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ORIGINAL PAPER

Why tazobactam and sulbactam have different intermediates population with SHV-1 β-lactamase: a molecular dynamics study

Rui Li · Yeng-Tseng Wang · Cheng-Lung Chen

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Abstract The imine intermediates of tazobactam and sulbactam bound to SHV-1 β-lactamase were investigated by molecular dynamics (MD) simulation respectively. Hydrogen bond networks around active site were found different between tazobactam and sulbactam acyl-enzymes. In tazobactam imine intermediate, it was observed that the triazolyl ring formed stable hydrogen bonds with Asn170 and Thr167. The results suggest that conformation of imine determined the population of intermediates. In imine intermediate of tazobactam, the triazolyl ring is trapped in Thr_Asn pocket, and it restricts the rotation of C5-C6 bond so that tazobactam can only generate trans enamine intermediate. Further, conformational cluster analyses are performed to substantiate the results. These findings provide an explanation for the corresponding experimental results, and will be potentially useful in the development of new inhibitors.

Keywords β-lactamases · Molecular dynamics simulations · Sulbactam · Tazobactam

Introduction

β-lactamase enzymes are the principal agents of bacterial resistance to β-lactam antibiotics [1, 2]. They have been divided into four classes (A–D) on the basis of the activity site differences [3]. Among the four β-lactamase classes, the class A enzymes are the most frequently encountered in the clinic. One of the most successful strategies to overcome β-lactamase resistance is to use β-lactamase inhibitors. Three class A β-lactamase inhibitors are commercially available: clavulanate, tazobactam, and sulbactam. The mechanism of class A β-lactamase inactivation by inhibitors has been studied extensively by means of enzyme kinetic, X-ray and Raman crystallography [4–13]. These studies suggested a common mechanism as follows. In the first step, the catalytic serine (Ser70) attacks the carbonyl carbon of the β-lactam ring, forming an acyl-enzyme intermediate (AEI) with β-lactam ring opening. In the second step, AEI undergoes further reaction to generate a linear imine intermediate with five-member ring opening. Lastly, a series of proton transfers can occur in which the imine AEI tautomerizes to yield a more stable cis or trans-enamine intermediate and thus providing transient inhibition.

Both tazobactam and sulbactam are penicillanic acid sulfone inhibitors (Fig. 1). However, they present different clinical efficacy, and tazobactam is more effective against β-lactamase than sulbactam. Recently, Kalp et al. [14] examined the reactions between the two inhibitors and SHV-1 β-lactamase in single crystals based on the result of Raman microscope. Their results showed tazobactam forms a predominant population of trans-enamine while sulbactam form a mixture of trans-enamine cis-enamine and imine. They indicated that tazobactam can form more stable population of trans-enamine, which leads to the superior performance in the clinic.

However, the reason why tazobactam and sulbactam show different AEI population is still under discussion and further studies on the mechanism of tautomerization from imine to enamine is certainly required. Herein, molecular modeling methodologies were employed to study the imine AEI of

R. Li
Department of Chemistry,
Liaocheng University, Liaocheng 252059 Shandong, China

Y.-T. Wang
National Center for High-Performance Computing, Hsin-Shi,
Tainan County, Taiwan

C.-L. Chen (✉)
Department of Chemistry, National Sun Yat-Sen University,
Kaohsiung 80424, Taiwan
e-mail: chen1@mail.nsysu.edu.tw

