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On the Absolute Configuration in 1,4-Dihydrothiazepine Covalent Complexes Derived from Inhibition of Class A and C β-Lactamases with 6-Methylidene Penems

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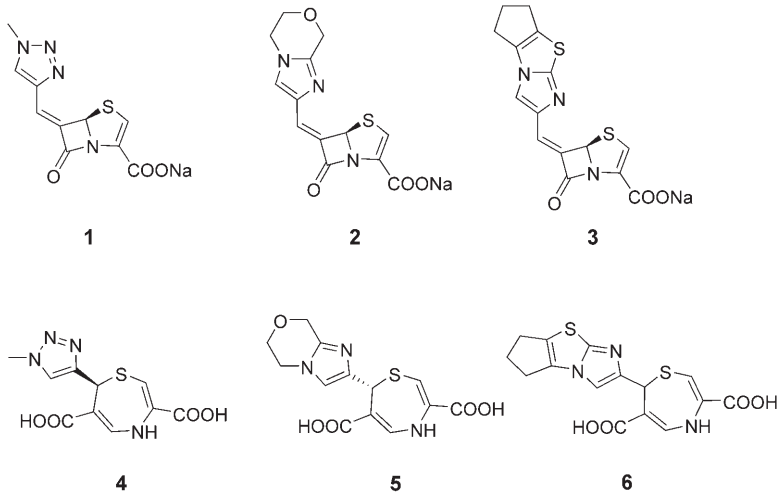
Serine and metallo β-lactamases catalyze the hydrolysis of β-lactam rings in all classes of β-lactam antibiotics which is a major cause of bacterial resistance to β-lactam antibiotics. Bacterial resistance is addressed clinically by combining a β-lactamase inhibitor, such as clavulanic acid, sulbactam, or tazobactam, with a β-lactam antibiotic (amoxicillin or piperacilin).[1–5] Whereas this strategy is effective with the class A β-lactamase inhibitors, there is an urgent need to extend the spectrum of activity to the other classes of serine β-lactamases including the class C enzymes.[6–8] Recently, new promising inhibitors of class C β-lactamases such as NXL104, AVE1330A, and diaroylphosphates have been disclosed.[9–11] Reports from our laboratories on 6-methylidene penems as mechanism-based inhibitors of serine-reactive class A and C β-lactamases disclosed extensive structure–activity relationships with penems containing monocyclic,[12] [6,5]-bicyclic,[13,14] and [5,5,5]-tricyclic[15,16] heterocycles that adopt the *Z* configuration at the C6 position.

The mode of action of penem inhibitors involves acylation by the catalytic serine residues followed by β-lactam ring opening and a sequence of transformations amounting to a remarkable 7-*endo trig* rearrangement reaction. Penems **1**–**3**[16–22] have been studied by a plethora of methods to establish the formation of the 1,4-dihydrothiazepine acyl–enzyme complex (Figure 1). The complex is stable to hydrolysis because of the displacement of water molecules. However, an issue concerns the absolute stereochemistry of the C7 moiety bearing the heterocyles. In dihydrothiazepine **4** bearing the methyltriazolyl heterocycle, the *S*-stereochemistry is evidenced by kinetic,[18–20] computational, and X-ray crystallographic studies[18,21,22] in class A and C enzymes. The dihydroimidazo[2,1-*c*]oxazine thiazepine **5** exists as the *R*-isomer in the crystal structure of both SHV-1 and GC1 enzymes.[13] A novel hydrophobic π-π stacking interaction between the C7 heterocycle with Tyr105 in SHV-1 and Tyr224 in GC1 was revealed. Furthermore, calculated interaction energy differences between C7*R* and C7*S* isomers of eight 6-methylidene penems bearing [6,5]-fused bicyclic heterocycles favor the formation of the C7*R* over the C7*S* enantiomer in both class A and C enzymes (SHV-1, GC1) respectively.[14] Insights into the binding sites in SHV-1 and GC1 revealed a certain degree of flexibility involving displacement of Tyr105 (SHV-1) and Tyr224 (GC1) residues. In the enzyme complex structure with **5**, the hydrophobic π-π stacking interactions with the [6,5]-fused imidazo[2,1-*c*]oxazine ring was clearly evident, which indeed was the case for the other [6,5]-fused heterocycles that were evaluated by the calculated interaction energy method.[14]



1 2 3 4 5 6

Figure 1. Structures of various penem inhibitors and rearranged seven-membered 1,4-dihydrothiazepine ring products.

