Division of Biochemistry
– Chemistry of Molecular Biocatalysts –

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Scope of Research

Our research interests are 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 useful *in vivo* active biochemical reagents and the lead compounds for pharmaceuticals and agrochemicals. Our current research includes the design, synthesis and applications of transition-state analog and mechanism-based inhibitors of the key enzymes in glutathione homeostasis, asparagine synthesis, and the acyl-activating enzyme superfamily that plays pivotal roles in plant hormone homeostasis, secondary metabolite biosynthesis and β-oxidation of fatty acids. The synthesis and evaluation of substrates and inhibitors of ABC transporters and the unique enzymes of Archaea hyperthermophiles are also studied.

KEYWORDS

Enzyme Reaction Mechanisms
Transition-State Analog Inhibitors
Mechanism-Based Inhibitors
Glutathione
Chemotherapeutic Agents

Selected Publications

Ikeuchi Y, Meyer ME, Ding Y, Hiratake J, Richards NGJ: 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).

Guitierrez JA, Pan Y.-X, Koroniak L, Hiratake J, Kilberg MS, Richards NGJ: An Inhibitor of Human Asparagine Synthetase Suppresses Proliferation of an L-Asparaginase Resistant Leukemia Cell Line, *Chem. Biol.*, **13,** 1339–1347 (2006).

Nakatsu T, Ichiyama S, Hiratake J, Saldanha A, Kobayashi, N, Sakata K, Kato H: Structural Basis for Spectral Difference in Luciferase Bioluminescence, *Nature*, **440,** 372–376 (2006).

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

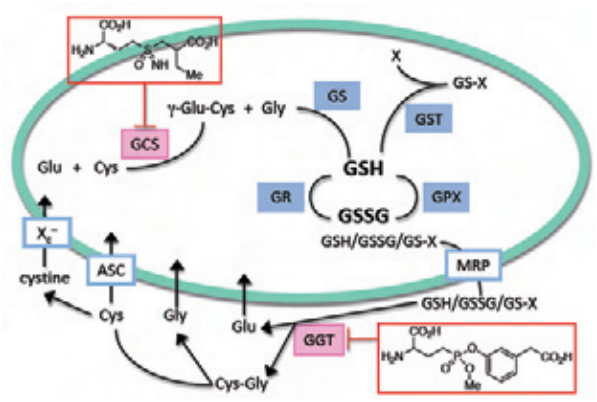


Figure 1. Biosynthesis, reaction and metabolism of glutathione (GSH) in cell. The specific inhibitors of GCS and GGT are shown (red squares).

Glutathione (GSH, γ-Glu-Cys-Gly) is an ubiquitous tripeptide found in many organisms, and 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 anti-oxidant enzymes. We are interested in regulating the cellular GSH level that leads to the modulation of cellular redox status 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). The compound **1**, designed as a transition-state analog, was found to serve as a potent mechanism-based inhibitor of GCS. The X-ray crystal structure of *E. coli* GCS in complex with **1** (Figure 2) revealed that the carboxy group (blue) as well as the side chain (magenta) is critical for the recognition by GCS, thereby the inhibition potency of **1** being 500-times higher than a classical GCS inhibitor, BSO, lacking the carboxy group. Furthermore, the introduction of a cyano group (compound **2**) as a mimic of SH further increased the inhibition potency by 31 and 55 times for the *E. coli* and the pathogenic *Streptococcus agalactiae* GCS, respectively. Notably, the inhibition potency of **2** is significantly higher (10,000 times) than BSO for the latter enzyme. The negatively charged carboxy group, however, appears to hinder the penetration of **1** and **2** through the cell membrane. Therefore a series of dipeptide-based inhibitors **3** was prepared for active transport through bacterial peptide transporters. This aspect of study is now under way to develop novel pharmaceuticals to combat multi-drug resistant pathogenic bacteria.

In glutathione biosynthesis, another limiting factor is

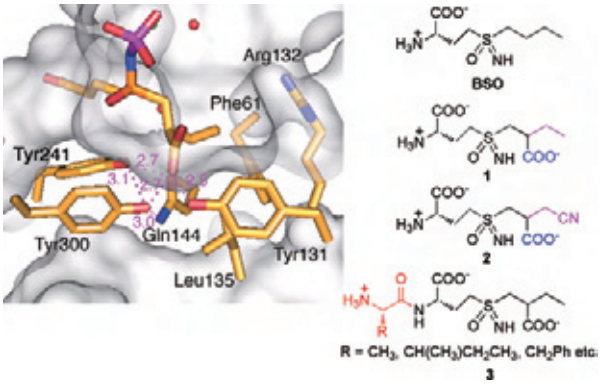
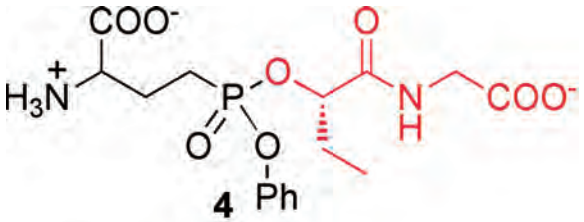


Figure 2. X-Ray crystal structure of the Cys binding site of *E. coli* GCS in complex with **1**.

the availability of Cys. γ-Glutamyl transpeptidase (GGT) is a key enzyme in supplying the cells with Cys by cleaving the γ-glutamyl bond of GSH, as well as in degrading GSH conjugates for detoxification. We have developed a series of phosphonate-based inactivators of GGT and found that a glutathione-like inhibitor **4** was highly efficient for human GGT. In light of the specific recognition of the C-terminal carboxy group by human GGT, a simplified peptide analog **5** was prepared and found to be a highly potent inactivator of human GGT. The inhibitor **5** was specific for GGT, non-toxic and rather stable compound, serving as a useful biochemical reagent to knock down GGT *in vivo*. Interestingly, compound **5** significantly increased the biosynthesis of collagen I of human skin fibroblasts and can be used, for example, as a novel anti-aging cosmetic ingredient.



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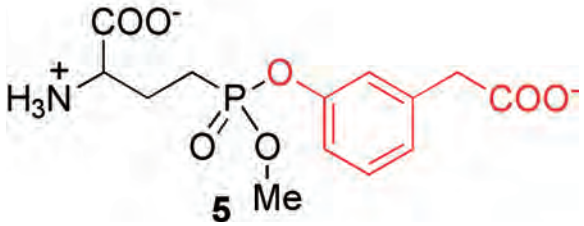

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