyl bromide (1.5 equiv) was added to the mixture of 2,4-dihydroxyacetophenone or paeonol (1.1 equiv), and Cs$_2$CO$_3$ (1.1 equiv) in acetone at room temperature. The reaction mixture was refluxed for 24 h and water was added. The solution was extracted three times with DCM and dried over anhydrous MgSO$_4$. The solvent was removed in vacuo and the residues were purified by silica gel column chromatography (DCM; EA: Hex = 1:6) to give propargylated compounds (5 and 6), respectively.

**General Procedure (C).** The following is a general reaction procedure for the synthesis of compound (1)-derivatives by the click reaction using a microwave reactor. Tryptamine azide (2, 1.1 equiv) was prepared by treating tryptamine with imidazole sulfonyl azide, which was added to a solution of propargylated compounds (3–10, 1.0 equiv) in acetone in the presence of Cu(PPh$_3$)$_3$Br (0.1 equiv). The reaction mixture was reacted in a microwave reactor at 65 °C for 30 min and then water was added. The solution was extracted three times with DCM and dried over anhydrous MgSO$_4$. The solvent was removed in vacuo and the tryptamine derivatives (11–18) were purified using silica gel column chromatography (DCM: MeOH = 9:1).

\[ (R)-N-\{(1-2-(1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl) methyl\}-5-(1,2-dithiolan-3-yl)pentanamide \] (11). Click reaction between compounds 3 and 2 resulted in compound 11 with 89% yield via general procedure (C).

**Compound 3:** White solid; yield: 85%; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.43 (m, 2H), 1.64 (m, 4H), 1.87 (m, 1H), 2.16 (m, 3H), 2.41 (m, 1H), 3.27 (m, 2H), 3.76 (m, 1H), 4.01 (m, 2H), 5.56 (s, 1H).

**Compound 11:** Yellow oily product; yield: 89%; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.38 (m, 2H), 1.62 (m, 4H), 1.85 (m, 1H), 2.12 (t, $J$ = 7.2 Hz, 2H), 2.43 (m, 1H), 3.09 (m, 2H), 3.33 (t, $J$ = 6.8 Hz, 2H), 3.51 (m, 1H), 4.38 (d, $J$ = 5.6 Hz, 2H), 4.60 (t, $J$ = 6.8 Hz, 2H), 6.15 (s, 1H), 6.87 (s, 1H), 7.08 (t, $J$ = 8.0 Hz, 1H), 7.18 (t, $J$ = 8.0 Hz, 1H), 7.19 (s, 1H), 7.36 (d, $J$ = 8.0 Hz, 1H), 7.43 (t, $J$ = 8.0 Hz, 1H), 8.24 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 25.3, 26.5, 28.8, 34.5, 34.8, 36.0, 38.4, 40.2, 50.9, 56.4, 110.6, 111.6, 118.1, 119.3, 122.0, 122.8, 122.9, 126.9, 136.3, 144.5, 173.2; ESI-MS: $m$/z [M]+ 460.63 (calcld 460.1).

\[ (R)-1-4-(1,2-dithiolan-3-yl)butyl)-3-(1-(2-(1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl)pentanamide \] (12). Compound...
was synthesized by Curtius rearrangement of lipic acid using diphenyl phosphorazidate (1.0 equiv) and propargyl amine. Click reaction between compound 4 and 2 resulted in compound 12 with 89% yield via general procedure (C).

Compound 4: White solid; yield: 87%; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.45 (m, 4H), 1.65 (m, 3H), 1.89 (m, 1H), 2.18 (s, 1H), 2.43 (m, 1H), 3.07 (m, 2H), 3.19 (m, 2H), 3.50 (m, 1H), 3.94 (m, 2H), 4.65 (bs, 1H), 4.73 (bs, 1H).