The [5,5,5]-tricyclic penem **3** rearranges to thiazepine **6** containing both *R* and *S* C7 configurations with 30% and 70% crystal occupancy, respectively (Figure 1) as observed in the GC1 enzymes but exists only as the *R* enantiomer in SHV-1 complex.[15] These results appear somewhat puzzling given the current insights into the mechanistic understanding of the function and structure of serine β-lactamases. As mechanism-based β-lactamase inhibitors that make stabilizing protein–ligand interactions and deacylate slowly can serve as an attractive synthetic strategy for novel β-lactamase inhibition, it was

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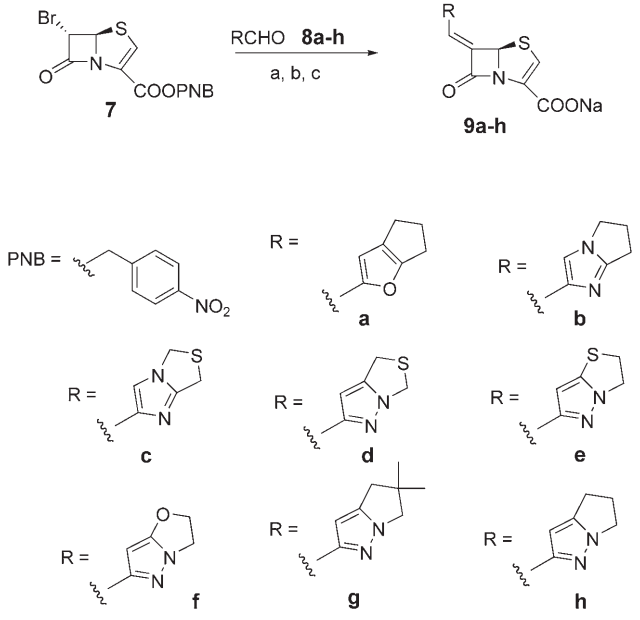
of interest to gain greater insights into understanding factors that control the stereochemical outcome at C7. Towards this end, we reasoned that the hydrophobic π-π stacking interaction between the C7 heterocycle and the side loop and Ω loop tyrosines plays a key role in orienting the heterocycle during the rearrangement events.

This hypothesis seemed reasonable as monocyclic rings smaller than [6,5] heterocycles lack this specific interaction and favor the opposite *S* stereochemistry.[18–20] Thus, penems with [5,5]-bicycles became of interest to us as synthetic targets to probe this hypothesis and clarify our understanding of the function of serine β-lactamase. In this communication we report our studies on the synthesis of novel and potent [5,5]-fused bicyclic 6-methylidene penems and their binding preference in class A and C β-lactamases. This series provides insights concerning the orientation of C7 heterocycle in the dihydrothiazepine–enzyme complexes.

The desired penems with furan, imidazole, and pyrazole-fused bicyclics at the 6-methylidene position were selected on the basis of modeling studies. Reaction between (5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo(3.2.0)hept-2-ene-2-carbocylic acid 4-nitrobenzyl ester **7** and aldehydes **8a–h** followed by a reductive elimination/deprotection step produced penems **9a–h** with *Z* configuration[23] (Scheme 1). The desired 5,5-bicyclic aldehydes **8a–h** required for these transformations were synthesized by utilizing different methods as described previously.[24]

The in vitro enzyme inhibition was carried out against TEM-1 (class A) and AmpC (class C) enzymes and the respective IC_{50} values of the bicyclic [5,5]-fused penems are listed in Table 1 with tazobactam as the standard and comparator.[25] These penems



Scheme 1. General synthesis of penems **9a–h**. Reagents: a) $MgBr_2/Et_3N$/THF:acetonitrile; b) Acetic anhydride/ 0–20 °C; c) Activated zinc/phosphate buffer pH 6.5/THF:acetonitrile/RT.

