Usage Report for Fiscal Year 2020

Project Title: Computational Structure-based Design of Protein Inhibitors

Name:

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1. Background and purpose of the project, relationship of the project with other projects

Alzheimer's disease (AD), a common chronic neurodegenerative disease, has become a major public health concern. Despite years of research, therapeutics for AD are limited. Overexpression of secretory glutaminyl cyclase (sQC) in AD brain leads to the formation of a highly neurotoxic pyroglutamate variant of amyloid beta, pGlu-Aß, which acts as a potential seed for the aggregation of full length Aß. Preventing the formation of pGlu-Aß through inhibition of sQC has become an attractive disease-modifying therapy in AD. We aim at discovering potent inhibitors of sQC and using them to develop therapeutics for treating AD.

2. Specific usage status of the system and calculation method

We have used a hierarchical virtual screening protocol to discover inhibitors of sQC from a library of chemicals in the Namiki-Shoji collection. We first generated a pharmacophore query from a known inhibitor, PDB-150 and used it to screening around 4 million compounds and selected 902,037 molecules. Applying Lipinski rules and custom filtering have further reduced the selected compounds to 333,214. Subsequently, three different docking methods, high-throughput virtual screen (HTVS), standard precision (SP), and extra precision (XP), were used not only to assess the binding modes but also to score the compounds, which have further reduced the number compounds to 183. We then used MM-GBSA calculations to estimate the binding free energy of these compounds to sQC and selected 54 compounds to be purchased for enzymatic assay.

3. Result

The enzymatic assay for the selected compounds at 100 µM have revealed that 8 of them are active and 6 of them having amide as their metal binding group (MBG). Further investigations showed that one compound, Cpd-41, has moderate activity against sQC (IC_{50} = 33.9 ± 5.1 µM). No inhibitor with amide as their MBG has yet been reported against sQC. Cytotoxicity evaluation using human neuroblastoma cell line RCB2108 NH-12 has shown that Cpd-41 has very low cytotoxicity even at 68 µM concentration. We performed 100 ns of MD simulations to critically assess the binding mode, affinity, and residence time of Cpd-41 in the active site of sQC. Furthermore, we have determined the crystal structure of the complex of Cpd-41 with sQC and found that crystallographic binding mode of Cpd-41 is fairly similar to the docked pose, except for the orientation of the thiazole and thiophene moieties.

4. Conclusion

We have discovered a novel sQC inhibitor (Cpd-41) with a piperidine-4-carboxamide moiety through our pharmacophore assisted high throughput virtual screening. The docking, MD simulation and crystallographic studies suggested that Cpd-41 anchors to the active site via a coordinate bond with Zn^{2+} ion located deep in the active site cleft of sQC. Based on the potential interactions made by Cpd-41 at the active site of sQC, it may be inferred that Cpd-41 prevents the entry of the substrate to the active site and makes the catalytic residues unavailable for the enzymatic reactions. The moderate toxicity of Cpd-41 even at higher

concentration (68 μM) makes the compound an attractive candidate for future studies.

5. Schedule and prospect for the future

Based on the binding modes of Cpd-41 in the theoretical and experimental structures and B-factor of Cpd-41 in the crystal structure, we propose that replacing piperidine with a pyridine moiety may not only maintain the cation-π interactions but also introduce additional stacking interactions with W207 and W329, which may improve the affinity of the inhibitor.

As revealed by our B-factor analysis, the flexibility of the Cpd-41 is high for the propyl, thiazole and thiophene moieties. Although there is a propyl moiety in the PBD-150, the flexibility is lower because it is trapped inside the active site and movement is impeded by the surrounding protein atoms. On the other hand, the propyl moiety in Cpd-41 is highly flexible because it is oriented towards the bulk solvent and there is no interaction with the protein atoms. Hence, we assume that by restricting the flexibility of this propane motif through substitution with bioisosteres may improve the affinity.

6. If no job was executed, specify the reason.

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Fiscal Year 2020 List of Publications Resulting from the Use of the supercomputer

[Paper accepted by a journal]

1. Janezic, M., Dileep, K. V., Zhang, K. Y. J. (2021) A multi-dimensional computational exploration of congenital myasthenic syndrome causing mutations in human choline acetyltransferase. *J. Cell. Biochem.*, https://doi.org/10.1002/jcb.29913.
2. Remya, C., Dileep, K. V., Variyar, E. J., Zhang, K. Y. J., Omkumar, R. V., Sadasivan, C. (2021) Chemical similarity assisted search for acetylcholinesterase inhibitors: Molecular modeling and evaluation of their neuroprotective properties. *Int. J. Biol. Macromol.*, **174**, 466-476. https://doi.org/10.1016/j.ijbiomac.2021.01.148.
3. Dileep, K. V., Sakai, N., Ihara, K., Kato-Murayama, M., Nakata, A., Ito, A., Sivaraman, D. M., Shin, J. W., Yoshida, M., Shirouzu, M., Zhang, K. Y. J. (2021) Piperidine-4-carboxamide as a new scaffold for designing secretory glutaminyl cyclase inhibitors. *Int. J. Biol. Macromol.*, **170**, 415-423. https://doi.org/10.1016/j.ijbiomac.2020.12.118.

[Conference Proceedings]

[Oral presentation]

[Poster presentation]

[Others (Book, Press release, etc.)]