Division of Biochemistry
– Chemistry of Molecular Biocatalysts –

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Scope of Research

Life is the integration of numerous chemical interactions conducted by low molecular weight compounds and proteins. Our interest is understanding the mechanisms of these interactions from the viewpoint of organic chemistry. Our current research focuses on the following: a) Design and synthesis of a series of chemical probes in order to reveal not only ligand–protein and protein–protein interactions, but also behavior of low molecular weight compounds *per se*. b) Unraveling biosynthetic pathways of bioactive natural products at enzyme level. c) Application of our chemical probes in development of novel practical bioactive compounds.

KEYWORDS

Enzyme Inhibitors
γ-Glutamyl Transpeptidase
Fructosyl Peptide Oxidase
Diabetes Diagnosis
Protein–Protein Interaction Inhibitors

Selected Publications

Watanabe, B.; Tabuchi, Y.; Wada, K.; Hiratake, J., Synthesis and Evaluation of the Inhibitory Activity of the Four Stereoisomers of the Potent and Selective Human γ-Glutamyl Transpeptidase Inhibitor GGsTop, *Bioorg. Med. Chem. Lett.*, **27,** 4920-4924 (2017).

Watanabe, B.; Morikita, T.; Tabuchi, Y.; Kobayashi, R.; Li, C.; Yamamoto, M.; Koeduka, T.; Hiratake, J., An Improved Synthesis of the Potent and Selective γ-Glutamyl Transpeptidase Inhibitor GGsTop together with an Inhibitory Activity Evaluation of Its Potential Hydrolysis Products, *Tetrahedron Lett.*, **58,** 3700-3703 (2017).

Kamiyama, A.; Nakajima, M.; Han, L.; Wada, K.; Mizutani, M.; Tabuchi, Y.; Kojima-Yuasa, A.; Matsui-Yuasa, I.; Suzuki, H.; Fukuyama, K.; Watanabe, B.; Hiratake, J., Phosphonate-Based Irreversible Inhibitors of Human γ-Glutamyl Transpeptidase (GGT). GGsTop is a Non-Toxic and Highly Selective Inhibitor with Critical Electrostatic Interaction with an Active-Site Residue Lys562 for Enhanced Inhibitory Activity, *Bioorg. Med. Chem.*, **24,** 5340-5352 (2016).

Watanabe, B.; Ichiyanagi, A.; Hirokawa, K.; Gomi, K.; Nakatsu, T.; Kato, H.; Kajiyama, N., Synthesis and Inhibitory Activity of Substrate-Analog Fructosyl Peptide Oxidase Inhibitors., *Bioorg. Med. Chem. Lett.*, **25,** 3910-3913 (2015).

Watanabe, B.; Minami, S.; Ishida, H.; Yoshioka, R.; Nakagawa, Y.; Morita, T.; Hayashi, K., Stereospecific Inhibitory Effects of CCG-1423 on the Cellular Events Mediated by Myocardin-Related Transcription Factor A., *PLoS One*, **10,** [e0136242-1]-[e0136242-16] (2015).

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 a 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.

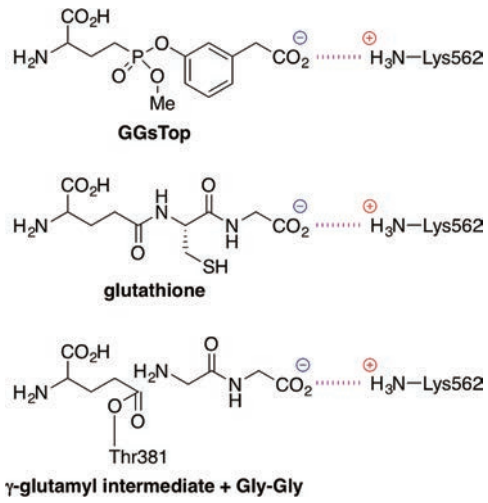

Figure 1. Proposed binding mode of GGsTop, glutathione, and acceptor substrate (Gly-Gly) to Lys562.

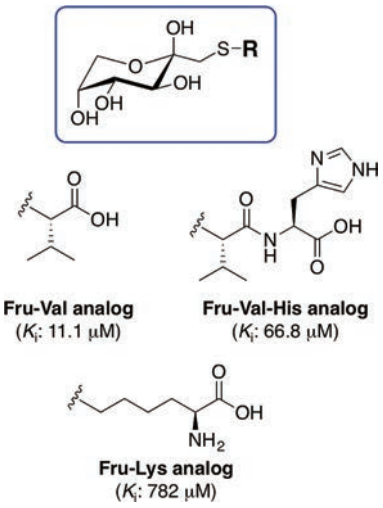

Figure 2. Chemical structures and inhibitory activity of FPOX inhibitors.

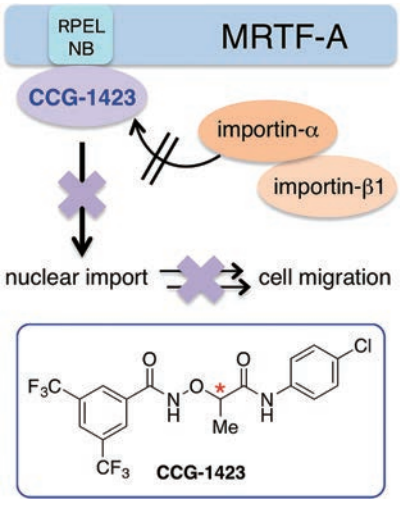

Figure 3. Molecular mechanism of MRTF-A inhibitor CCG-1423.