Compound 12: Yellow oily product; yield: 89%; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 1.30 (m, 3H), 1.48 (m, 1H), 1.60 (m, 1H), 1.81 (m, 1H), 2.34 (m, 1H), 2.93 (m, 2H), 3.06 (m, 1H), 3.11 (m, 1H), 3.18 (t, $J$ = 7.6 Hz, 2H), 3.54 (m, 4H), 4.14 (d, $J$ = 5.6 Hz, 2H), 4.54 (t, $J$ = 7.6 Hz, 2H), 5.88 (t, $J$ = 5.6 Hz, 1H), 6.17 (t, $J$ = 5.6 Hz, 1H), 6.93 (t, $J$ = 7.4 Hz, 1H), 7.02 (t, $J$ = 7.4 Hz, 1H), 7.05 (d, $J$ = 2.4 Hz, 1H), 7.28 (d, $J$ = 8.0 Hz, 1H), 7.49 (d, $J$ = 8.0 Hz, 1H), 7.65 (m, 1H), 7.85 (s, 1H), 10.82 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 21.61, 25.16, 25.20, 29.27, 29.73, 30.64, 33.25, 35.28, 34.52, 45.99, 51.75, 105.72, 106.82, 113.26, 114.61, 117.24, 118.14, 121.97, 125.08, 128.53, 131.48, 153.97; ESI-MS: $m/z$ [M]$^+$ 445.1 (calcd 445.1).

1-4-((1-(2-(1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl) methoxy)-2-hydroxyphenyl)ethene (13). Click reaction between compounds 5 and 2 resulted in compound 13 with 79% yield via general procedure (C). Analytical data can be found in Ref. 17.

1-(1-((2-(1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl) methoxy)-4-methoxyphenyl)ethene (14). Click reaction between compounds 6 and 2 resulted in compound 14 with 92% yield via general procedure (C). Analytical data can be found in Ref. 17.

4-((1-(2-(1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl) methylcarbamoyl)-2,6-dimethoxyphenyl acetate (15). Click reaction between compound 7 and 2 resulted in compound 15 with 70% yield via general procedure (C). Analytical data can be found in Ref. 17.

(E)-N-((1-(2-(1H-indol-3-yl)ethyl)-1H-1,2,3-triazol-4-yl) methyl)-3-(3,4-dimethoxyphenyl)acrylamide (16). Click reaction between compounds 8 and 2 resulted in compound 16 with 85% yield via general procedure (C).

Compound 8: Analytical data can be found in Ref. 14.

Compound 16: White solid; yield: 85%; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 3.19 (t, $J$ = 7.6 Hz, 2H), 3.73 (s, 6H), 4.35 (d, $J$ = 2.4 Hz, 2H), 4.55 (t, $J$ = 7.6 Hz, 2H), 6.49 (d, $J$ = 15.6 Hz, 1H), 6.93 (s, $J$ = 4.0 Hz, 1H), 6.94 (s, 1H), 7.02 (t, $J$ = 7.6 Hz, 1H), 7.05 (d, $J$ = 12.0 Hz, 1H), 7.06 (s, 1H), 7.08 (d, $J$ = 12.0 Hz, 1H), 7.28 (d, $J$ = 7.6 Hz, 1H), 7.39 (d, $J$ = 15.6 Hz, 1H), 7.49 (d, $J$ = 7.6 Hz, 1H), 7.95 (s, 1H), 8.42 (t, $J$ = 2.4 Hz, 1H), 10.83 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ 26.5, 34.8, 50.3, 55.8, 56.0, 110.5(2C), 111.9, 112.2, 118.6, 118.9, 120.1, 121.5, 121.7, 123.3, 126.3, 127.3, 128.1, 136.6, 139.5, 145.1, 149.3, 150.6, 165.6; ESI-MS: $m/z$ [M]$^+$ 432.49 (calcd 432.2); mp: 160 °C.

