Usage Report for Fiscal Year 2019

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

Glycosyl hydrolase family 18 chitinases catalyze the degradation of chitin, a homopolymeric form of *β*-(1,4)-linked *N*-acetyl-D-glucosamine (GlcNAc). Chitinases play essential roles in the growth and development of chitin-containing organisms as well as immunologically defensive roles in non-chitin containing organisms such as human and plants. Human contains two chitinases, chitotriosidase (Chit1) and acidic mammalian chitinase (AMCase). A number of studies indicate that elevation of Chit1 in plasma or tissues is associated with several diseases. Small molecule drug-like inhibitors of human chitinases played significant part in understanding their biological role and implications of inhibiting these proteins. However, investigations on the role of Chit1 and/or AMCase in immune response are much more challenging.

Via hierarchical virtual screening, we have discovered a series of chitinase inhibitors with a novel scaffold that have high inhibitory activity and selectivity against human and insect chitinases. We further demonstrated the in vivo efficacy of most potent human Chit1-selective inhibitor using a murine model of bleomycin-induced pulmonary fibrosis, which suggested a protective role of Chit1 in inflammation under experimental conditions.

2. Specific usage status of the system and calculation method

Structure-based virtual screening (SBVS) was carried out following a hierarchical virtual screening protocol. Initially, a library of about four million commercially available compounds was filtered based on their shape and electrostatic potential similarities with three units of *β*-1,4-linked *N*-acetylglucosamine, $(GlcNAc)_3$. The bioactive conformation of $(GlcNAc)_3$ was used as a query for shape and electrostatic comparisons (PDB code: 3WL1). The shape similarity calculations were performed using ROCS program. Ligand conformations for shape similarity calculations were generated employing Omega program. The 1000 top-ranked compounds in their ROCS aligned conformations were then subjected to electrostatic potential comparisons using the EON program. The top ranking 500 compounds were then selected for further prioritization using molecular docking based on their interactions with the active site. Molecular docking was performed using Glide program. Ligands for molecular docking were prepared using LigPrep from Schrodinger. Ionization and tautomeric states of compounds were generated at a target pH of 7.0 ± 2.0 using Epik program. Protein structure for molecular docking was prepared using protein preparation utility in Maestro (Schrodinger Inc.). Grid for molecular docking was generated using the coordinates of $(GlcNAc)_3$ bound to *Of*Cht1 (PDB code: 3WL1). Glide 'docking score', which is a Glide score with Epik penalties, was used as a scoring function to rank-order compounds.

3. Result

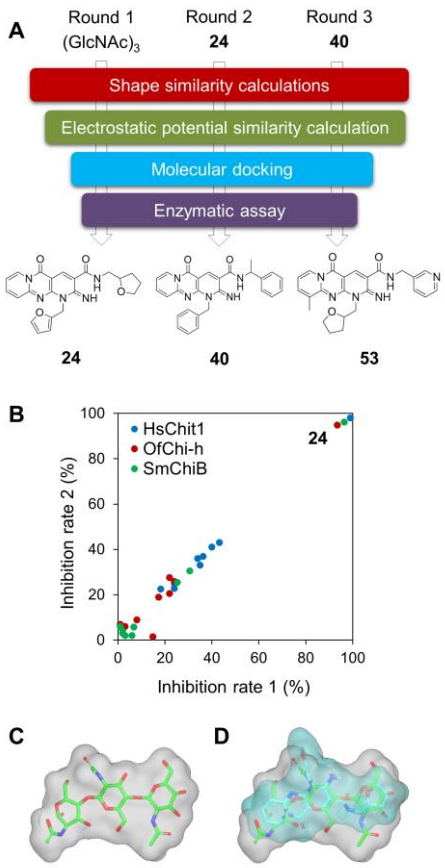
Structure-based virtual screening facilitated the identification of dipyrido-pyrimidine based scaffold that exhibited inhibitory activity against chitinases from various species such as human, insect and bacterium.

The most potent human chitotriosidase inhibitor,

compound **40** exhibited a K_i of 49 nM and the most potent inhibitor of the insect pest chitinase *Of*Chi-h, compound **53** exhibited a K_i of 9 nM. The binding of these two most potent inhibitors was confirmed by X-ray crystallography. In a murine model of bleomycin-induced pulmonary fibrosis, compound **40** was found to suppress the chitotriosidase activity by 60%, leading to a significant increase in inflammatory cells.

Among the dipyrido-pyrimidine derivatives, **40** was found to be the most potent *Hs*Chit1 inhibitor and to the best of our knowledge, **40** is the most potent synthetic *Hs*Chit1 inhibitor reported to date.

