**Searching for New GSK3 Kinase Inhibitors for Treatment of Alzheimer’s Disease: Computer Modeling, Enzyme and Cell Assays**

Chia-Jen Hsu †, Wen-Chi Hsu†, An-Lun Liu† , Chia-Ming Chang†, Chih-Hsin Lin‡, **Ying-Chieh Sun**†,\*, Guan-Chiun Lee‡,\* , Guey-Jen Lee-Chen‡,\*

*Department of Chemistry† and Department of Life Science‡, National Taiwan Normal University, 88, Tingzhou Road Sec. 4, Taipei 116, Taiwan*

Email: sun@ntnu.edu.tw

Glycogen synthase kinase 3β (GSK-3β) is widely known as a critical target protein for treating Alzheimer’s disease (AD). We utilized virtual screening to search databases for compounds with the potential to be used in drugs targeting GSK-3 kinase, and kinase as well as cell assays to investigate top-scored, selected compounds. Virtual screening of more than 1.1 million compounds in the ZINC and in-house databases was conducted using an optimized computational protocol in the docking program GOLD. Of the top-ranked compounds, 16 underwent a luminescent kinase assay and a cell assay using HEK293 cells expressing DsRed-tagged ΔK280 in the repeat domain of tau (tauRD). The compounds COMP-003 and COMP-008, with determined IC50 values of 0.25 and 5.4 μM, respectively, were identified as reducing tau aggregation. Both compounds increased expression of phospho-GSK-3β (Ser9) and reduced endogenous tau phosphorylation at the sites of Ser202, Thr231, and Ser396. In the ∆K280 tauRD-DsRed SH-SY5Y cells, COMP-008 enhanced HSPB1 and GRP78 expression, increased ∆K280 tauRD-DsRed solubility, and promoted neurite outgrowth. These compounds may guide the identification and synthesis of potential inhibitors analogous to these compounds and may be useful for further animal testing.

In addition, high-accuracy MD simulation was employed to compute relative binding free energy of ligand-GSK3 complexes for 10 analogous ligands to a reference ligand. 3 of them, analogs **8**-**10**, underwent kinase assay as well. The measurements returned IC50 values of 61 nM, 65 M, and 0.4 M, respectively. Examination of structures from simulations showed all 3 analogs have hydrogen bonds (h-bond) with Val135 of GSK3 kinase, which is a fingerprint h-bond of inhibitor-kinase complexes located within the hinge segment. The predicted binding modes should aid in designing the derivatives of these compounds in order to enhance binding affinity in the future.

□ Oral presentation

□ Poster presentation