

Division of Biochemistry – Chemistry of Molecular Biocatalysts –

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Scope of Research

Our research interests focus on the molecular design and synthesis of specific inhibitors of physiologically important enzymes (biocatalysts) for use as chemical probes to understand the reaction mechanisms, three-dimensional structures and physiological roles of the enzymes. The finely designed inhibitors are further elaborated to develop pharmaceuticals and agrochemicals. Our research includes: Design, synthesis and applications of transition-state analogue and mechanism-based inhibitors of the key enzymes in glutathione homeostasis, asparagine synthetase, and the development of intermediate analogue inhibitors of acyl-activating enzyme superfamily that plays pivotal roles in plant hormone and secondary metabolite biosynthesis.

KEYWORDS

Enzyme Reaction Mechanisms
Transition-State Analogue Inhibitors
Glutathione Homeostasis
Bioactivity Substance
Plant Secondary Metabolite Biosynthesis



Selected Publications

- Joyce-Brady, M.; Hiratake, J., Inhibiting Glutathione Metabolism in Lung Lining Fluid as a Strategy to Augment Antioxidant Defense, *Current Enzyme Inhibition*, **7**, 71-77 (2011).
- Koeduka, T.; Watanabe, B.; Suzuki, S.; Hiratake, J.; Mano, J.; Yazaki, K., Characterization of Raspberry Ketone/Zingerone Synthase, Catalyzing the Alpha, Beta-Hydrogenation of Phenylbutenones in Raspberry Fruits, *Biochem. Biophys. Res. Commun.*, **412**, 104-108 (2011).
- Ikeuchi, H.; Meyer, M. E.; Ding, Y.; Hiratake, J.; Richards, N. G. J., A Critical Electrostatic Interaction Mediates Inhibitor Recognition by Human Asparagine Synthetase, *Bioorg. Med. Chem.*, **17**, 6641-6650 (2009).
- Hiratake, J., Novel Inhibitor of γ -Glutamyl Transpeptidase (GGT): Unique Chemical Tools to Probe the Physiological Significance of GGT, *Wako Chemicals Jihou*, **76 (No. 3)**, 2-6 (2008) (in Japanese).
- Han, L.; Hiratake, J.; Kamiyama, A.; Sakata, K., Design, Synthesis and Evaluation of γ -Phosphono Diester Analogues of Glutamate as Highly Potent Inhibitors and Active Site Probes of γ -Glutamyl Transpeptidase, *Biochemistry*, **46**, 1432-1447 (2007).

Design, Synthesis and Applications of Specific Inhibitors of γ -Glutamyl Transpeptidase for Modulating Cellular Redox Status

Glutathione (GSH, γ -Glu-Cys-Gly) is an ubiquitous tripeptide containing Cys found in many organisms. It plays central roles in the redox status of cells not only by detoxification of reactive oxygen species, but also by regulating the transcription of specific genes such as phase II antioxidant enzymes. We are interested in regulating the cellular GSH level by controlling the activities of its biosynthetic enzyme, γ -glutamylcysteine synthetase (GCS), and its metabolic enzyme, γ -glutamyl transpeptidase (GGT), by using specific inhibitors (Figure 1). GGT is a key enzyme in supplying the cells with Cys by cleaving the γ -glutamyl bond of extracellular GSH, as well as in degrading GSH conjugates for detoxification. We developed a series of phosphonate-based inactivators of GGT and found that an inhibitor was highly efficient for human GGT. Interestingly, this inhibitor significantly increased the biosynthesis of type I collagen and elastin of human skin fibroblasts and can be used, for example, as a novel antiaging cosmetic ingredient. The study on its mode of action and its application as a novel “cosmeceutical” are in progress.

