Usage Report for Fiscal Year 2022

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

GH18 chitinases hydrolyze ß-1,4-glycosidic bonds in chitin and chitooligosaccharides. As chitin is present in the eggshell, cuticle, pharynx and microfilarial sheath of nematodes, nematode chitinases have been shown to play an important role in various physiological processes, including egg hatching, larva molting, and reproduction. Downregulating the expression level of nematode chitinases led to hatching delay and molting defects in many nematode species. The importance of nematode chitinases indicates that they may be promising nematicide targets for the development of small-molecule inhibitors for nematode pest control. Many GH18 chitinase inhibitors with diverse scaffolds have been reported so far, and some showed potential applications as antifungal agents, pesticides, and drugs. However, the inhibition of nematode chitinases is rarely studied, and only few inhibitors have been reported to be effective on nematode chitinases. We aim to discover novel and potent inhibitors of chitinase from nematode *Caenorhabditis elegans* (*Ce*Cht1) by computational structure-based drug design method for the control of agricultural pests.

2. Specific usage status of the system and calculation method

A hierarchical virtual screening strategy was used for the discovery of *Ce*Cht1 inhibitors. At first, structural analogs to active hits were identified from a subset of commercially available compounds from ZINC database employing substructure search and shape similarity calculations. Substructure search was performed using OEChem toolkit (OpenEye Scientific Software). Shape similarity calculations were performed using ROCS. The conformational database used for shape similarity calculations was prepared using OMEGA. Compounds in the screening library were scored using "TanimotoCombo" score. Structural analogs were then prioritized for the evaluation of *Ce*Cht1 inhibitory activity using molecular docking. The crystal structure of *Ce*Cht1-CAD was used for molecular docking calculations. The protein structure for molecular docking was prepared using Maestro (Schrodinger Inc.), where all water molecules were removed, hydrogens were added and protonation states of all charged residues were assigned at neutral pH. Ligands for molecular docking were prepared using LigPrep (Schrodinger Inc.). Tautomeric and ionization states of all ligands were determined using Epik program at neutral pH. Molecular docking was performed using Glide program in extra precision mode. Grids for molecular docking calculation were prepared by including the catalytic residues and residues in both "+" and "-" GlcNAc binding subsites. Ligands were scored using Glidescore with Epik penalties and a single pose per compound was saved.

3. Result

Screening of an in-house collection of compounds resulted in the identification of several compounds that showed moderate *Ce*Cht1 inhibitory activity. Among these compounds, there were three compounds bearing a similar (R)-3,4-diphenyl-4,5-dihydropyrrolo[3,4-c]pyrazol-6(2H)-one scaffold, and the best one (**PP3**) inhibited *Ce*Cht1 with a K_i value of 38.3 µM. As compounds with this scaffold have not been previously described

to possess activity against any chitinase, we decided to proceed with compound **PP3**. To identify compounds with improved *Ce*Cht1 inhibition, a hierarchical virtual screening was performed. Initially, structural analogs were identified employing substructure search and shape similarity calculations using compound **PP3** as the starting structure. Finally, molecular docking was used to prioritize compounds for the evaluation of *Ce*Cht1 inhibitory activity. A set of compounds (**PP4**–**PP26**) were identified, and most of these compounds showed improved inhibitory activity over the starting compound. **PP7** is the most potent among these compounds, with a K_i value of 0.76 μM. We performed another round of virtual screening and obtained several derivatives (**PP27**-**PP32**). The inhibitory activity analysis showed that four compounds were better than **PP7** (**Table 3**). Among these, compound **PP28** exhibited the most potent activity with a K_i value of 0.18 μM, which was a 4-fold increase than that of **PP7**.

4. Conclusion

We have identified a series of *Ce*Cht1 inhibitors bearing a novel scaffold. Structure–activity relationship analyses and crystallographic studies clearly elucidated the inhibitory mechanism of these compounds. The crystal structures of enzyme-inhibitor complexes provided clues to develop compounds with improved inhibitory activity. This work presents an efficient strategy, which combined computational and experimental studies, to discover potent inhibitors. In addition, this work may promote further development of nematicides to deal with the increasing damages caused by nematode pests.

5. Schedule and prospect for the future

We plan to further improve the potency and selectivity of the **PP** 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.

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Fiscal Year 2022 List of Publications Resulting from the Use of the supercomputer

[Paper accepted by a journal]

1. Okuda, K., Nakahara, K., Ito, A., Iijima, Y., Nomura, R., Kumar, A., Fujikawa, K., Adachi, K., Shimada, Y., Fujio, S., Yamamoto, R., Takasugi, N., Onuma, K., Osaki, M., Okada, F., Ukegawa, T., Takeuchi, Y., Yasui, N., Yamashita, A., Marusawa, H., Matsushita, Y., Katagiri, T., Shibata, T., Uchida, K., Niu, S.-Y., Lang, N. B., Nakamura, T., Zhang, K. Y. J., Lipton , S. A., Uehara, T. (2023) Pivotal role for S-nitrosylation of DNA methyltransferase 3B in epigenetic regulation of tumorigenesis. *Nat. Commun.*,**14**:621. https://doi.org/10.1038/s41467-023-36232-6.
2. Dileep, K. V., Ihara, K., Mishima-Tsumagari, C., Kukimoto-Niino, M., Yonemochi, M., Hanada, K., Shirouzu, M., Zhang, K. Y. J. (2022) Crystal structure of human acetylcholinesterase in complex with tacrine: implications for drug discovery. *Int. J. Biol. Macromol.*, **210**, 172-181. https://doi.org/10.1016/j.ijbiomac.2022.05.009.