Division of Biochemistry
– Chemistry of Molecular Biocatalysts –

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Scope of Research

Plant hormones are a group of small molecules that are synthesized by plants and control their growth, development and environmental responses. This laboratory aims at elucidating how plant hormones are made and act in plants. Towards this goal, we combine chemical (organic chemistry, biochemistry, and analytical chemistry) and biological (molecular genetics, physiology, molecular biology, genomics) approaches. We are also looking for new hormone-like compounds by using mutant plants that show morphological phenotypes.

KEYWORDS

Plant Hormone
Strigolactone
Biosynthesis
Cytochrome P450
Receptor

Selected Publications

Watanabe, B.; Kirikae, H.; Koeduka, T.; Takeuchi, Y.; Asai, T.; Naito, Y.; Tokuoka, H.; Horoiwa, S.; Nakagawa, Y.; Shimizu, B.; Mizutani, M.; Hiratake, J., Synthesis and Inhibitory Activity of Mechanism-Based 4-Coumaroyl-CoA Ligase Inhibitors, *Bioorg. Med. Chem.*, **26,** 2466-2474 (2018).

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Yao, J.; Mashiguchi, K.; Scaffidi, A.; Akatsu, T.; Melville, K. T.; Morita, R.; Morimoto, Y.; Smith, S. M.; Seto, Y.; Flematti, G. R.; Yamaguchi, S.; Waters, M. T., An Allelic Series at the *KARRIKIN INSENSITIVE 2* Locus of *Arabidopsis thaliana* Decouples Ligand Hydrolysis and Receptor Degradation from Downstream Signaling, *Plant J.*, **96,** 75-89 (2018).

Fujikura, U.; Jing, R.; Hanada, A.; Takebayashi, Y.; Sakakibara, H.; Yamaguchi, S.; Kappel, C.; Lenhard, M., Variation in Splicing Efficiency Underlies Morphological Evolution in Capsella, *Dev. Cell*, **44,** 192-203 (2018).

Determination of a Key Residue of γ-Glutamyl Transpeptidase for Substrate Recognition

γ-Glutamyl transpeptidase (GGT) plays a central role in homeostasis of antioxidant tripeptide glutathione, and has been implicated in a vast array of physiological disorders. In this study, we synthesized a series of mechanism-based GGT inhibitors to probe electrostatic interactions between the acceptor site residues of GGT and substrates. Our chemical, enzymological, and molecular biological approaches revealed that 3-hydroxyphenylacetic acid is an excellent mimic of the cysteinylglycine moiety of glutathione, and Lys562 of human GGT strongly recognizes their negative charge on the carboxy group (Figure 1). We demonstrated that this interaction considerably enhances the human GGT specificity of our inhibitor named GGsTop. GGsTop exhibited no inhibitory activity at 10 mM on a representative member of glutamine-dependent amidotransferases essential for a wide range of biosynthetic pathway, and showed no cytotoxicity toward human fibroblasts and hepatic stellate cells up to 1 mM.

Substrate-Analog Fructosyl Peptide Oxidase Inhibitors

Fructosyl peptide oxidase (FPOX) is widely used in the area of diabetes diagnosis today. In this study, we designed and synthesized its substrate-analog inhibitors in order to unveil the substrate recognition mechanism of FPOX by X-ray diffraction analysis of enzyme-inhibitor co-crystals. Kinetic study revealed that our substrate analogs act as competitive inhibitors with K_i values ranging from 11.1 to 782 μM (Figure 2). Co-crystallization of the enzyme with our inhibitors in order to determine the three-dimensional structure of FPOX is now in progress.

Molecular Mechanism of Myocardin-Related Transcription Factor A Inhibitors

Myocardin-related transcription factor A (MRTF-A) plays a pivotal role in epidermal-msenchymal transition. Inhibition of its nuclear transport is regarded as one of the attractive therapeutic targets since MRTF-A is closely associated with cancer and tissue fibrosis. In this study, we revealed that CCG-1423, originally developed as a Rho inhibitor, binds to the nuclear localization signal of MRTF-A and inhibits its nuclear transport mediated by importin-α/β1 (Figure 3). We also demonstrated that CCG-1423 inhibits migration of melanoma cells triggered by MRTF-A activation, and the potency is affected by the stereochemistry of CCG-1423. The difference is elucidated by the binding manner of each stereoisomer to MRTF-A that speculated by a molecular modeling approach.

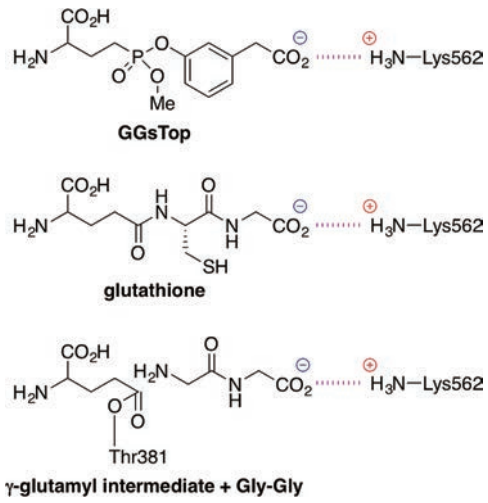

Figure 1. Proposed binding mode of GGsTop, glutathione, and acceptor substrate (Gly-Gly) to Lys562.

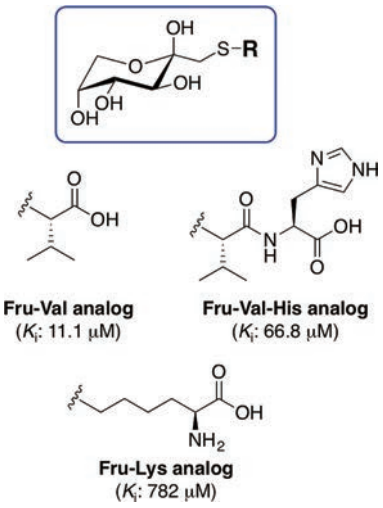

Figure 2. Chemical structures and inhibitory activity of FPOX inhibitors.

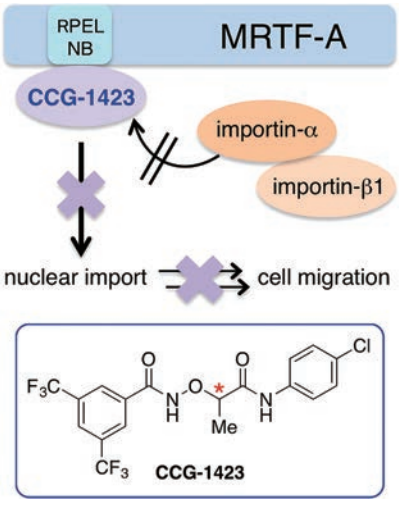

Figure 3. Molecular mechanism of MRTF-A inhibitor CCG-1423.