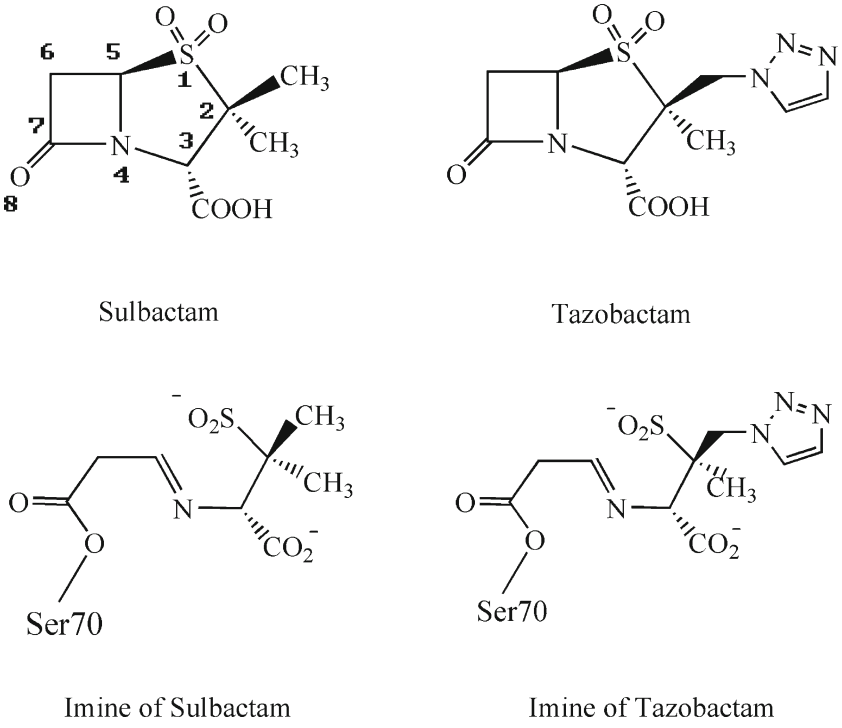

Fig. 1 The structure of sulbactam and tazobactam

tazobactam and sulbactam respectively. Our studies reveal imine AEI is a key intermediate, and its dynamic behaviors affect the population of intermediates. Understanding the dynamic details of different inhibitors AEI can aid the design of improved mechanism-based β-lactamase inhibitors.

Models and methods

Two systems were computed in the current study, namely **imine_taz** and **imine_sul** respectively. The initial coordinates for tazobactam bound to SHV-1 was taken from the crystal structural data 1VM1 [15] of the Protein Data Bank (PDB). Based on this structure, the imine configuration of tazobactam bound to Ser70 was constructed (**imine_taz**). Then, the imine structure of sulbactam (**imine_sul)** was constructed by removing the triazolyl ring from tazobactam.

In both of the computed systems, the complex was surrounded by a periodic box containing TIP3P water molecules that were extended 8 Å from the protein. Na^+ counterions were placed in the simulation box by LEaP module of the AMBER 10 computational program package [16]. Atomic charges for substrates and substrate-bound Ser70 were determined using RESP module. The electrostatic potential at points selected according to the Merz-Shigh-Kollman scheme for use in the RESP module was calculated at the RHF/6-31G** level by Gaussian 03 software program [17]. Force field parameters for the protein were assigned from the "parm99" set of parameters, while the parameters of substrates were obtained from the "gaff" parameters within AMBER 10.

At the start of each of the MD run, the energy minimization was carried out first. The steepest descent method followed by conjugate gradient method was performed for the minimization. After that, MD simulation with position-restrain, which restrained the atomic positions of the macromolecule while it allowed the solvent molecules to move, was carried out for 20 ps. Finally, a 5 ns MD production run was performed for the system. In the MD process, the SHAKE procedure was applied to constrain all bonds involving hydrogen atoms. The Langevin dynamics [18] was used to control the temperature at 300 K using a collision frequency of 1.0 ps^{-1}. Isotropic position scaling was used to maintain the pressure at 1 atm and a relaxation time of 2 ps was used. Periodic boundary conditions were used with a particle mesh Ewald (PME) [19] implementation of the Ewald sum for the description of long-range electrostatic interactions. A cutoff of 10 Å was used for other nonbonded interactions.

Table 1 Important average distance (in Å) in active site

System	imine_taz	imine_sul
Nδ@Asn170-Oε1@Glu166	2.92	2.90
Oε2@Glu166-Nζ@Lys73	2.80	2.82
Nζ@Lys73-O@Ser130	3.31	2.86
Oγ@Ser70-Nζ@Lys73	3.18	3.29
Nζ@Lys73-Oδ@Asn132	2.82	2.84
Nδ@Asn132-O @Asp104	2.88	2.87
N@Ser70-O8@TAZ/SUL	3.08	3.00
N@Ala237-O8@TAZ/SUL	2.98	3.02

To further validate our conclusions, we perform fixed radius clustering based on the mutual root-mean-square deviations (rmsd) of inhibitor atoms using MMTSB [20]. In both systems, the MD snapshots at every 1 ps were abstracted to yield a total of 5000 structures that were clustered together. By setting the clustering radius to 1 Å, three subclusters were obtained in both systems. The conformation with the lowest rmsd from the centroid of each cluster was used as the representative structure.

