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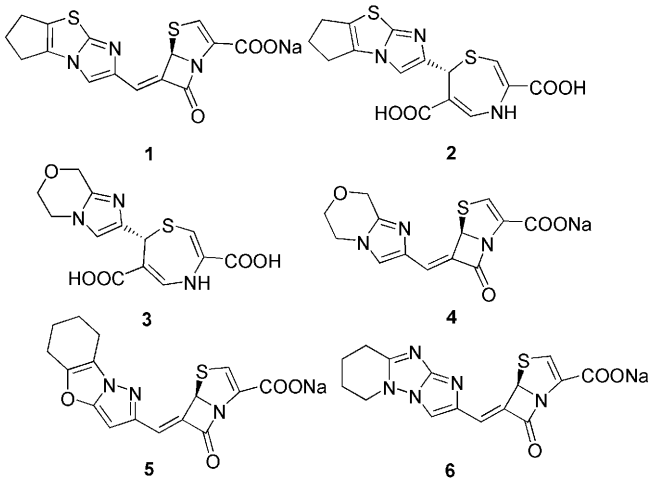
Targeting Val 216 in Class A β-Lactamases with Tricyclic 6-Methylidene Penems

Aranapakam Venkatesan,[a] Atul Agarwal,[a] Takao Abe,[b] Hideki Ushirogochi,[b] Tsuyoshi Takasaki,[b] Ado Mihira,[b] and Tarek S. Mansour*[a]

In memory of Hassan Elokdah, Ronald Magolda and John Morin

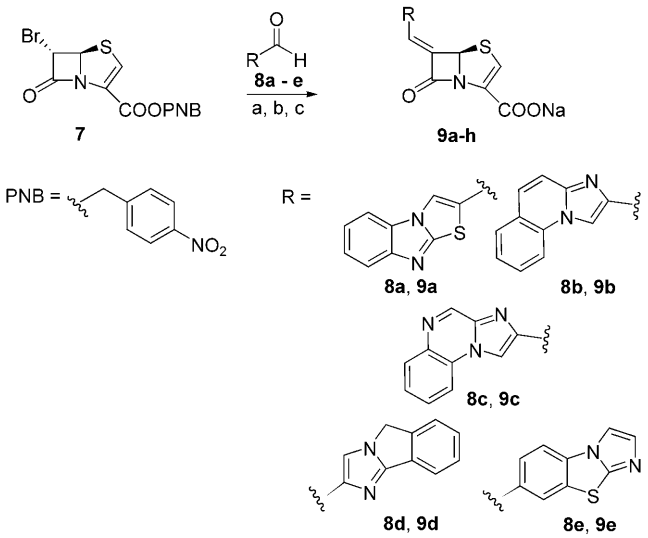
Recently, we reported on the discovery and biological activities of tricyclic 6-methylidene penems as mechanism-based inhibitors of serine β-lactamases.[1–3] In combination with piperacilin, these inhibitors proved to be highly potent in inhibiting the hydrolysis of β-lactam rings, which is a major cause of bacterial resistance to β-lactam antibiotics. Earlier efforts focused on [5,5,5]-tricyclic penems, such as compound **1**, which rearranges



to thiazepine **2** upon binding to serine β-lactamases, with C_7 *R* configuration in the SHV-1 complex (class A enzyme).[1] The complex structure indicates similar interactions with the protein as thiazepine **3**, derived from the bicyclic penem **4** involving Ser 70, Ala 237, Asn 132 and π–π stacking interactions between the C_7 tricycle with Tyr 105.[4] Further modifications of the tricyclic core structure by incorporating terminal lipophilic 6-membered rings in penems **5** and **6** provided potent inhibitors of TEM-1 and Amp-C enzymes (classes A and C) with IC_{50} values of 4.0, 5.5 nM and 2.4, 1.4 nM, respectively.[5] Herein, we report our studies on the design, synthesis and activity of novel potent and broad spectrum tricyclic 6-methylidene penems containing a terminal aromatic ring.

The design of new tricyclic penem inhibitors took into consideration the specific amino acids interactions found in the co-crystal structure of compound **1** with SHV-1. Docking the 1,4-dihydro-thiazepines with novel C_7 tricycles into the active site of SHV-1 (PDB code 1Q2P) identified certain important features for binding including the interaction of the acyl bond with Ser 70, Ala 237-N4 and Asn 132-S. Terminal aromatic rings as new structural features were deemed worthy of further investigation. Our choice in designing inhibitors based on the SHV-1 complex structure with compound **1**, and not the GC1 complex (PDB code 1Q2Q), is due to the formation of two thiazepines differing at the C_7 configuration in the latter complex.

Reaction of compound **7** ((5*R*, 6*S*)-6-bromo-7-oxo-4-thia-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylic acid 4-nitrobenzyl ester) with aldehydes **8a**–**e**, followed by a reductive elimination/deprotection step, produced penems **9a**–**e** with the *Z* configuration[6] (Scheme 1). Aldehydes **8a**–**e** were prepared by different methods as depicted in Schemes 2–5.



Scheme 1. General synthesis of penems **9a–e**. Reagents and conditions: a) $MgBr_2$, Et_3N, THF:MeCN (1:1); b) Ac_2O, 0 °C–20 °C; c) activated zinc, phosphate buffer (pH 6.5), THF:MeCN (1:1), RT, 2–4 h.

