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Theoretical Study on the Inhibition Mechanism of the Proteasome by Bortezomib

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Abstract: Bortezomib can form covalent bonds with the proteasome to kill myeloma tumor cell, but the detailed covalent inhibition reaction mechanism remains unclear. In this study, we first established the parameters for boron atom in bortezomib and explored the possible reaction pathways of inhibition of the proteasome by bortezomib through molecular dynamics (MD) simulation and Quantum Mechanics/Molecular Mechanics (QM/MM) calculation. The computational results indicate that the most energetically favorable pathway includes two reaction steps. The first step involves a proton abstraction from the -OH group to -NH_2 group of Thr1 residue, which is coupled with the nucleophilic attack on the boron atom of bortezomib by the newly formed O^- ion. The second step is the proton transfer from the protonated -NH_3^+ group to one of the hydroxyl group connected to the boron atom, forming a bortezomib-proteasome complex associated with a water molecule coordinated with the boron atom. The rate-determining step of the inhibition reaction is the first step with an energy barrier of 19.1 kcal/mol, which is close to the activation energy of ~20.8 kcal/mol derived from kinetic experiments. This work provides detailed mechanistic insights for proteasome inhibition by the boron compounds.

Key words: bortezomib, proteasome, inhibition mechanism, QM/MM calculation.

1. Introduction

The proteasome is a large protein complex (2500 kDa) that regulates intracellular proteolysis. With a sedimentation coefficient of 26S as determined by density gradient centrifugation, it is also known as the 26S proteasome [1-3]. The 26S proteasome consists of the 19S regulatory particle and the 20S core particle, and many researches have shown that degradation can occur without the regulation of the 19S particle and can be mediated solely by the 20S proteasome, which is thus identified as the catalytic core of the proteasome [1,2,4-6]. As we all know, proteins in living cells are constantly being degraded and synthesized, and the proteasome generally plays an indispensable role in the cells for degrading misfolded and useless proteins into amino acids [7-9]. If the proteasome is inhibited, the cells will die quickly. Although scientists have not fully demonstrated the reasons why proteasome

inhibitors can selectively kill cancer cells while they are harmless to normal cells, this does not affect the potential applications of proteasome inhibitors as anti-tumor drugs [10-12]. Noteworthy, it has been discovered that proteasome inhibitors possess excellent anticancer activity, and have already begun to be applied in the medical field [13-15].

Up to now, a great number of inhibitors have been reported in the literature to inhibit the proteasome [16-23]. Based on their structural characteristics and different modes of action with the proteasome, they can be roughly divided into two main categories: covalent inhibitors and non-covalent inhibitors depicted in **Figure 1**. Covalent inhibitors include several types, such as aldehyde peptides [24-25], boronic acid peptides [26-29], β-lactones [20,30-32], vinyl sulfone peptides [33-35], and epoxyketones [36-38] and others [19]. Non-covalent inhibitors include cyclic peptide inhibitors (such as TMC-95A and its derivatives) [38-42].

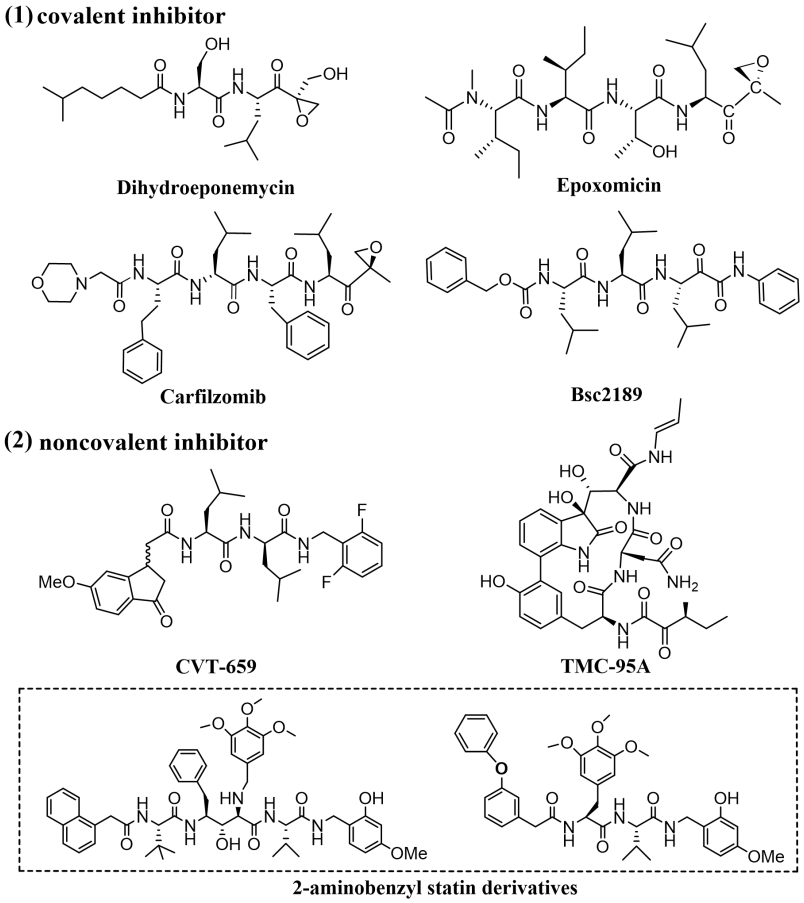

Figure 1. Examples of covalent and non-covalent inhibitors of proteasome.

Each of proteasome active site contains an N-terminal threonine (Thr1) residue, whose terminal amine serves as the general basis for Thr1-OH activation [3,43-45]. Then the activated Thr1-O^- ion can act as a nucleophile in proteasome-catalyzed hydrolysis of the peptide bond of the protein, and it can also attack the corresponding inhibitor molecules to form a covalent bond. Different types of inhibitors may have different covalent-binding modes and reaction mechanisms at the proteasome active site. For example, vinyl sulfone inhibitors (such as Z-L3VS) [46] form covalent bonds through nucleophilic attack by Thr1-O^- on the olefinic carbon, β-lactone inhibitors such as NPI-0047 [47] can react with Thr1-O^- to form covalent bonds. Peptide aldehyde inhibitors such as Ac-Leu-Leu-nLeu-al [48], form a hemiacetal bond between the aldehyde group and the Thr1-O^- at the active site depicted in **Figure 2**. For many years, scientists have studied several types of inhibition mechanism of proteasome through theoretical calculations [17,18,49-53]. However, the inhibition mechanism of some special types of covalent inhibitors such as bortezomib, remains to be unexplored in theory.

The proteasome inhibitor bortezomib has been approved by the U.S. Food and Drug Administration (FDA) in 2003 for the treatment of multiple myeloma [54,55]. It can inhibit the tumor activity by interacting with the proteasome. Although the catalytic rate constant of this reaction has been characterized [56], the fundamental reaction pathway remains unclear due to limitations in the parameters of boron atom during the computational process. This issue and our interest in mechanistic studies of proteasome prompt us to contribute this computational work on the bortezomib inhibition reaction. Based on the previous theoretical studies on the proteasome inhibition by other kinds of inhibitors [18,49], we have proposed a possible pathway for the bortezomib inhibition depicted in **Scheme 1**. The pathway involves intramolecular proton transfer of Thr1 in the proteasome coupled with B-O bond formation, and a second intramolecular proton transfer. In this work, we aim to clarify the detailed mechanism of the inhibition reaction of bortezomib and the rate-determining step of the reaction. In the