Division of Biochemistry
– Chemistry of Molecular Biocatalysts –

http://www.scl.kyoto-u.ac.jp/~hiratake/index-j.html

Prof
HIRATAKE, Jun
(D Agr)

Assist Prof
WATANABE, Bunta
(D Agr)

Assist Prof
KOEDUKA, Takao
(D Agr)

Students

KAWANISHI, Daisuke (M2)
LI, Chunjie (M2)
JING, Dongyu (M2)
OZAKI, Ayane (M1)
YIN, Ziwei (RS)

Scope of Research

Our research focuses on the molecular design and synthesis of specific inhibitors of physiologically important enzymes (biocatalysts). The enzyme inhibitors are used for probing the reaction mechanisms, three-dimensional structures and identifying the physiological roles of the enzymes. The finely designed inhibitors are further elaborated to develop useful bioactive substances that could knockout the specific enzyme *in vivo* to develop lead compounds for novel pharmaceuticals, agrochemicals and cosmetic ingredients. Our current research includes the design, synthesis and applications of transition-state analogue and/or mechanism-based inhibitors of such enzymes as γ-glutamyl transpeptidase, a key enzyme in glutathione metabolism, asparagine synthetase, an important enzyme for cancer chemotherapy, and 4-coumaroyl CoA ligase that plays a pivotal role in the biosynthesis of a vast array of phenylpropanoid in plants. The identification of the genes of hitherto unknown enzymes for biosynthesis of phenylpropanoid volatiles in plants are also pursued to shed light on the detailed reaction mechanisms and the physiological function of the biosynthetic enzymes in plant secondary metabolites.

KEYWORDS

Enzyme Reaction Mechanisms
Transition-State Analogue Inhibitors
Mechanism-Based Enzyme Inhibitors
Glutathione Homeostasis
Bioactive Substance

Selected Publications

Ida, T.; Suzuki, H.; Fukuyama, K.; Hiratake, J.; Wada, K., Crystal Structure of *Bacillus subtilis* γ-glutamyltranspeptidase in Complex with Acivicin: Diversity of the Binding Mmode of a Classical and Electrophilic Active-site Directed Glutamate Analogue, *Acta Crystallogr. Section D*, **70,** 607-614 (2014).

Koeduka, T.; Sugimoto, K.; Watanabe, B.; Someya, N.; Kawanishi, D.; Gotoh, T.; Ozawa, R.; Takabayashi, J.; Matsui, K.; Hiratake, J., Bioactivity of Natural *O*-prenylated Phenylpropenes from *Illicium anisatum* Leaves and Their Derivatives Against Spider Mites and Fungal Pathogens, *Plant Biol. (Stuttg.)*, doi: 10.1111/plb.12054 (2013).

Hiratake, J.; Suzuki, H.; Fukuyama, K.; Wada, K.; Kumagai, H., γ-Glutamyltranspeptidase and Its Precursor, *Handbook of Proteolytic Enzymes, 3rd Ed.*, **820,** 3712-3719 (2013).

Ikeuchi, H.; Ahn, Y. M.; Otokawa, T.; Watanabe, B.; Hegazy, L.; Hiratake, J.; Richards, N. G. J., A Sulfoximine-Based Inhibitor of Human Asparagine Synthetase Kills L-Aspraginase-Resistant Leukemia Cells, *Bioorg. Med. Chem.*, **20,** 5915-5927 (2012).

Joyce-Brady, M.; Hiratake, J., Inhibiting Glutathione Metabolism in Lung Lining Fluid as a Strategy to Augment Antioxidant Defense, *Curr. Enz. Inhibit.*, **7,** 71-78 (2011).

Development and Applications of Specific Inhibitors of γ-Glutamyl Transpeptidase, a Key Enzyme in Glutathione Metabolism

Glutathione (GSH, γ-Glu-Cys-Gly) is a ubiquitous redox active tripeptide containing Cys and plays central roles in detoxification of reactive oxygen species (ROS) and toxic xenobiotics in the front line of cellular defense system. γ-Glutamyltranspeptidase (GGT) is a key enzyme in GSH metabolism that catalyzes the cleavage of γ-glutamyl peptide bond of extracellular GSH to supply cells with Cys, a rate-limiting substrate for intracellular GSH biosynthesis. Hence GGT is implicated in many physiological disorders such as drug resistance of cancer cells, cardiovascular diseases and asthma. We have developed a phosphonate-based mechanism-based inhibitor, GGsTop™, that was a highly specific and non-toxic inhibitor of GGT. A series of phosphonate-based GGT inhibitors with a peptidyl side chain have also been synthesized for evaluation as inhibitors of human and *E. coli* GGTs to probe the Cys-Gly binding site (Figure 1).