Results and discussion

Hydrogen bond network in active site

Based on the MD trajectories, we found that several amino acid residues (Ser70 Ala237, Lys73, Ser130, Glu166, Thr167, Asn170, Asn132 and Asp104) around the active site formed hydrogen bonds with substrates or with each other. The

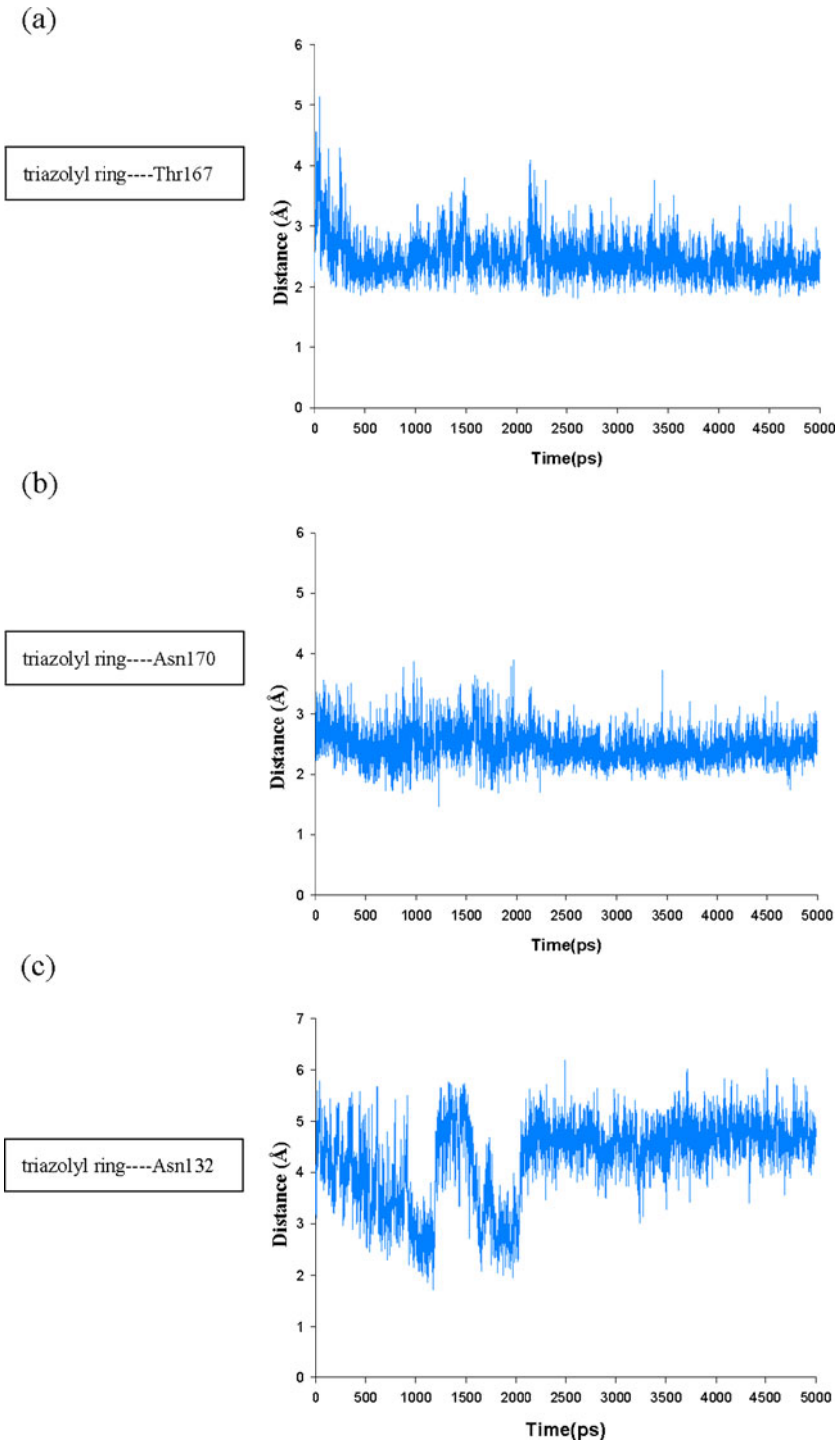

Fig. 2 Change of distance between triazolyl ring of tazobactam and (**a**) Thr167 (**b**) Asn170 (**c**) Asn132 during MD simulation