Cholinesterase Assay. Measurements of inhibitory activity against AcChE and BuChE were performed using the Ellman method.18

Homology Modeling. Amino acid sequence for Equus caballus BuChE (horse BuChE, accession no: P89190) was obtained from UniProtKB (http://www.uniprot.org/). Template structure was searched on PSI-BLAST and sequence alignment with the selected template was performed using Discovery Studio (DS) 2016 (BIOVIA, San Diego, CA, USA). The homology model structure was generated by
using the Build Homology Models protocol available in DS. The energy minimization for the model structure was performed using GROMACS 5.0.6 package with CHARMM 27 force field. The steepest descent algorithm was used and the maximum number of minimization steps was set to 10,000. The stereochemical quality of the predicted structure was checked by PROCHECK\(^{19}\) ProSA (Protein Structure Analysis),\(^{20}\) and ERRAT.\(^{21}\)

**Molecular Docking Calculation.** Molecular docking calculation of 11 to BuChE was performed using Genetic Optimization for Ligand Docking (GOLD) version 5.2.\(^{22}\) The X-ray crystal structure of human BuChE (PDB ID: 4AQD)\(^{23}\) from the RCSB Protein Data Bank (www.rcsb.org) and the homological model structure of horse BuChE were used. All co-crystal water and ligand molecules were removed from the structure. The protonation states of all titratable residues in the protein were set to pH 7 using Clean Protein tool in DS 2016. The 3D structure of 11 was prepared by DS. The geometry of the compound was optimized by energy minimization through Minimize Ligands tool in DS. All atoms within 15 Å from the center of the binding site were defined as docking sites. The number of docking runs was set to 100. The most populated conformations were selected for further investigation.

**Molecular Dynamics Simulation.** Molecular dynamics (MD) simulation of the protein-ligand complex was performed with AMBER03 force field\(^{24}\) using GROMACS 5.0.6 package. The complex structure obtained from the molecular docking calculation was used as the initial structure for MD simulation. The ligand topology was generated by ACPYPE (AnteChamber Python Parser interface).\(^{25}\) The complex structure was immersed in a water box of TIP3P water molecules\(^{26}\) to make an aqueous environment. Counter-ions were added to neutralize the system and energy minimization with steepest descent algorithm was carried out until the maximum force converged to less than 1000 kJ/mol. The system was then subjected to 100 ps NVT equilibration followed by 100 ps NPT equilibration. Finally, a 10 ns production run was carried out under NPT ensemble. A constant temperature of 300 K and a pressure of 1 bar was controlled by Nose–Hoover thermostat\(^{27}\) and Parrinello–Rahman barostat,\(^{28}\) respectively. All bond lengths and the geometry of water molecules were restrained by LINCS\(^{29}\) and SETTLE algorithms,\(^{30}\) respectively. Cut-off values for short-range electrostatic and van der Waals interactions were 1.2 nm while long-range electrostatic interactions were calculated using particle mesh Ewald method.\(^{31}\) The simulation was performed under periodic boundary conditions to avoid edge effects. The time step of the simulation was 2 fs and the coordinates were saved to trajectory files every 1 ps.

**Results**

**Chemistry.** The structures of tryptamine azide (2) and propargylated derivatives (3–10) of lipoic acid, polyphenols, and paeonol used in the click reactions are shown in Figure 2. The representative triazole-linked tryptamine derivatives 11–18 were, respectively, synthesized by the click reactions between tryptamine-azide (2) and the propargyl compounds 3–10 using a microwave reactor (Scheme 1). All tryptamine hybrid compounds synthesized in this work are listed in Figure 3.

**Inhibitory Results (IC\(_{50}\) Value).** The inhibitory effects (IC\(_{50}\) value) of tryptamine derivatives on AChE and BuChE are shown in Table 1 and galantamine was used as a control.

![Figure 2](image_url)  
**Figure 2.** The structures of tryptamine azide and propargylated compounds prepared for click reaction.

![Scheme 1](image_url)  
**Scheme 1.** Synthesis of triazole-linked tryptamine hybrid compounds by click reaction.
positive control compound. The starting compounds (tryptamine, lipoic acid, paenol, syringic acid, ferulic acid, and caffeic acid) did not show any inhibitory effect for cholinesterases (data not shown). All tryptamine hybrid compounds had the same pattern as compound 1. They selectively inhibited BuChE compared to AChE.

The Binding Mode of Compound 11 in Horse BuChE.

Homology Modeling of Horse (Equus caballus) BuChE. A homology model of horse BuChE was constructed using the 3D structure of human BuChE as a template (PDB ID: 4AQD). Sequence alignment displayed that horse BuChE was highly homologous to human BuChE with a sequence identity of 90.2% and the similarity of 94.3% (Figure 4(a)).