Table 1. Enzyme activity of penems **9a–h** against different β-lactamase.[a]

Inhibitor	TEM-1	AmpC
Tazobactam	100 ± 8	84 000 ± 300
9a	2 ± 1	2 ± 1
9b	1 ± 0.5	2 ± 1
9c	3 ± 1	3 ± 1
9g	0.75 ± 0.5	2 ± 1
9h	1 ± 0.2	1 ± 0.5
9d	1 ± 0.4	1 ± 0.5
9e	2 ± 1	2 ± 1
9f	1 ± 0.1	1 ± 0.2

[a] IC_{50} [nM].

with *Z*-configuration **9a–h** proved to be potent inhibitors of TEM-1 with IC_{50} values between 0.75 and 3 nM against both TEM-1 and AmpC enzymes. With potent inhibitors in hand in this series, the preference for either the C7*R* or C7*S* stereochemistry in the 1,4-dihydrothiazepine complexes was determined by comparing calculated interaction energy differences of 1,4-dihydrothiazepines **10–12** (Figure 2) in SHV-1 and GCI

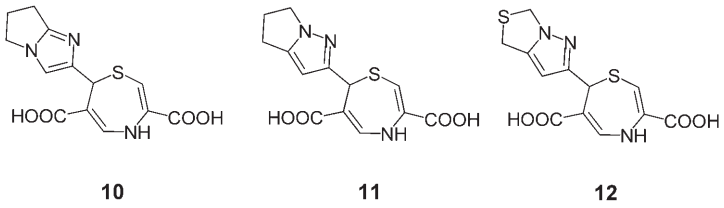


Figure 2. Structures of 1,4-dihydrothiazepines **10–12**.

enzymes. The enzymes were chosen because the high resolution crystal structure complexes for penems **2** and **3** were obtained in these systems.[13,15] This method was established earlier with the [6,5]-fused heterocycle inhibitors and corroborated well with data from X-ray crystallography for penem **2**.

The interaction energies in SHV-1 indicate that the *S*-enantiomers of all three thiazepines are preferred by 16, 18, and 23 kcal mol^{-1} respectively, over the corresponding *R*-enantiomers (Table 2). Interestingly, all stereoisomers of thiazepines **10–12** form π-π stacking interactions with Tyr105 in SHV-1, however, the *S*-enantiomers make additional nonpolar interactions with Ala237 and Tyr105 side chains through the thiazepine ring. In regards to H-bonding interactions, the *R*-enantiomers (Figure 3a) make two strong H-bond interactions (thiazepine-NH/Ala237-CO and imidazole-N/Ser130-OH) and two

Table 2. Calculated interaction energy differences in SHV-1 and GC1 enzymes.[a]

Thiazepine	Δ*IE* SHV-1	Δ*IE* GC1
10	16	−45
11	18	−41
12	23	−18

[a] Δ*IE* = *IE R*-stereoisomer-*IE S*-stereoisomer in kcal mol^{-1}.