hydrogen bond network is similar for these two systems. The catalytically and structurally important interatomic average distances of two models were listed in Table 1. Glu166 is known to be the catalytically important residue. The distances indicate that Oε1 and Oε2 of the Glu166 make two rigid hydrogen bonds with the side chains of Asn170 and Lys73. The ammonium group of Lys73 was located on the center of the hydrogen bond network, and it can form four hydrogen bonds with Glu166, Ser70, Ser130 and Asn132 respectively. This is consistent with previous reported experimental results [21, 22]. The Asn132 also made contact with the backbone carbonyl group of Asp104. The entire hydrogen bond network represents a crisscross shape. Besides, the carboxyl oxygen of the sulbactam (O8) was trapped by an "oxyanion hole" that consists of two NH groups of the main chains in Ser70 and Ala237. The average distance of the two hydrogen bonds were about 3 Å in all trajectories. It indicates the conformation of the oxyanion hole is quite stable, and it plays an important role to stabilize the negative charge on the O8 atom.

Different from sulbactam, tazobactam has a triazolyl ring attached to the C2 atom. It means tazobactam has more chance to contact with protein. We have checked the hydrogen bonds between the substrate and enzyme, and found that tazobactam have formed more hydrogen bonds with SHV-1 than sulbactam. In **imine_taz**, it was observed that the triazolyl ring has very close contact with Asn170, Thr167 respectively (Fig. 2). The distance between triazolyl ring and Asn170 was retained rigidly through the simulation. Such a contact also was observed between Thr167 and triazolyl ring, and the average distance was 2.56 Å. We also found a close contact between Asn132 and triazolyl ring at the beginning of the simulation. However, it was not steady and fluctuated from 1.71 to 5.79 Å. Moreover, after 2200 ps the distance was long and the average distance was 4.67 Å. It indicated that Asn132 did not form stable hydrogen bond with triazolyl ring.

The tautomerization from imine to enamine

Previous experimental studies suggested the imine AEI tautomerize to yield a more stable cis or trans-enamine intermediate and thus provide transient inhibition. However, the mechanism and details are still unclear. Herein, we consider conformation of imine intermediate as a key for the subsequent tautomeritions. We felt that the distribution of the trans and cis enamine intermediates must relate to the dynamic behavior of imine. If the imine represents trans conformation (dihedral angle N4-C5-C6-C7 close to 180°), an trans-enamine can be generated by proton transfer from C5 to N4. On the other hand, if the imine represents cis conformation (dihedral angle N4-C5-C6-C7 close to 0°), a cis-enamine can be generated by proton transfer from C5 to N4.

It is interesting to compare the distributing of dihedral angle N4-C5-C6-C7 in **imine_taz** and **imine_sul** (Fig. 3). In **imine_taz**, the average value of dihedral angle N4-C5-C6-C7 is 160°, and the standard deviation is 16.7. The distribution range is from about 90° to 210°, and most of them are located between 150° and 180°, which represents trans conformation. In **imine_sul**, the average value of dihedral angle N4-C5-C6-C7 is 91°, and the standard deviation is 30.2. The range of N4-C5-C6-C7 is about from 30° to 180°, and shows an even distribution. It suggests that the motion of imine AEI of tazobactam was restricted, and the C5-C6 bond cannot rotate freely. This can be ascribed to the interaction of triazolyl ring with Thr167, Asn170. It is obvious that the triazolyl ring was trapped in the Asn_Thr pocket so that it cannot move to the opposite of the active site. Besides, we also found the distance between Asn132-triazolyl ring is related to the change of dihedral angle N4-C5-C6-C7. At the beginning of simulation, when the dihedral angle N4-C5-C6-C7 tends to trans conformation (1200–1500 ps), the distance of Asn132-triazolyl ring was increased. On the other hand, When C5-C6 rotated

