Usage Report for Fiscal Year 2021

Project Title: Computational Structure-based Design of Protein Inhibitors

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

Nematode chitinases play crucial roles in various processes of the nematode lifecycle, including hatching, molting and reproduction. Small-molecule inhibitors of nematode chitinases have shown promise for controlling nematode pests. However, only limited nematicides are currently available and most of them are confronted with the growing resistance problem, which further necessitates the need to develop novel anti-nematode agents. Chitin, a naturally synthesized homopolymer of N-acetyl-β-D-glucosamine (GlcNAc), is known to be present in the eggshell, cuticle, pharynx and microfilarial sheath of nematodes. Because chitin is absent in vertebrates and plants, enzymes associated with chitin metabolism, such as chitinase, might be potential targets for the development of novel nematicides. We aim to discover novel and potent nematode chitinase inhibitors by computational structure-based drug design method for the control of agricultural pests.

2. Specific usage status of the system and calculation method

In order to maximize our chances to identify *Ce*Cht1 inhibitors, hierarchical virtual screening was carried out using two separate virtual screening strategies. In the first strategy, a combined ligand and structure based virtual screening approach was followed. In the second virtual screening strategy, a purely structure-based virtual screening approach was followed and no inhibitor information from the nematode chitinases was used. A subset of commercially available compounds from ZINC database was docked to *Ce*Cht1 employing a two-step molecular docking protocol. The docking predicted binding mode of compound in complex with *Ce*Cht1 was used as input for the molecular dynamics (MD) simulation using Desmond program (Schrodinger Inc.). The protein ligand system for the MD simulation was prepared using the "system builder" utility of Maestro (Schrodinger Inc.). Molecular dynamics trajectories were processed and analyzed using Maestro.

3. Result

Evaluation of *Ce*Cht1 enzymatic activity at a concentration of 20 μM revealed two compounds with moderate inhibitory activity. Among these, compound **BP1** with a 3-(*N*-(benzo[*d*]thiazol-2-yl)acetamido) -*N*,*N*-dimethylpropan-1-aminium scaffold showed the most potent inhibitory activity, with a K_i value of 11.3 μM. It was interesting to note that the most potent compound **BP1** came from virtual screening strategy 2, where no nematode chitinase inhibitor information was used. Compound 14 that showed moderate *Ce*Cht1 inhibitory activity with a K_i value of 30.5 μM was identified from virtual screening strategy 1 using the most potent compound from 4-hydroxy-1,2,3-triazoles[20] scaffold as the query for ligand-based virtual screening. Out of these compounds, we decided to proceed with compound **BP1**, not only because it is more potent but also compounds with this scaffold have not been previously described to possess activity against any chitinase. To identify compounds with an improved *Ce*Cht1 inhibition, a third round of screening was performed using compound **BP1** as the starting structure. A set of compounds (**BP2**–**BP12**) were identified and most of these compounds showed improved inhibitory activity over the starting compounds.

4. Conclusion

We identified a series of novel inhibitors by hierarchical virtual screening. Analysis of the structure–activity

relationships of these compounds provided insight into their interactions with the enzyme active site, which may inform future work in improving the potencies of their inhibitory activities. This work gives an insight into the structural features of nematode chitinases, and provides a solid basis for the development of inhibitors.

5. Schedule and prospect for the future

We plan to further improve the potency and selectivity of the **BP** series of inhibitors using computational structure-based drug design paradigm. We will first computationally screen for analogs in the chemical library and test their inhibitory activities. Using the structure–activity relationships of these compounds, we will computationally design novel compounds to be chemically synthesized and tested against *Ce*Cht1.

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

Fiscal Year 2021 List of Publications Resulting from the Use of the supercomputer

[Paper accepted by a journal]

1. Rajpoot, S., Solanki, K., Kumar, A., Zhang, K. Y. J., Pullamsetti, S. S., Savai, R., Faisal, S. M., Pan, Q., Baig, M. S. (2022) In-silico design of a novel tridecapeptide targeting spike protein of SARS-CoV-2 variants of concern. *Int. J. Pept. Res. Ther.*, **28**, 28. https://doi.org/10.1007/s10989-021-10339-0.
2. Remya, C., Dileep, K. V., Reddy, E. K., Mantosh, K., Lakshmi, K., Jacob, R. S., Sajith, A. M., Variyar, E. J., Anwar, S., Zhang, K. Y. J., Sadasivan, C., Omkumar, R. V. (2021) Neuroprotective derivatives of tacrine that target NMDA receptor and acetyl cholinesterase - Design, synthesis and biological evaluation. *Comput. Struct. Biotechnol. J.*, **19**, 4517–4537. https://doi.org/10.1016/j.csbj.2021.07.041.
3. Chen, W., Chen, Q., Kumar, A., Jiang, X., Zhang, K. Y. J., Yang, Q. (2021) Structure-based virtual screening of highly potent inhibitors of the nematode chitinase CeCht1. *J. Enzyme Inhib. Med. Chem.*, **36**, 1198-1204. https://doi.org/10.1080/14756366.2021.1931862.
4. Muhammad, E. F., Kumar, A., Wahab, H. A., Zhang, K. Y. J. (2021) Identification of 1,2,4-triazolylthioethanone scaffold for the design of new acetylcholinesterase inhibitors. *Molecular Informatics*, **40**, 2100020. https://doi.org/10.1002/minf.202100020.
5. Berenger, F., Kumar, A., Zhang, K. Y. J., Yamanishi, Y. (2021) Lean-Docking: Exploiting Ligands' Predicted Docking Scores to Accelerate Molecular Docking. *J. Chem. Inf. Model.,* **61**, 2341−2352. https://doi.org/10.1021/acs.jcim.0c01452.
6. Chen, Q., Chen, W., Kumar, A., Jiang, X., Janezic, M., Zhang, K. Y. J., Yang, Q. (2021) Crystal structure and structure-based discovery of inhibitors of the nematode chitinase CeCht1. *J. Agric. Food Chem.*, **69**, 3519−3526. https://dx.doi.org/10.1021/acs.jafc.1c00162.
7. Balan, S., Iwayama, Y., Ohnishi, T., Fukuda, M., Shirai, A., Yamada, A., Weirich, S., Schuhmacher, M. K., Vijayan, D. K., Endo, T., Hisano, Y., Kotoshiba, K., Toyota, T., Otowa, T., Kuwabara, H., Tochigi, M., Watanabe, A., Ohba, H., Maekawa, M., Toyoshima, M., Sasaki, T., Nakamura, K., Tsujii, M., Matsuzaki, H., Zhang, K. Y. J., Jeltsch, A., Shinkai, Y., Yoshikawa, T. (2021) A loss of function variant in SUV39H2 identified in autism spectrum disorder causes altered H3K9-trimethylation and dysregulation of protocadherin ß cluster genes in the developing brain. *Molecular Psychiatry*, https://doi.org/10.1038/s41380-021-01199-7.

[Conference Proceedings]

[Oral presentation]

[Poster presentation]

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