Furthermore, **40** demonstrated high selectivity against hAMCase and is the most selective *Hs*Chit1 inhibitor known to date. Recently, a highly potent inhibitor of mouse Chit1 (**17**) with 143-fold selectivity against mAMCase was also reported, but its inhibitory effect on *Hs*Chit1 is not known. Dipyrido-pyrimidine based scaffold possessed broad chitinase inhibitory activity and also inhibited chitinases from insect pests and bacteria with high potency. Compound **53** was especially interesting inhibiting *Of*Chi-h and *Sm*ChiB with potency in the low nM range. Importantly, this compound was only a weak inhibitor of *Hs*Chit1, making it an excellent candidate for agrochemical development.



4. Conclusion

By utilizing a combination of in silico, in vitro and in vivo experiments, we have discovered and validated a chemical scaffold, which potently inhibited chitinases from human, insect and bacterium. Combining X-ray crystallography and computational modeling, we further revealed the molecular basis of inhibition and selectivity. The identified scaffold can be utilized to develop highly efficient and selective molecular tools to regulate chitinase which is vital in human immunity, pathogenic fungi and pest insect development.

5. Schedule and prospect for the future

Although **40** is the most selective inhibitors of HsChit1 over hAMCase, there is significant inhibitory activity against hAMCase. To understand the role of HsChit1 in human inflammation, we plan to design a potent HsChit1 inhibitor that is completely devoid of any inhibition towards hAMCase. Furthermore, our selectivity profiling of **40** against a panel of human proteins revealed that it inhibits hERG and PDE4D2. We plan to design

derivatives of **40** to remove their off-target activities. Although **40** doesn't violate Lipinski's rule of five, properties such as molecular weight, log P are towards the higher end and should be either reduced or maintained during optimization. Efforts should also be made to improve the solubility of compounds for oral bioavailability. Safety is also the most important factor in the development of pesticides and future development efforts should be focused on improving the selectivity towards insect chitinase while avoiding any activity against human chitinase and other human and animal proteins.

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

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Fiscal Year 2019 List of Publications Resulting from the Use of the supercomputer

[Paper accepted by a journal]

1. Jiang, X., Kumar, A., Motomura, Y., Liu, T., Zhou, Y., Moro, K., Zhang, K. Y. J., Yang, Q. (2020) A series of compounds bearing a dipyrido-pyrimidine scaffold acting as novel human and insect pest chitinases inhibitors. *J. Med. Chem.*, doi:10.1021/acs.jmedchem.9b01154.
2. Nair, H. B., Santhamma, B., Dileep, K.V., Binkley, P., Acosta, K., Zhang, K. Y. J., Schenken, R., Nickisch, K. (2019) EC313-a tissue selective SPRM reduces the growth and proliferation of uterine fibroids in a human uterine fibroid tissue xenograft model. *Sci. Rep.*, **9**, 17279. doi:10.1038/s41598-019-53467-w.
3. Kumar, A., Zhang, K. Y. J. (2019) Improving ligand 3D shape similarity-based pose prediction with a continuum solvent model. *J. Comput-Aided Mol. Des.*, **33**, 1045-1055. doi:10.1007/s10822-019-00220-0.
4. Viswanadhapalli, S., Luo, Y., Sareddy, G. R., Santhamma, B., Zhou, M., Li, M., Ma, S., Sonavane, R., Pratap, U. P., Altwegg, K. A., Li, X., Chang, A., Chávez-Riveros, A., Dileep, K. V., Zhang, K. Y. J., Pan, X., Murali, R., Bajda, M., Raj, G. V., Brenner, A., Manthati, V., Rao, M., Tekmal, R. R., Nair, H. B., Nickisch, K. J., and Vadlamudi, R. K. (2019) EC359-A first-in-class small molecule inhibitor for targeting oncogenic LIFR signaling in triple negative breast cancer. *Mol. Cancer Ther.*, **18**, 1341-1354. DOI:10.1158/1535-7163.MCT-18-1258.

[Conference Proceedings]

[Oral presentation]

1. Computational and Mathematical Bioinformatics and Biophysics (CMBB2019), Dec. 9-13, 2019, Sanya, China, Invited Speaker, “Computational Structure Based Drug Design Using Ligand 3D Shape Similarity”.

[Poster presentation]

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

1. Vijayan, D. K. and Zhang, K. Y. J. (2019) Human glutaminyl cyclase: Structure, function, inhibitors and involvement in Alzheimer’s disease. *Pharmacol. Res.* **147**, 104342. doi:10.1016/j.phrs.2019.104342.
2. Kumar, A., Zhang, K. Y. J. (2019) Human Chitinases: Structure, Function, and Inhibitor Discovery. In: Yang, Q. and Fukamizo, T. (eds.), *Targeting Chitin-containing Organisms*, pp. 221–251. Singapore: Springer Nature. doi:10.1007/978-981-13-7318-3_11