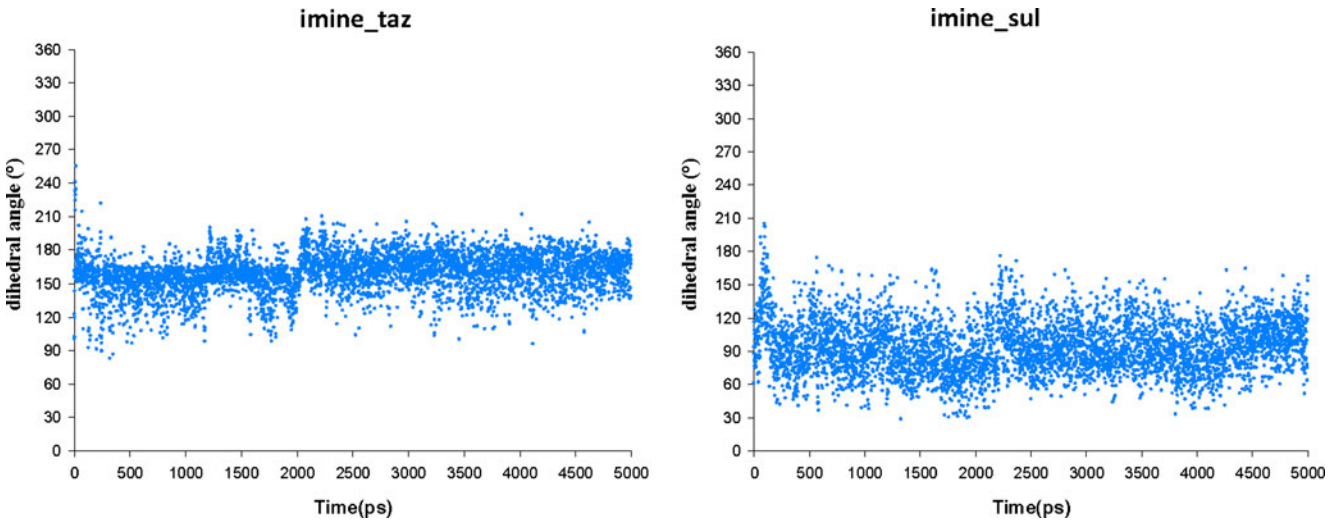

Fig. 3 The distribution of dihedral angle N4-C5-C6-C7 along MD simulations of **imine_taz** and **imine_sul**