The structural alignment of horse BuChE to the template showed that the overall tertiary structures are very similar with a root-mean-square deviation (RMSD) of 0.16 Å for the backbone atoms in the proteins (Figure 4(b)). They shared the same active site residues, except that Ala277 of human BuChE is replaced by Val277 in horse BuChE. The homology model was subjected to energy minimization to refine intramolecular contacts followed by the assessment of its stereochemical quality. Ramachandran plot from PROCHECK revealed that 91.2% of the residues were in the most favored regions, 8.4% were in additional allowed regions, and only two residues were found in disallowed regions (Figure 4(c)). The ProSA Z-Score value of -10.6 was within the range of scores typically found for native proteins of similar size. Moreover, a quality factor score obtained from ERRAT was 95.93, which represents a high quality model (Figure 4(d)). As a result, the quality of our homology model for horse BuChE was evaluated using three different criteria. These results indicated that the model structure is reliable for further modeling studies.

Binding Conformation of 11 at the Active Site of Horse BuChE.

Molecular docking of 11 to horse BuChE was also performed. The GOLD fitness score for all the generated 100 poses ranged from 59.08 to 82.1. The result showed that 62 conformations were similar to that of human BuChE. The top-scored conformation was selected as a starting structure for MD simulation. To check the stability of the system, RMSD and potential energies were measured during the 10 ns simulation time. The Cα RMSD value for the protein and the potential energies were constant and remained less than 0.15 nm and −1 030 000 kJ/mol, respectively (Figure 5(a) and (b)). Structural analysis revealed that the binding mode of 11 in horse BuChE was analogous to that of human BuChE (Figure 5(c) and (d)).

Table 1. ChE inhibition IC₅₀ values for tryptamine hybrid compounds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>LogP value</th>
<th>AChE inh. IC₅₀(µM)</th>
<th>BuChE inh. IC₅₀(µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal.</td>
<td>1.41</td>
<td>0.64 ± 0.1</td>
<td>8.4 ± 0.1</td>
</tr>
<tr>
<td>1</td>
<td>3.88</td>
<td>91.30 ± 0.97</td>
<td>0.72 ± 0.11</td>
</tr>
<tr>
<td>11</td>
<td>3.32</td>
<td>&gt;480</td>
<td>0.42 ± 0.29</td>
</tr>
<tr>
<td>11ᵇ</td>
<td>&gt;480</td>
<td>1.96 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>±12</td>
<td>2.98</td>
<td>&gt;480</td>
<td>7.69 ± 0.82</td>
</tr>
<tr>
<td>13</td>
<td>2.48</td>
<td>229.8 ± 1.26</td>
<td>&gt;450</td>
</tr>
<tr>
<td>14</td>
<td>2.75</td>
<td>&gt;450</td>
<td>5.49 ± 0.41</td>
</tr>
<tr>
<td>15</td>
<td>2.16</td>
<td>&gt;450</td>
<td>9.04 ± 0.62</td>
</tr>
<tr>
<td>16</td>
<td>2.92</td>
<td>&gt;450</td>
<td>&gt;450</td>
</tr>
<tr>
<td>17</td>
<td>2.63</td>
<td>&gt;450</td>
<td>41.79 ± 1.30</td>
</tr>
<tr>
<td>18</td>
<td>2.34</td>
<td>&gt;450</td>
<td>&gt;450</td>
</tr>
</tbody>
</table>

ᵇ AChE (from human serum) and BuChE (from human serum) were used.
⁻ BuChE (from human serum). IC₅₀ values represent the inhibitor concentration necessary to decrease enzyme activity by 50% and are calculated using the mean of the triplicate measurements.
⁻ LogP values were calculated by using ChemDraw Program.
Binding Conformation of 11 at the Active Site of Human BuChE.

