

Division of Biochemistry – Chemistry of Molecular Biocatalysts –

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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

- 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 Cysteiny-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 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, GGSTopTM, 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* GGSTs to probe the Cys-Gly binding site (Figure 1).

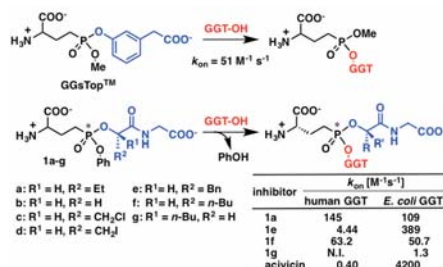


Figure 1. Mechanism-based inhibition of GGT by GGSTopTM and peptidyl phosphonate inhibitors **1a-g**.

Interestingly, GGSTopTM, 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 GGSTopTM to serve as a novel active ingredient for anti-ageing cosmetics. This compound are now marketed under a trade name of “Nahlsagen[®]” and has attracted significant interests from the cosmetic market.

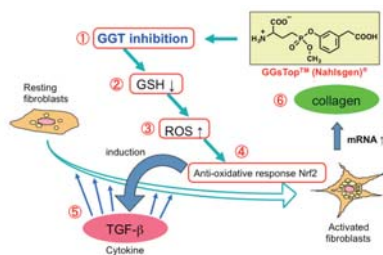


Figure 2. Mechanism for activation of human fibroblasts by GGT inhibitor, GGSTopTM.

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, anti-oxidative and anti-tumor activities. Eugenol and its derivatives such as estragole, *O*-methyleugenol, 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 amount in leaves of Japanese star anise (*Illicium anisatum*) and exhibits unique activity of suppression of oviposition of mites, whereas 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 eugenol such as estragole, methyleugenol and safrole did not show any oviposition suppression activity at 2 mM, whereas *O*-allyl-based alkenyl derivatives include DMAE solely 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 and the formation of *p*-quinone methide is inferred as an active entity.