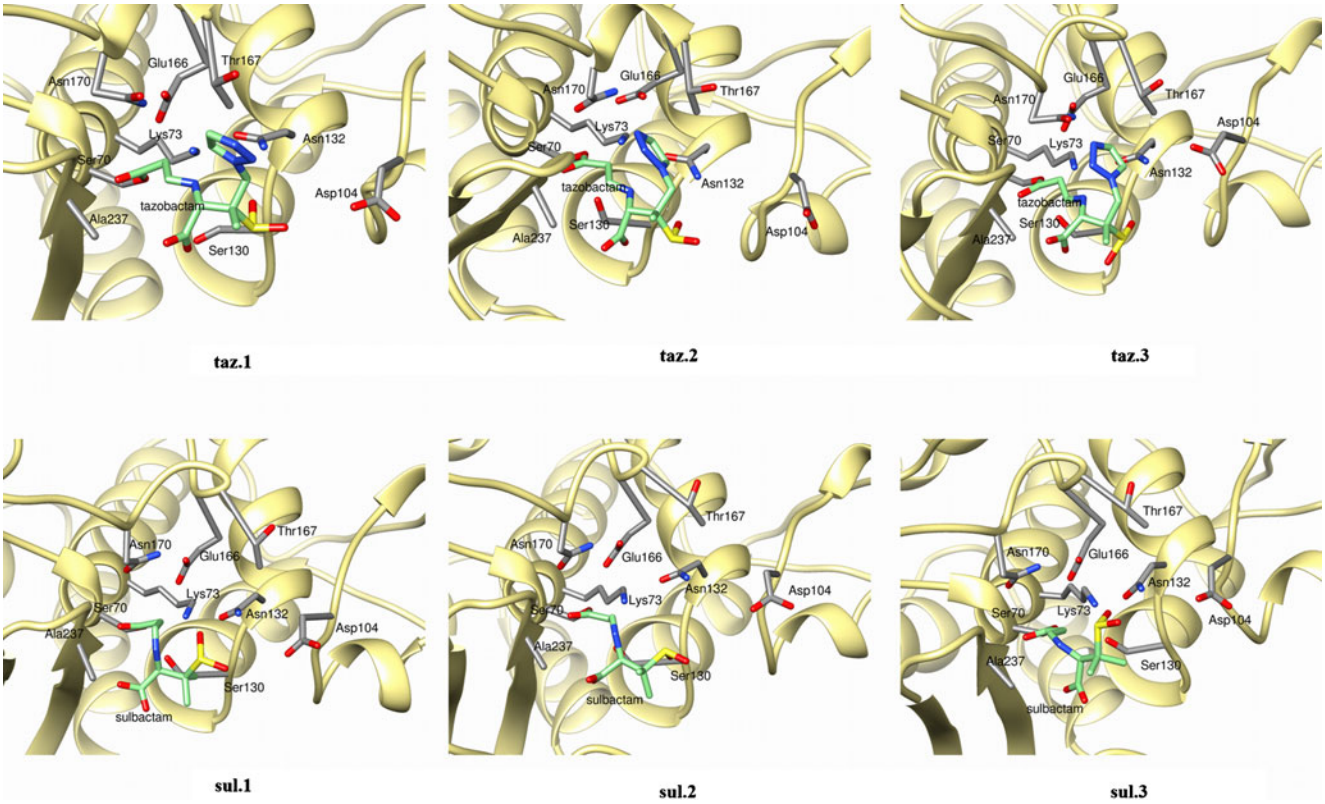

Fig. 4 The representative cluster structures of the two systems

forward cis conformation (1600–2000 ps), the distance between Asn132 and triazolyl ring became short (Figs. 2 and 3). It indicated that Asn132 located on the bottom of the pocket, and has some steric restriction for triazolyl ring motion which made the ring unable to move out from the Asn_Thr pocket. After 2.2 ns, the dihedral angle N4-C5-C6-C7 was stably around 170°, and the distance of Asn132-triazolyl ring was stably around 4.7 Å. It indicated the triazolyl ring had been fully restricted by the Asn_Thr pocket. Based on this, tazobactam is very difficult to yield cis enamine intermediate.

The same feature could be visually investigated by clustering analyses. In **imine_taz** system, three clusters were obtained, and the representative structures are **taz.1**, **taz.2** and **taz.3** (Fig. 4) respectively. Two main clusters (1 and 2) account for more than 99 % of the sampled states. The important hydrogen bonds around the active site of the representative structures from different clusters of two systems are shown in Table 2. It is obvious that the hydrogen-bond networks are consistent in different structures. Besides, hydrogen bonds between triazolyl ring and Asn_Thr pocket were observed in all of the structures. The dihedral angle N4-C5-C6-C7 shows little difference in three structures. The dihedral angle in **taz.1** is 146°, that for **taz.2** is 167°, and that for **taz.3** is 220°. It is obvious that the imine of tazobactam represents trans conformation. In the second system, **imine_sul**, the conformations were also clustered into three, and the representative structures are **sul.1**, **sul.2** and **sul.3** (Fig. 4) respectively. The hydrogen-bond networks are also consistent in

Table 2 Hydrogen bond networks (in Å) in active site of representative structures

Representative structure	taz.1	taz.2	taz.3	sul.1	sul.2	sul.3
Nδ@Asn170-Oε1@Glu166	2.93	2.92	2.89	2.90	2.90	2.91
Oε2@Glu166-Nζ@Lys73	2.84	2.81	2.82	2.81	2.82	2.80
Nζ@Lys73-O@Ser130	3.31	3.31	3.30	2.86	2.87	2.86
Oγ@Ser70-Nζ@Lys73	3.18	3.16	3.20	3.29	3.29	3.30
Nζ@Lys73-Oδ@Asn132	2.80	2.83	2.83	2.84	2.84	2.83
Nδ@Asn132-O @Asp104	2.88	2.88	2.87	2.87	2.88	2.87
N@Ser70-O8@TAZ/SUL	3.08	3.07	3.10	3.01	3.00	2.98
N@Ala237-O8@TAZ/SUL	3.00	2.97	2.96	2.99	3.02	2.98

different structures. Compared with **imine_taz**, the dihedral angle N4-C5-C6-C7 shows some difference. In **sul.1** and **sul.2**, which account for more than 96 % of the sampled states, the dihedral angles N4-C5-C6-C7 are 83° and 110° respectively. In **sul.3**, the dihedral angle N4-C5-C6-C7 is 147°. It indicates that, due to the absence of triazolyl ring, sulbactam has difficulty keeping the trans conformation.

Conclusions

In this work, we have compared the different dynamic behavior of imine AEI of tazobactam and sulbactam. Both tazobactam and sulbactam have similar hydrogen bond networks. However, the triazole group of tazobactam can provide for hydrogen bonding interactions with the enzyme. It is evident that such interactions have an effect on the conformation of tazobactam. The triazole ring is trapped in the Asn_Thr pocket which restricts the C5-C6 bond rotation, so that the imine AEI of tazobactam can just tautomerize to trans-enamine. On the other hand, sulbactam which lacks such restriction can have both cis and trans enamine intermediates.

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