Screening of P450 Enzyme Inhibitors for Controlling Multiple-Herbicide-Resistant Weeds Using Molecular Docking and Molecular Dynamics Simulation

Natchaphon Fo-ud[1] , Kowit Hengphasatporn[2*] and Pattarasuda Chayapakdee[3*]

[1] Student special project in B.Sc. (Biology) student code 640510325, Email : natchaphon_foud@cmu.ac.th
[2*] Center for Computational Sciences University of Tsukuba, Email : kowith@ccs.tsukuba.ac.jp
1-1-1 Tennodai, Tsukuba 305-8577, Japan
[3*] Lecturer Dr., Department of Biology, Faculty of science, Chiang Mai University, Email : pattarasuda.c@cmu.ac.th
239 Huay Kaew Road, Mueang District, Chiang Mai, Thailand, 52000

University of Tsukuba
Center for Computational Sciences

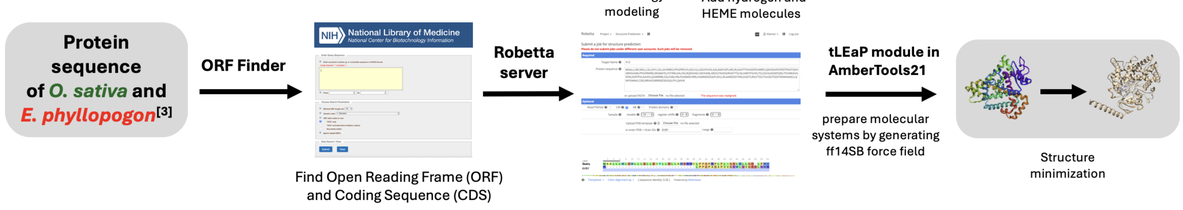
FACULTY OF SCIENCE
CHIANG MAI UNIVERSITY

Abstract

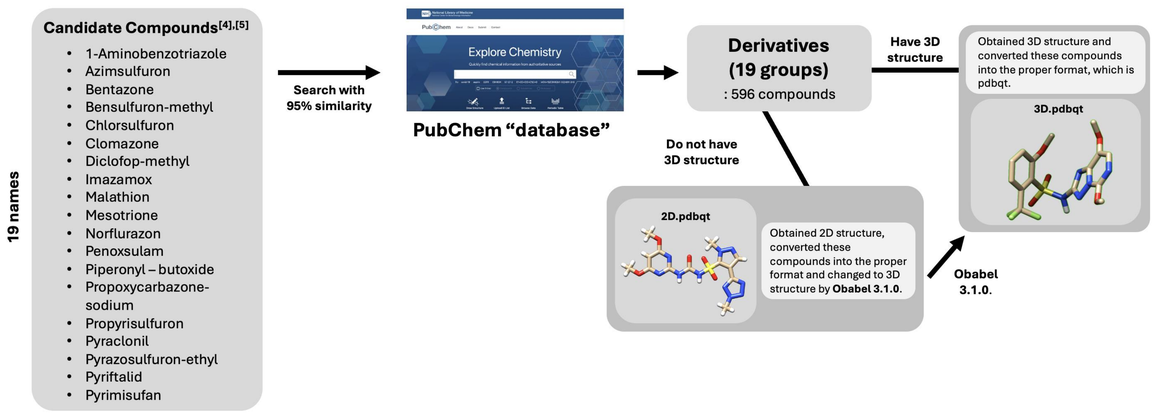
Cytochrome P450 monooxygenase enzymes (CYP81A) play a crucial role in herbicide detoxification in plants, including rice crop (*Oryza sativa*) and late watergrass weed (*Echinochloa phyllopogon*)[1]. Since CYP81As contribute to multiple herbicide resistance in late watergrass, inhibiting their function could enhance herbicide effectiveness, leading to targeted plant mortality after herbicide application in rice cultivation. This research aimed to identify a novel selective inhibitor targeting CYP81As in late watergrass. First, the 3D structures of CYP81A6 from rice, CYP81A12, A21, and A24 from late watergrass were constructed *in silico* from their amino acid sequences due to the absence of experimentally determined structures. A set of 596 compounds was selected from the PubChem database based on 95% similarity to 19 reported substrates with high binding preference for these CYP81As and was then subjected to molecular docking analysis using AutoDock Vina 1.2.3. The top five potent compounds identified through molecular docking results and binding specificity analysis were two derivatives of penoxsulam, along with derivatives of propyrisulfuron, pyraclonil, and pyrazosulfuron-ethyl. These top-ranking inhibitors were further analyzed using molecular dynamics simulations to evaluate their binding efficiency and stability. Among them, ethyl1-[(4,6-dimethoxypyrimidin-2-yl) carbamoyl]-3-sulfamoylpyrazole-4-carboxylate exhibited the most promising interaction with CYP81A12, A21, and A24 of late watergrass while showing minimal interaction with CYP81A6 of rice, suggesting highly selective inhibitory properties. Based on these findings, the identified compound may serve as a potential candidate for future herbicide development.

