Supporting Information

New 1,2,3-Triazole-genipin Analogues and Their Anti-Alzheimer’s Activity

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Molecular docking

To perform the docking of the compounds, saved in pdb format and the energy was minimized using AutoDock 4.2. The molecular docking was performed using AutoDock 4.2. To rationalize the observed structure–activity relationship and identify the binding interactions between the synthesized compound with the target enzymes BuChE (PDB code: 4BDS). The water molecules were removed from these structures and the addition of hydrogen atoms. The target enzymes BuChE were docked to experimentally synthesized compounds using AutoDockTools-1.5.7. The search space of 60 x 60 x 60 in x, y, and z dimensions was used for enzymes BuChE centered on the binding site of protein. The docking results were then visualized using Discovery Studio Visualizer.

Commonly, the docking configuration of the target compound was basically consistent with that of the target enzyme. 1,4-Disubstituted genipin-triazole (8a-10) showed a good fit in the pocket site of the enzyme by interaction with important amino acid residues and exhibited a binding free energy of -9.77 kcal/mol with BuChE. In the binding mode, the carbonyl group of acetoxy formed hydrogen bonds with the Trp82 (a key residue in the CAS of BuChE) and the triazole group also formed a hydrogen bond interaction with the Tyr332 as a key residue in the PAS region. On the other hand, the binding of 1,5-disubstituted genipin-triazole (8a-10′) to target enzyme BuChE formed only hydrogen bonds at key residue in the CAS region but was not binding at key residue in the PAS region (Figure S1). Also, 1,5-disubstituted genipin-triazole (8a-10′) showed binding energy higher than 1,4-disubstituted (8a-10) and was calculated as -7.15 kcal/mol. Therefore, 1,4-disubstituted genipin-triazole (8a-10) is a suitable regioisomer for interaction with the target enzyme BuChE.

Figure S1 Proposed binding mode of compound 8a-10 compared with 8a-10′ in the active site of BuChE (PDB code: 4BDS).
Measurement of cell viability using MTT assays

To investigate the neuroprotective activity of all 1,2,3-triazolegenipin based compounds 8a and 8b, we used an MTT assay to explore cell viability. SK-N-SH cells were cultured in a 96-well plate at a density of 5 x 10^5 cells/mL for 24 h at 37 °C in a CO₂ incubator. The cells were pretreated with 0.075, 0.15, 0.3 or 0.6 µM of 1,2,3-triazole-genipins 8a and 8b for 2 h and then treated in the presence or absence of 250 µM H₂O₂. After 24 h of incubation, 100 µL of MTT solution (10 mg/mL) was added to each well and incubated at 37 °C for 2 h. The medium was aspirated and 100 µL dimethylsulfoxide (DMSO) was then added to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader (Bio-tek, Instruments, Winoaski, VT, USA).
$^1$H and $^{13}$C NMR spectra of 10-triazolyl-genipin analogues

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