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)

Students

AKASHI, Takuya (M2)
KURODA, Kou (M2)
YIN, Ziewi (M2)
ITO, Shota (M1)
SAITO, Shingo (M1)
NAESHIRO, Kazuki (M1)
LI, Xian (M1)

Scope of Research

Our research focuses on the molecular design and synthesis of specific inhibitors of physiologically important enzymes (biocatalysts). Enzyme inhibitors are used for probing reaction mechanisms, three-dimensional structures, and identifying the physiological roles of the enzymes. Finely designed inhibitors are further elaborated to develop useful bioactive substances that could knock out specific enzymes *in vivo* to develop lead compounds for novel pharmaceuticals, agrochemicals, and cosmetic ingredients. Our current research includes the design, synthesis, and application of transition-state analogues 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, which plays a pivotal role in the biosynthesis of a vast array of phenylpropanoids 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 physiological functions of the biosynthetic enzymes for plant secondary metabolites.

KEYWORDS

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

Selected Publications

Tuzova, M.; Jean, J.-C.; Hughey, R. P.; Brown, L. A. S.; Cruikshank, W. W.; Hiratake, J.; Joyce-Brady, M., Inhibiting Lung Lining Fluid Glutathione Metabolism with GGsTop as a Novel Treatment for Asthma, *Front. Pharmacol*, **5,** 1-8 (2014).

Saino, H.; Shimizu, T.; Hiratake, J.; Nakatsu, T.; Kato, H.; Sakata, K.; Mizutani, M., Crystal Structures of β-Primeverosidase in Complex with Disaccharide Amidine Inhibitors, *J. Biol. Chem.*, **289,** 16826-16834 (2014).

Nakajima, M.; Watanabe, B.; Han, L.; Shimizu, B.; Wada, K.; Fukuyama, K.; Suzuki, H.; Hiratake, J., Glutathione-Analogous Peptidyl Phosphorus Esters as Mechanism-Based Inhibitors of γ-Glutamyl Transpeptidase for Probing Cysteinyl-Glycine Binding Site, *Bioorg. Med. Chem.*, **22,** 1176-1194 (2014).

Kodan, A.; Yamaguchi, T.; Nakatsu, T.; Sakiyama, K.; Hipolito, C. J.; Fujioka, A.; Hirokane, R.; Ikeguchi, K.; Watanabe, B.; Hiratake, J.; Kimura, Y.; Suga, H.; Ueda, K.; Kato, H., Structural Basis for Gating Mechanisms of a Eukaryotic P-Glycoprotein Homolog, *Proc. Natl. Acad. Sci. U.S.A.*, **111,** 4049-4054 (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.*, **16,** 451-456 (2013).

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 the cellular defense system. γ-Glutamyl transpeptidase (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 mechanism-based inhibitor, named 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).

Interestingly, GGsTop™, a highly efficient inhibitor of human GGT, induces the 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 is now marketed under the trade name Nahlsgen® and has attracted significant interest from the cosmetic market.

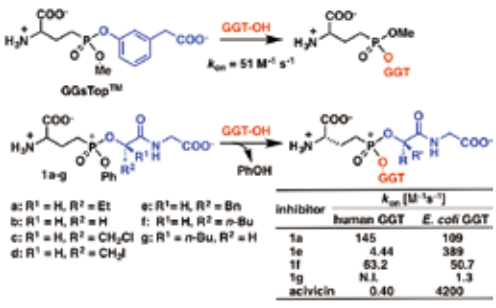

Figure 1. Mechanism-based inhibition of GGT by GGsTop™ and peptidyl phosphonate inhibitors **1a–g**.

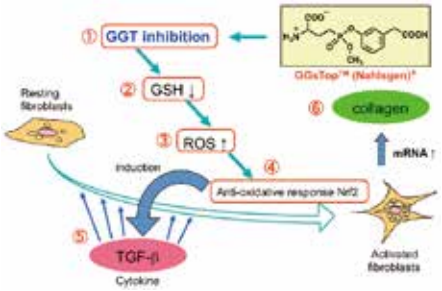

Figure 2. Mechanism for activation of human fibroblasts by GGT inhibitor, GGsTop™.

Biological Activities of Plant Second Metabolite Phenylpropenes and Their Mode of Action

Phenylpropenes having a C6–C3 unit carbon skeleton with a variety of substituents on the benzene ring (C6) and the propene side chain (C3) are one of the most prevalent plant secondary metabolites that exhibit various biological activities such as bacteriocidal, anti-fungal, anti-viral, antioxidative, and anti-tumor activities. Eugenol and its derivatives such as estragole, *O*-methyleugenol, and safrole are typical volatile phenylpropenes found widely across the plant kingdom and are considered to be a part of the chemical self-defense system of plants. Among them, *O*-dimethylallyleugenol (DMAE) is a unique eugenol derivative found in enormous quantities in leaves of Japanese star anise (*Illicium anisatum*) and exhibits the unique activity of suppression of ovipositon of mites, which its parent compound eugenol does not. We therefore are interested in the mode of action of DMAE and performed structure–activity relationship studies.

O-alkylated eugenols like estragole, methyleugenol, and safrole did not show any ovipositon suppression activity at 2 mM; only *O*-allyl-based alkenyl derivatives including DMAE exhibited significant activity. Furthermore, the activity was observed only for the allyl benzene with *O*-allyl substituent at the para position. Interestingly, the activities of DMAE and *O*-allyleugenol (AE) were totally abolished in the presence of piperonyl butoxide (PBO), a competitive inhibitor of cytochrome P450 enzyme, suggesting that the metabolic activation of *O*-allyleugenols involving P450 is responsible for the biological activity. The formation of *p*-quinone methide is inferred as an active entity.