Methodology

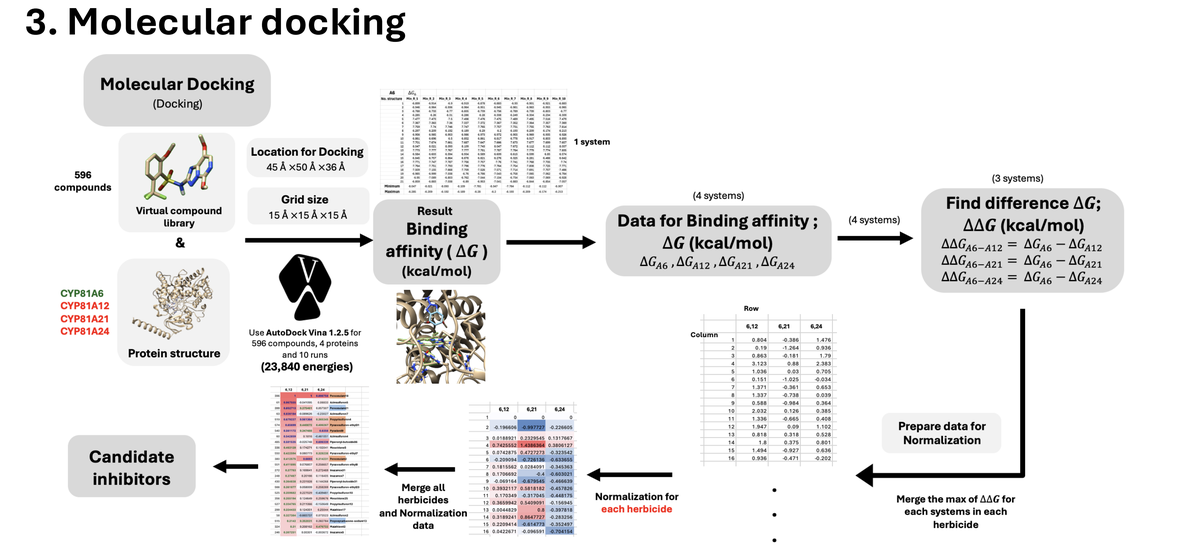
1. Protein structural modeling



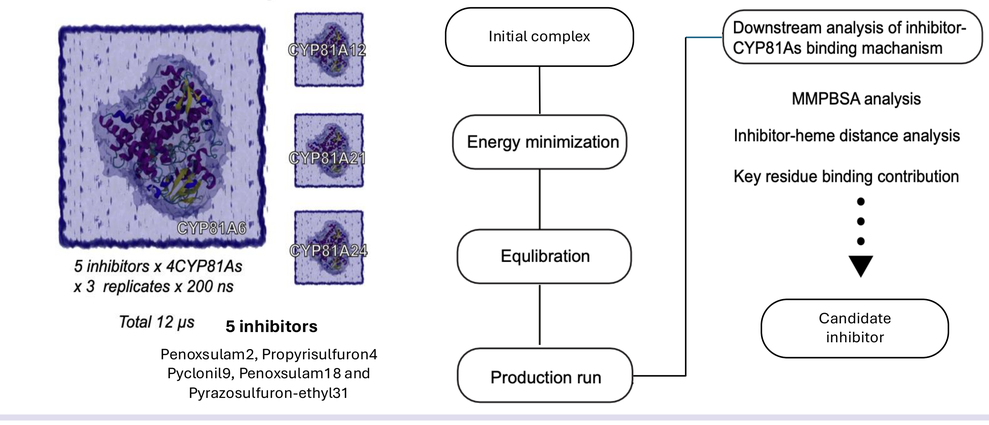
2. Virtual library preparation



3. Molecular docking



4. Molecular dynamics simulation



Result and Discussion

Molecular docking

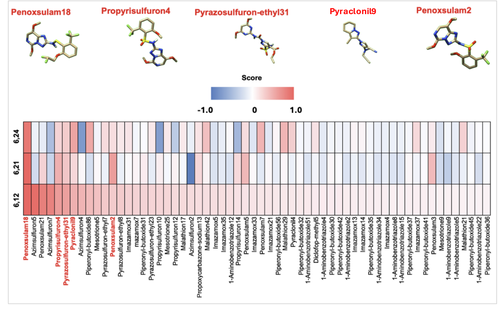


Figure 1 Heatmap of molecular docking results. The score was judged by the lower binding energy and the probability of CYP81As and inhibitor formation.

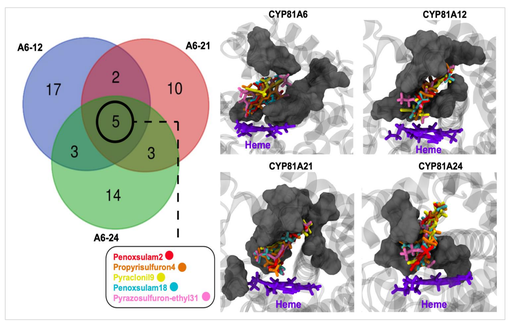


Figure 2 Venn diagram showing the overlap of inhibitor compounds based on their binding energies to the crop (CYP81A6) and weed enzymes (CYP81A12, A21, A24), including both the binding affinity and the orientation of the inhibitor in the active sites of CYP81As.