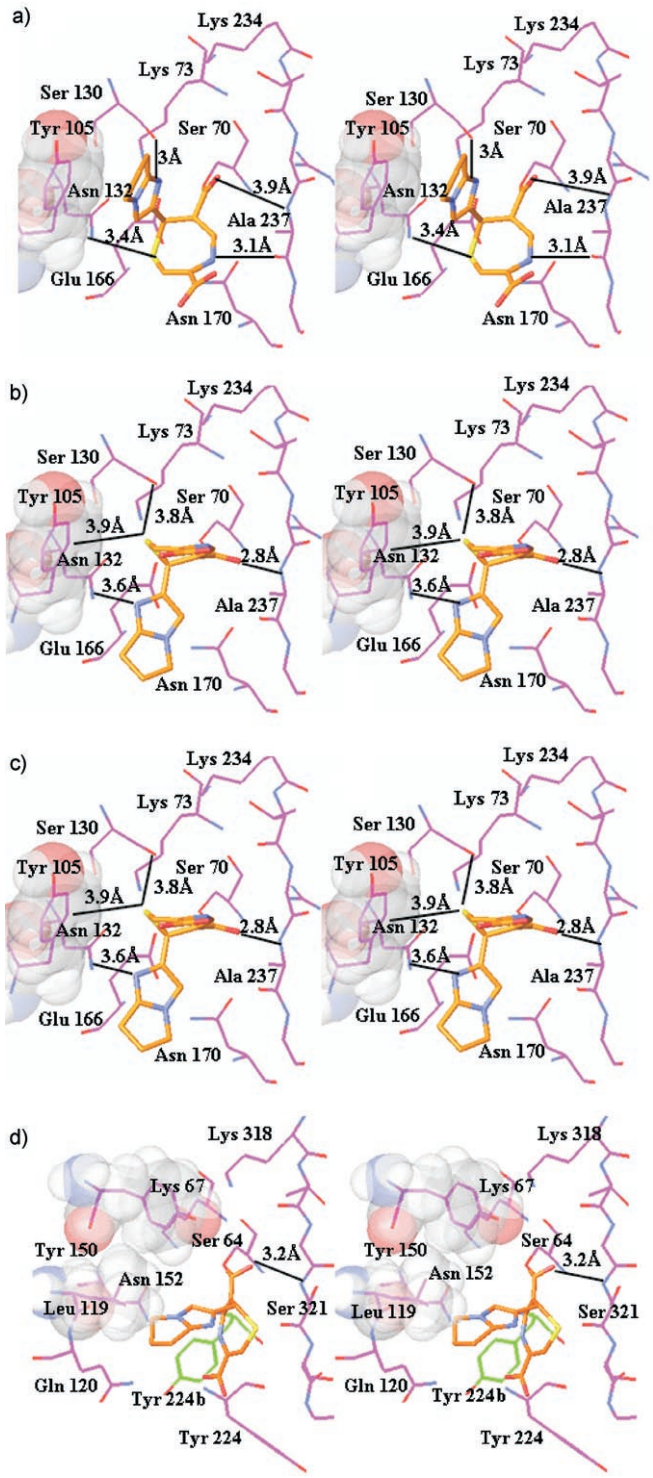


Figure 3. a) SHV-1/compound **10**-*R*-complex; b) SHV-1/compound **10**-*S*-complex; c) GC1/compound **10**-*R*-complex; d) GC1/compound **10**-*S*-complex.

weaker H-bonds (thiazepine-S/Asn132-NH_2 and thiazepine-CO/Ala237-NH) whereas, the *S*-enantiomers form two H-bond interactions (thiazepine-NH/Ala237-CO and imidazole-N/Asn132-NH_2) and one weaker H-bond (thiazepine-S/Ser130-OH) as shown in Figure 3b. It is likely that the *S*-enantiomer's additional nonpolar interactions with the enzyme contributed to its larger interaction energy.

In contrast to SHV-1, the interaction energies in GC1 of thiazepines **10**–**12** indicate that the *R*-enantiomers are favored by 45, 41, and 18 kcal mol^{-1}, respectively over the corresponding *S*-enantiomers (Table 2). In these cases, none of the thiazepines form a hydrophobic π-π stacking interaction with Tyr224. The preference for *R*-stereochemistry is based on strong H-bond interactions through the carboxylate anion, thiazepine-NH, and thiazepine-CO (Figure 3c). The *S*-enantiomer makes only one H-bond interaction with the enzyme (thiazepine-CO/Ser321-NH) as depicted in Figure 3d. As for nonpolar interactions, *R*- and *S*-enantiomers make almost the same contacts with the enzyme but the interacting ligand regions, heterocycle and thiazepine are interchanged.