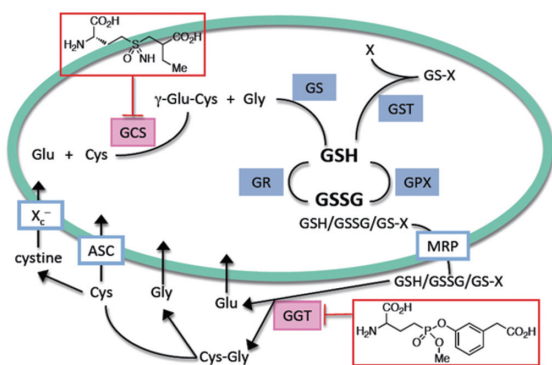


Figure 1. Biosynthesis, reaction, and metabolism of glutathione (GSH) in cell. The specific inhibitors of GCS and GGT are shown in red squares.

Inhibitors Targeting Human Asparagine Synthetase for Cancer Chemotherapy

Human asparagine synthetase (hAS) catalyzes the ATP-dependent synthesis of Asn from Asp using Gln as an ammonia source. The inhibition of hAS is highly important in enhancing and broadening the efficacy of asparaginase chemotherapy of leukemia and cancer. We previously developed the first nano molar *in vitro* hAS inhibitor **1** ($K_i^* = 24$ nM) and succeeded in suppression of proliferation of asparaginase-resistant cancer cell line, but at higher concentrations (100–1000 μ M). The bioavailability of **1**

was increased by removing a carboxy group to eliminate net negative charge. Surprisingly, the new compound **2** inhibited hAS even stronger ($K_i^* = 7.6$ nM) than the original **1** and not only suppressed cell proliferation in a dose-dependent manner, but also induced cell death irrespective of the presence of asparaginase. This result suggests that hAS in itself is a novel target without the aid of asparaginase, which might open a new paradigm for anti-cancer chemotherapy.

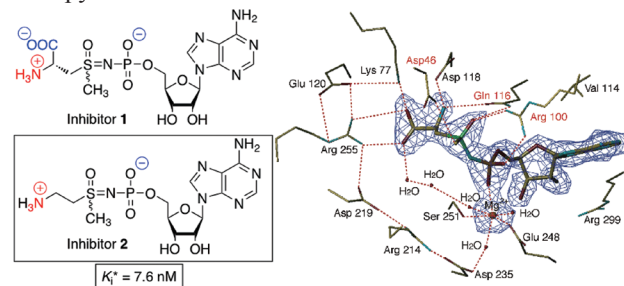


Figure 2. (Left) The structure of the original inhibitor **1** and newly synthesized inhibitor **2**. (Right) X-Ray crystal structure of *E. coli* AS in complex with **1**.

Specific Inhibitors of Acyl-activating Enzymes for “Chemical Knockout”

Acyl-activating enzymes constitute a large enzyme superfamily that contains a number of such important enzymes as for fatty acid β -oxidation and biosynthesis of plant secondary metabolites. In light of their common mechanistic features involving acyl-adenylate intermediate, we designed and synthesized *N*-acyl adenosyl sulfamides as intermediate-analogue inhibitors to probe the physiological impact of a key enzyme 4-coumaric acid:CoA ligase (4CL) on phenylpropanoid biosynthesis. The synthetic compounds inhibited 4CL *in vitro*, and the substituents on benzene ring significantly affected their potency. Administration of the inhibitors to *Arabidopsis* caused decrease of the phenylpropanoids contents. This result implied that the inhibitors were up-taken by plants and inhibited 4CL *in vivo*. The inhibitors are promising tools for chemical biology of plants for controlling plant secondary metabolites including the phenylpropene volatiles and lignin biosynthesis.

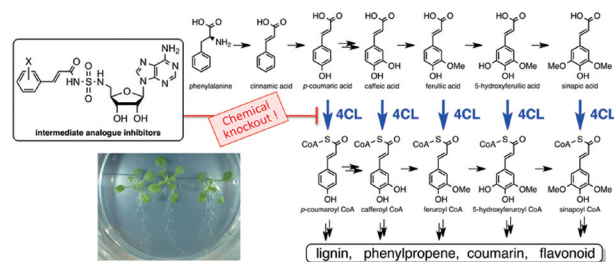


Figure 3. The outline of phenylpropanoid biosynthesis and the structure of intermediate analogue inhibitors.