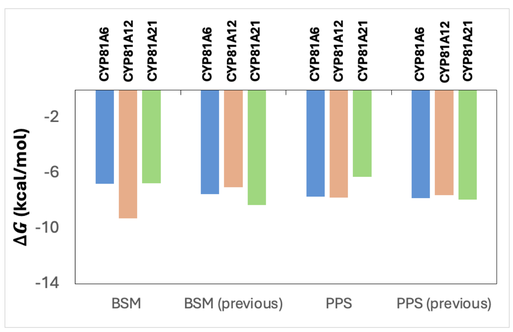


Figure 3 Comparison of binding affinity of this research and previous research[2], which show the similarity of binding free energy in CYP81As – BSM (Bensulfuron-methyl) and CYP81As – PPS (Propyrisulfuron) complex.

Molecular dynamics simulation (MD simulation)

Figure 4 Result of MD simulation when five compound inhibitors and four CYP81As were performed with three times by GROMACS which used NPT ensemble, pressure 1.0 bar, temperature 300K and 200 ηs

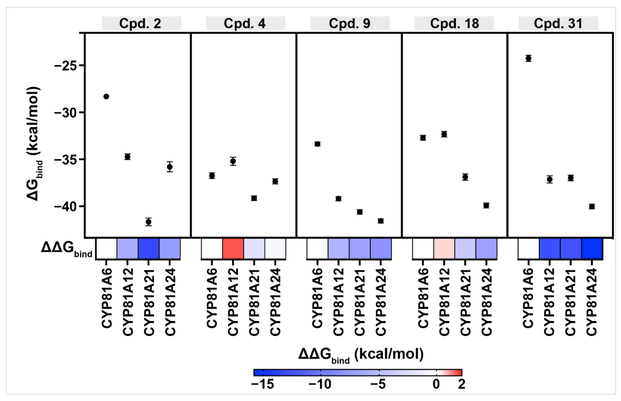


Figure 5 The calculated binding free energies (kcal/mol) using MM/GBSA for the CYP81As - inhibitor complexes. Only the last 40 ns snapshots are calculated.