Our studies aim at understanding the mechanism of inhibition and function in class A and C β-lactamases upon inhibition by 6-methylidene penems. The [5,5]-fused heterocyclic penems offer the possibility to probe the hypothesis of the significance of π-π stacking with Ω and side loop tyrosines in these enzymes which could orient the base in a certain stereochemical preference. Interaction energy calculations demonstrate that despite the existence of π-π stacking with the side loop Tyr105 in SHV-1, the base orientation is influenced by additional inhibitor–enzyme interactions. In GC1 such π-π stacking interactions do not seem to play an important factor for this particular class of penems in contrast to [6,5]-bicyclic or [5,5,5] tricyclic inhibitors.[14,15]

The flexibility in the binding site is due in large part to the movement of side loop tyrosine Tyr 105 (SHV-1) and Ω loop tyrosine Tyr 224 (GCI) which led to the dominance of van der Waals and stacking interactions for binding. This work clearly demonstrates that the binding mode and the stereochemistry of the C7 heterocycle of a [5,5]-bicyclic 6-methylidene penem may be different between class A and class C enzymes. It is of interest to note that the size difference in the terminal ring in [5,5] relative to [6,5]-bicyclic heterocycles results in a dramatic binding mode difference in class A but not class C enzymes. Our results are also consistent with the findings for penem **1**[18–22] which carries a monocyclic moiety (Table 3). In class C enzymes, major contributions to the interaction energies in dihydrothiazepines **10**–**12** is achieved by ionic interactions over hydrophobic ones. This was not expected given the precedents in the [6,5]-bicyclic and [5,5,5]-tricyclic systems.

In conclusion, the [5,5]-fused bicyclic penems are potent inhibitors of the class A and C enzymes. On the basis of computational and modeling methods,[26] 1,4-dihydrothiazepines **10**–

Table 3. Stereochemistry of 1,4-dihydrothiazepine acyl complexes.

Thiazepine	Stereochemistry Class A	Stereochemistry Class C	Method/Reference
4	*S*	*S*	Kinetics[18–20], computational studies[18,21] X-ray crystallography[22]
5	*R*	*R*	X-ray crystallography[13]
6,5-bicyclic	*R*	*R*	Computational studies[14]
10, **11**, **12**	*S*	*R*	Computational studies
6	*R*	*R*:*S* (30:70)	X-ray crystallography[15]

12 form π-π stacking with the side loop tyrosine residues in class A but not Ω loop tyrosine in class C enzymes. This series provided further insights concerning the mechanism of inactivation and the stereochemical question of the C7 absolute configuration in serine β-lactamases by 6-methylidene penems. In this set of [5,5]-fused bicyclic inhibitors, opposite preference of C7 stereochemistry in SHV-1 and GC1 enzymes is predicted. This work reconciles differences in the literature reported in this class of penem inhibitors.

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[26] Computational Methods: Protein–ligand complexes of the *R*-enantiomers were built by modifying the ligand in 1ONG (SHV1/**2** X-ray structure) and 1ONH (GC1/**2** X-ray structure). The *S*-enantiomers were modeled by modifying the ligand in 1Q2Q (GC1/**3** X-ray structure) and replacing the *R*-enantiomers in the above written models with modeled *S*-enantiomers. Hydrogen atoms were added in all the models using Sybyl7.0/Biopolymer module and solvated with the Tripos water model by applying periodic boundary conditions. All protein–ligand complexes were energy minimized at a constant dielectric of one with the conjugated gradient method and the MMFF94 force field. Protein-ligand interaction energies were calculated by subtracting the internal energies of the protein and ligand from the internal energy of the protein–ligand complex. Preference for *R* or *S*-enantiomer was determined by comparing calculated interaction energies in SHV-1 or GC1 enzymes.

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