Molecular docking calculation was performed to explore the binding mode of 11 in the active site of human BuChE. From the top cluster including 48 out of 100 docking poses, a pose with a GOLD fitness score of 80.45 was selected as a representative pose of the compound based on their molecular interactions (Figure 6). The binding conformation of 11 showed that the indole group of the compound was headed for Trp82 and the end of lipoamide group was toward to Ser198. The complex structure was subjected to MD simulation in order to alleviate unfavorable contacts and check the binding stability. Prior to trajectory analysis, the RMSD for the Cα atoms in the protein and the potential energy of the system were calculated. The RMSD was converged within 0.15 nm during 10 ns MD simulation (Figure 7(a)). The potential energy was maintained at about $-1075000 \text{ kJ/mol}$ (Figure 7(b)). Both plots showed that there were no abnormal behaviors in the structure during the entire simulation time. A snapshot with the lowest potential energy was taken as a representative structure for further analysis. Compound 11 bound to human BuChE by forming a hairpin or U-shaped structure in the active site (Figure 7(c) and (d)).

Discussion

Compound 11 exhibited slightly higher inhibitory activity against BuChE (from horse serum, IC$_{50}$ = 0.42 ± 0.29 μM) than compound 1. The IC$_{50}$ value (1.96 ± 0.15 μM) was a little higher for the BuChe originating from human serum compared to horse serum. Molecular modeling study for BuChE originating from both human and horse were also done on compound 11.

Lipoic acid transferred to compound 4, which has one less carbon, through Curtius rearrangement reaction quenching with propargyl amine. The click reaction between 4 and 2 resulted in compound 12 (IC$_{50}$ = 7.69 ± 0.82 μM). Compound 11, which has an amide functional group, had an IC$_{50}$ value 10 times less than compound 12, which has an urea functional group.

Compounds 13 and 14 are derivatives of paeonol. When anti-inflammatory activity was measured using compounds 13, 14, and 15, compound 13 and 14 independently inhibited NO (nitric oxide) production in BV2 cells. However, compound 15 suppressed NO generation to a minimum. This means that the paeonol moiety plays an important role in the anti-inflammatory effect. Starting with....

Figure 4. Homology modeling for horse BuChE. (a) Sequence alignment between horse and human BuChEs. (b) Superimposed structure of homology model (green) and template (gray) of BuChE. The catalytic triad of horse BuChE was displayed as gray stick models. (c) Ramachandran plot of the backbone dihedral angles (φ and ψ) for the model structure of horse BuChE. (d) ProSA plot of model structure. The Z-score of −10.6 was represented as a black dot.
compound 5 was synthesized from the selective propargylation between 2,4-dihydroxyacetophenone and propargyl chloride, which replaced the methyl group of 4-methoxy group of paeonol with a propargyl group. Starting compound 6 was obtained from the propargylation of paeonol. Compounds 13 and 14 were synthesized by the
click reactions between compounds 5 or 6 and tryptamine azide, respectively. Compound 13 exhibited weak inhibitory activity against AChE, but there was no inhibitory activity against BuChE. Compound 14 showed moderate inhibitory activity against BuChE only (IC₅₀ = 5.49 ± 0.41 μM). Compounds 15–18 coupled between tryptamine azide and propargylated polyphenols such as acetyl syringic acid, 3,4-dimethoxy cinnamic acid, acetyl ferulic acid, and diacetyl caffeic acid showed moderate or no inhibitory activity against BuChE. This may be due to the size effect of polyphenol. Compound 15 has the benzoic acid moiety, but compounds 16–18 have the cinnamic acid moiety. The size of cinnamic acid is bigger than that of benzoic acid. When anti-inflammatory activity was measured using compounds 13, 14, and 15, compound 13 has the benzoic acid moiety, but compounds 16–18 have the cinnamic acid moiety. The size of cinnamic acid is bigger than that of benzoic acid. When anti-inflammatory activity was measured using compounds 13, 14, and 15, compound 13 showed moderate inhibitory activity against BuChE only (IC₅₀ = 5.49 ± 0.41 μM). Compounds 15–18 showed similar inhibitory effects on LPS-induced NO production, while compound 15 had little anti-inflammatory activity. However, compound 14 was less cytotoxic than 13.¹⁷ In the measurement of cholinesterase inhibitory activity using these compounds, compound 13 weakly inhibited AChE, while compounds 14 and 15 selectively inhibited BuChE.