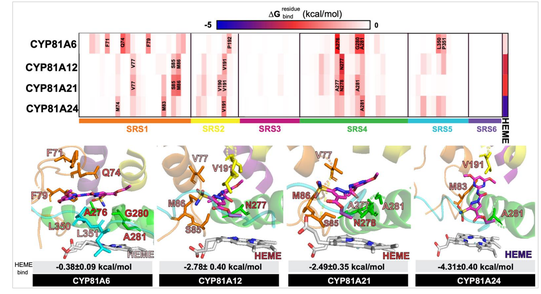


Figure 6 The residues in six substrate recognition sites (SRS1-6) and heme binding free energy with Pyrazosulfuron-ethyl31. SRS representative color is displayed (red—SRS1, pink—SRS2, blue—SRS3, green—SRS4, yellow—SRS5 and orange—SRS6). The Pyrazosulfuron-ethyl31 and heme are represented in the licorice model. Colored according to atom type: cyan—carbon; blue—nitrogen; red—oxygen; yellow—sulfur. The binding free energy value of heme to Pyrazosulfuron-ethyl31 is shown.

Conclusion

Ethyl1-[(4,6-dimethoxypyrimidin-2-yl)carbamoyl]-3-sulfamoylpyrazole-4-carboxylate (Cpd. 31), a pyrazosulfuron-ethyl derivative, exhibited strong binding specificity to weed CYP81As while showing minimal interaction with the rice enzyme, indicating its potential to selectively disrupt herbicide detoxification in weeds without harming crops.

Acknowledgements

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Reference

[1] Guo F, Iwakami S, Yamaguchi T, Uchino A, Sunohara Y, Matsumoto H. Role of CYP81A cytochrome P450s in clomazone metabolism in Echinochloa phyllopogon. 2019;283:321–8. Available from: https://www.sciencedirect.com/science/article/pii/S0168945218311142

[2] Ha, W., Yamaguchi, T., Iwakami, S., Sunohara, Y. and Matsumoto, H. (2022), Comparison of herbicide specificity of CYP81A cytochrome P450s from rice and a multiple-herbicide resistant weed, Echinochloa phyllopogon. Pest Manag Sci 78 : 4207-4216.

[3] Iwakami, S., Endo, M., Saika, H., Okuno, J., Nakamura, N., Yokoyama, M., Watanabe, H., Toki, S., Uchino, A., & Inamura, T. (2014). Cytochrome P450 CYP81A12 and CYP81A21 Are Associated with Resistance to Two Acetolactate Synthase Inhibitors in Echinochloa phyllopogon. *Plant physiology*, *165*(2), 618–629. Available from: https://doi.org/10.1104/pp.113.232843

[4] Oliveira, M. C., Gaines, T. A., Dayan, F. E., Patterson, E. L., Jhala, A. J., & Knezevic, S. Z. (2018). Reversing resistance to tembotrione in an Amaranthus tuberculatus (var. rudis) population from Nebraska, USA with cytochrome P450 inhibitors. *Pest management science*, *74*(10), 2296–2305. Available from: https://doi.org/10.1002/ps.4697

[5] Dimaano, N. G., Yamaguchi, T., Fukunishi, K., Tominaga, T., & Iwakami, S. (2020). Functional characterization of cytochrome P450 CYP81A subfamily to disclose the pattern of cross-resistance in Echinochloa phyllopogon. *Plant molecular biology*, *102*(4-5), 403–416. Available from: https://doi.org/10.1007/s11103-019-00954-3