[S2-3] *[4/21/2000 (Fri) 11:10~11:50/Room B112, B1 Floor, Bldg 26]*

Structure- and Mechanism-Based Enzyme Inhibitor Design

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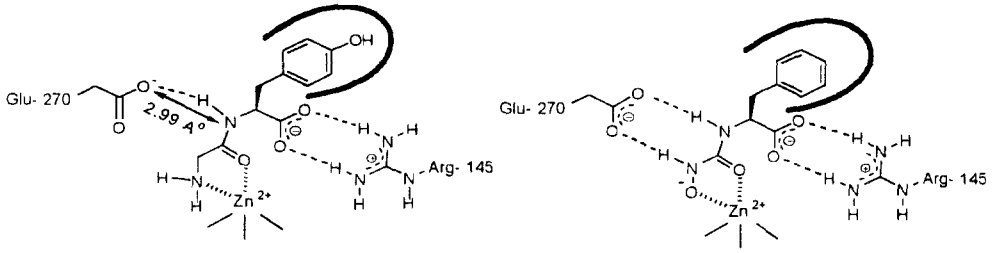
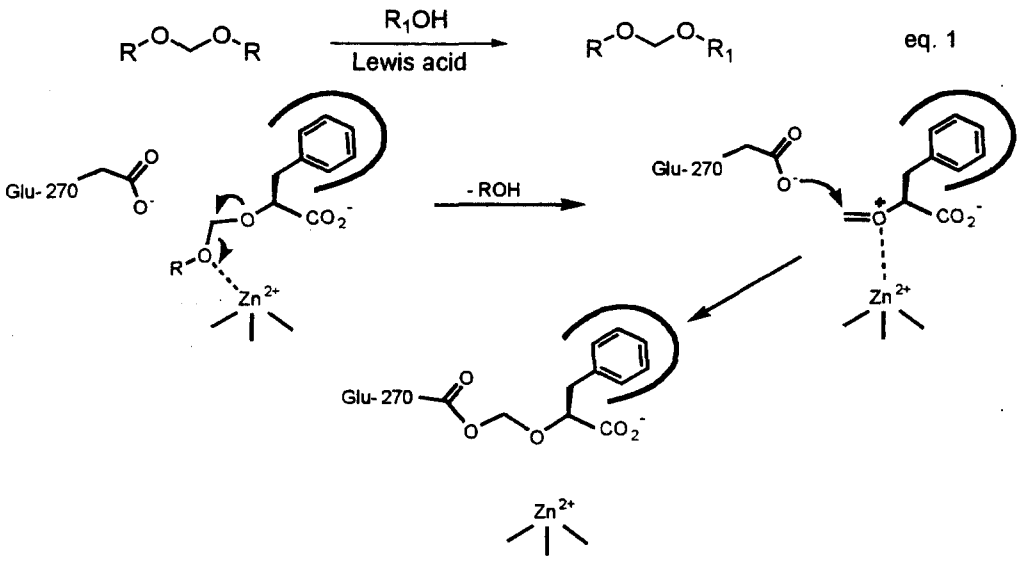
Carboxypeptidase A (CPA), a prototypic zinc-containing exo-monopeptidase, has been served as a model enzyme for a large family of zinc proteases. Inhibitor design strategies developed for CPA can be utilized to design the inhibitors that are effective for other zinc proteases of medical interest such as angiotensin-converting enzyme (ACE), enkephalinase, and matrix metalloproteases (MMP). Recent advance in the area of x-ray crystallography and molecular modeling has garnered a lot of structural information of enzymes, and thus popularized structure-based enzyme inhibitor design. In addition to the structural information, understanding of enzyme reaction mechanism has also afforded an insight into a selective inhibitor design of the enzyme.

N-Hydroxyurea derivatives were designed as potential CPA inhibitors by a simple structural modification of the substrate observed in a known crystal structure of CPA-substrate complex (Scheme 1). The designed inhibitors were easily prepared from commercially available α-amino acids in three steps, and evaluated as CPA inhibitors giving a potent inhibitory activity against the catalytic activity of CPA (K_i = 1.6 ~ 3.3 μM). This represents the first successful example to make use of *N*-hydroxyurea in the development of protease inhibitor.



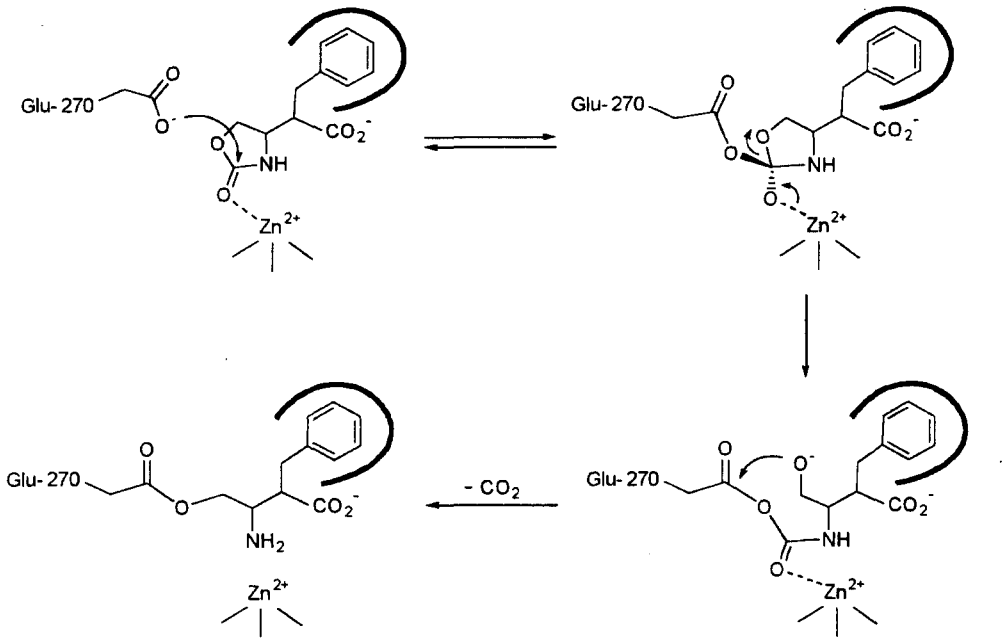
Scheme 1. Design of *N*-hydroxyurea derivatives as CPA inhibitors

It is well known that Lewis acid makes acetal functionality susceptible to an attack by nucleophiles such as water or alcohol (eq.1). Based on that CPA has a Lewis acid (Zn^{2+}) and a possible nucleophile (the carboxylate of Glu-270) at the active site, therefore, acetal derivatives were designed as potential irreversible inhibitors of CPA (Scheme 2). The acetal compounds prepared from 3-phenyllactic acid showed a potent irreversible inhibition of CPA.



Scheme 2. Design rationale of acetal derivatives as CPA inhibitors

Mechanism-based inactivators of enzymes are of very interest because of their ability to inhibit selectively a target enzyme and usefulness to prove the catalytic mechanism of the target enzyme. Oxazolidinone was incorporated into potential inhibitors as a functional group to react with the catalytic residue of CPA. All four stereoisomers that were synthesized from L- and D-aspartic acids showed an irreversible inhibition against CPA activity. Molecular modeling suggested that the inactivation may follow the mechanism proposed in Scheme 3.



Scheme 3. A proposed inactivation mechanism of CPA by oxazolidinone derivatives