Previous we synthesized hybrid molecules between (R)-lipoic acid (ALA) and acetylated or methylated polyphenol compounds and evaluated their in vitro cholinesterases [acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)] inhibitory activity.³² In ALA-polyphenol compounds, ALA-acetylated caffeic acid compounds showed the highest BuChE inhibitory activity, while in the tryptamine derivatives acetylated syringic acid derivative showed the highest activity. In ALA-polyphenol compounds, activity was analyzed using various QSAR parameters [hydrophobicity parameter (π), Hammett electronic substituent constant (σ m & σ p), and molar refractivity (MR)].³²

In the tryptamine derivative, the size of the propargyl moiety and the position of its functional group appear to be important for anti-inflammatory activity and cholinesterase inhibitory activity.

The binding mode of compound 11 was analyzed. It was observed that the indole group interacted with Trp82 and Tyr332 though π–π stacking interactions with horse BuChE. The middle triazole ring also formed π–π stacking with Phe329. The lipoamide group was stabilized by hydrophobic interactions with the active site residues including Glu197, Ser198, Trp231, Leu285, Leu286, and Val288. The middle NH moiety and carbonyl group in lipoamide group made hydrogen bonds with Leu285 and Gln119, respectively. Furthermore, the sulfur atom in a terminal dithiolane ring of lipoamide group formed hydrogen bond interactions with Gly116 and Gly117. The middle amide group of compound 11 was toward the outside, and indole...
and lipoamide groups at both ends were buried inside of human BuChE. The indole group of compound 11 was placed near a catalytic anionic site (CAS) and anionic subsite (AS). It showed hydrophobic interactions with Trp82 and Tyr128 on the AS and His438 on the CAS. The NH group of the indole ring formed a hydrogen bond with the carboxyl group of Glu197. On the other hand, the lipoamide group was positioned within the pocket surrounded by residues that belong to the peripheral anionic subsite (PAS), acyl binding pocket (ABP), and oxanion hole (OH). It formed hydrophobic interactions with Gln119 and Tyr332 on PAS, Leu286, Val288, and Trp231 on ABP, as well as Gly116 and Gly117 on OH subsites. Additionally, a terminal dithiolane ring in the lipoamide group was involved in sulfur–π interactions with Phe329 and Phe398. The middle NH moiety and sulfur atom in the dithiolane ring were hydrogen bonded to the backbone carbonyl group of Pro285 and the hydroxyl group of Ser198, one of the catalytic triad, respectively.

A kinetic study of BuChE was performed at different concentrations of compound 11 to explore inhibition mechanisms (Figure 8), and the Lineweaver–Burk plot suggested a mixed type of inhibition. The mixed type of inhibition corresponds well with the molecular modeling study, in which compound 11 was bound to the CAS, AS, PAS, ABP, and OH.

**Conclusion**

In conclusion, a series of tryptamine–triazole hybrid compounds 11–19 were synthesized via click reactions between tryptamine azide and propargylated derivatives. They generally showed butyrylcholinesterase inhibitory activity. Compound 11 showed the most potent inhibitory activity (IC$_{50}$ = 0.42 ± 0.29 for equine BuChE and 1.96 ± 0.15 for human BuChE) among the 10 compounds and its inhibitory mechanism against BuChE was a mixed type of inhibition.

In order to investigate the binding mode of compound 11 to the active site of human and horse BuChE, molecular docking and MD simulation was performed using human BuChE determined by crystal structure and horse BuChE generated by homology modeling. The results indicate that the binding pattern of compound 11 is almost identical in both structures. Compound 11 forms hydrogen bond interactions with Glu197, Ser198, and Pro285 in human BuChE while compound 11 interacts with Gly116, Gly117, Gln119, and Leu285 in horse BuChE. The detailed structural analysis demonstrates that compound 11 is bound to the active site by forming various molecular interactions with the residues across all the subsites in both human and horse BuChEs. Taken together, our molecular modeling studies provide valuable information to design potential inhibitors of BuChE.

**Acknowledgments.** This research was supported by the Next-Generation BioGreen 21 Program (PJ01106202) from Rural Development Administration (RDA) of Republic of Korea and by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A1B04028757).

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