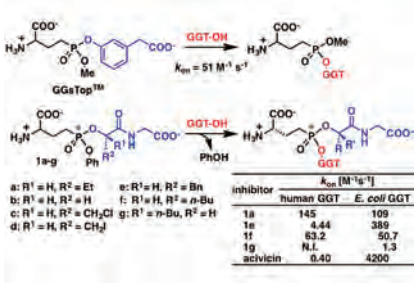

Figure 1. Mechanism-based inhibition of GGT by GGsTop™ and peptidyl phosphonate inhibitors **1a-g**.

Interestingly, GGsTop™, a highly efficient inhibitor of human GGT, induces cellular anti-oxidative stress response. As a result, this compound exhibited interesting biological activities such as increasing the biosynthesis of type I collagen, elastin and HSP47 of human dermal fibroblasts (Figure 2). These properties, along with its non-toxic nature, allowed GGsTop™ to serve as a novel active ingredient for anti-ageing cosmetics. This compound are now marketed under a trade name of "Nahlsgen®" and has attracted significant interests from the cosmetic market.

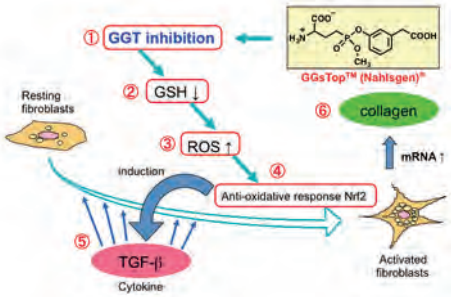

Figure 2. Mechanism for activation of human fibroblasts by GGT inhibitor, GGsTop™.

Inhibitors Targeting Human Asparagine Synthetase for Cancer Chemotherapy

A standard current clinical protocol for acute lymphoblastic leukemia (ALL) is the use of the enzyme L-asparagine amidohydrolase (ASNase) that catalyzes the hydrolysis of L-Asn to deplete circulating L-Asn in blood. The efficacy of ASNase chemotherapy, however, is hampered by the emergence of ASNase-resistant leukemic blasts; 10–12% of patients who initially go into remission undergo subsequent relapse. One widely-accepted hypothesis for ASNase resistance is the up-regulation of glutamine- and ATP-dependent asparagine synthetase (ASNS) that enables the leukemia cells to synthesize L-Asn necessary for their growth. Hence the inhibition of human ASNS is highly important in cancer chemotherapy, and we have synthesized a specific inhibitor of human ASNS. According to the proposed reaction mechanism of ASNS in which the β-carboxy group of L-Asp is activated by adenylation followed by nucleophilic substitution by ammonia to yield L-Asn, AMP and PPi, an *N*-adenylated sulfoximine **1** was synthesized as a transition-state analogue inhibitor of ASNS (Figure 3).

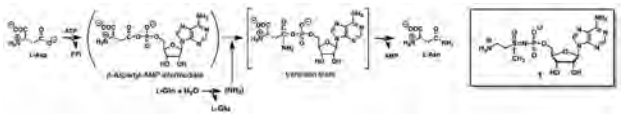

Figure 3. Reaction mechanism of asparagine synthetase and its transition-state analogue 1.

Compound **1** was found to be an extremely potent inhibitor of human ASNS; it inhibited the enzyme in a time-dependent manner with an overall K_i* of 8 nM. Furthermore, the inhibitor **1** suppressed cell proliferation of ASNase-resistant MOLT-4R leukemia cells (Figure 4) with an IC_{50} of ca. 0.1 mM. Surprisingly, compound **1** induced cell death as well as suppression of cell proliferation at higher concentrations. ASNase-sensitive leukemia cells with low ASNS activity were much less sensitive toward compound **1**, suggesting that ASNS in itself was an essential enzyme for leukemia cells and was an important drug target for cancer chemotherapy.

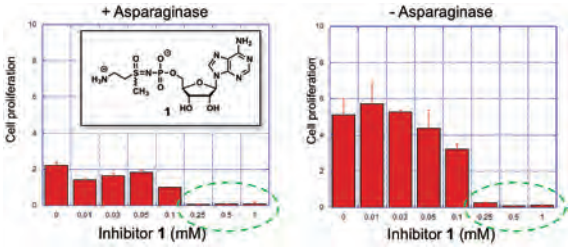

Figure 4. The effect of ASNS inhibitor **1** on the proliferation of ASNase-resistant MOLT-4 leukemia cells in the presence and absence of 1U asparaginase. (Left) MOLT-4 cells with ASNase; (right) MOLT-4R cells without ASNase. Cell proliferation is defined as the number of viable cells after 48 h incubation.