EXPLORING UREASE INHIBITION: COMPUTATIONAL ANALYSIS OF CINNAMOYL HYDROXAMIC ACIDS

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ABSTRACT

Ureases are nickel (II)-dependent enzymes present in bacteria, archaea, plants, algae, and fungi, known for catalyzing the hydrolysis of urea into ammonia and carbamic acid, which subsequently decomposes into additional ammonia and carbonic acid. In the medical field, ureases function as virulence factors in various pathogenic bacteria, contributing to conditions such as kidney stones, pyelonephritis, gastric ulcers, and other illnesses. Over the past decade, hydroxamic acids have shown promising potential as urease inhibitors in the treatment of *H. pylori* and *P. mirabilis* infections. For the in silico study, the co-crystallized structure of jack bean urease (PDB entry: 4H9M) was retrieved from the RCSB PDB. Preliminary molecular dynamics (MD) simulations were conducted to maintain the native conformation of the enzyme, using a TIP3P water model and an orthorhombic simulation box with periodic boundary conditions in Desmond. A 150 ns MD simulation at 300 K and 1.01325 bar stabilized the system, with a 0.15 M NaCl solution neutralizing the net charge. The most stable urease conformation was subsequently used for docking, with the grid centered on the co-crystallized ligand's coordinates. Ligands (AHA and **4a-k**) were drawn in ChemDraw, minimized using the AM1 method, and docked with GOLD software employing the CHEMPLP scoring function. Throughout the 100 ns MD simulation, the protein–ligand complexes showed a consistent fitting mechanism. For compounds **4a-i** and **k**, the hydroxamic acid groups played a crucial role by coordinating their carbonyl and hydroxylamine oxygen atoms with Ni ions, forming stable ligand-enzyme complexes essential to their inhibitory effects. However, derivative **4j** demonstrated distinct behavior, where a nitro group in the meta position induced a reorientation within the active site, directly coordinating with the Ni ions and diverging from the role of hydroxamic acid groups observed in other derivatives. MD simulations of cinnamoyl hydroxamic acid **4i** at the catalytic site revealed coordination with Ni ions and hydrogen-bond interactions with Cys592 in 60% of the simulation, suggesting the potential for covalent bond formation and irreversible inhibition. To further explore this, a covalent bond was formed between the thiol group of Cys and the β-carbon of **4i**, followed by an additional 150 ns MD simulation of the complex. Unlike the open flap conformation observed with native thiourea, the covalent complex prevented flap closure, with **4i** inducing steric hindrance at the catalytic site entrance, consistent with mixed-type inhibition. In conclusion, experimental and in silico analyses of binding modes indicate that compounds **4a** and **4i** can interact with nickel atoms at the urease active site and with Cys residues in an allosteric site adjacent to the active site, contributing to their inhibitory potential.