Aldehyde **8a**, required to prepare the final compound **9a**, was prepared by a two step procedure. The commercially available 2-mercapto benzimidazole **10**, on reaction with 2-bromomalonaldehyde, gave the uncyclized S-alkylated compound **11** in 85% yields. Cyclization was affected by heating

[a] *Dr. A. Venkatesan, Dr. A. Agarwal, Dr. T. S. Mansour*
Chemical and Screening Sciences, Wyeth Research
401 North Middletown Road, Pearl River, NY 10965 (USA)
Fax(+1) 845-602-5580
E-mail: mansout@wyeth.com

[b] *Dr. T. Abe, Dr. H. Ushirogochi, T. Takasaki, A. Mihira*
Medicinal Chemistry, Wyeth KK, Saitama (Japan)

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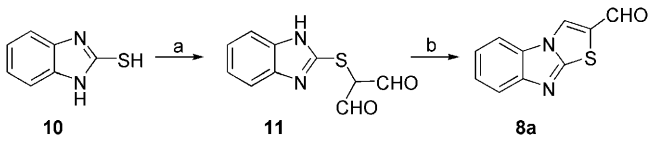
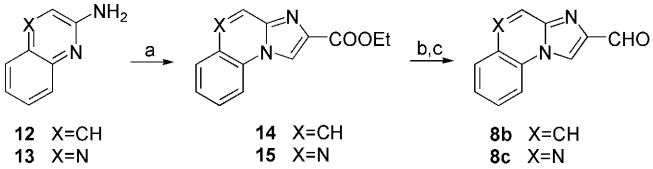
compound **11** in DMF at 100 °C in the presence of a catalytic amount of acetic acid to yield aldehyde **8a** (Scheme 2). Aldehydes **8b** and **8c** were prepared from 2-aminoquinoline **12**

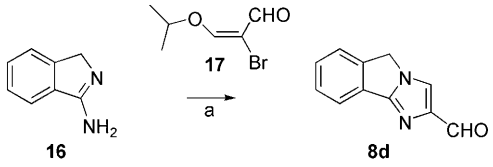


Scheme 2. Synthesis of aldehyde **8a**. Reagents and conditions: a) K_2CO_3, $BrCH(CHO)_2$, DMF, 80 °C; b) DMF, AcOH, 100 °C, 2 h.

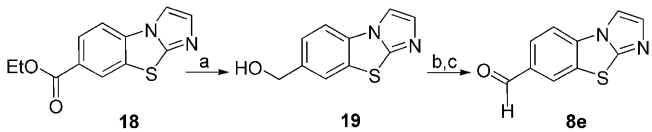
and 2-aminoquinoxaline **13**, respectively, by condensation with ethyl bromopyruvate to afford imidazo esters **14** and **15** in a regioselective manner. The regiochemistry in the cyclization to **14** and **15** results in the formation of the less encumbered esters. Reduction of esters **14** and **15** to the corresponding alcohols followed by MnO_2 oxidation afforded **8b** and **8c** in good yields (Scheme 3). In the case of aldehyde **8d**, direct condensation of 1*H*-isoindol-3-amine **16** with compound **17** produced **8d** regioselectively (Scheme 4). Commercially available imidazothiazole **18** was reduced to alcohol **19**, followed by MnO_2 oxidation to produce aldehyde **8e** (Scheme 5).



Scheme 3. Synthesis of aldehydes **8b** and **8c**. Reagents and conditions: a) $BrCH_2COCO_2Et$, DME, sealed tube, reflux ; b) $LiAlH_4$, THF, 0 °C; c) MnO_2, $CHCl_3$, reflux, 24 h.



Scheme 4. Synthesis of aldehyde **8d**. Reagents and conditions: a) MeCN, reflux, 8 h.



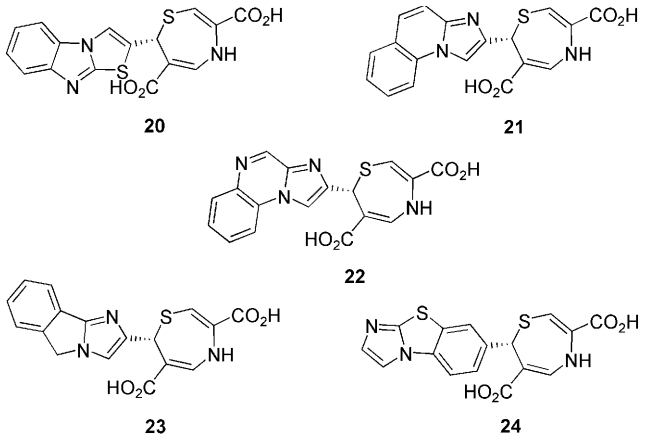
Scheme 5. Synthesis of aldehyde **8e**. Reagents and conditions: a) Bromo acetaldehyde diethyl acetal, $LiAlH_4$, THF, 0 °C; b) MnO_2, $CHCl_3$, reflux.

The in vitro enzyme inhibition assay was carried out against TEM-1 (class A similar to SHV-1) and AmpC (class C similar to GCI) enzymes and the respective IC_{50} values of the penems are listed in Table 1, with tazobactam as the standard agent for comparison.[2] Penems **9a–e** proved to be potent inhibitors with IC_{50} values of 2.5–14.5 nM against TEM-1, and 1.2–46.9 nM against AmpC (Table 1).

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

Inhibitor	TEM-1 IC_{50} [nM]	Amp-C IC_{50} [nM]
Tazobactam	100	84,000
9a	14.5	46.9
9b	3.4	2.1
9c	2.5	1.2
9d	2.6	2.8
9e	4.5	9.5
1	1.4	2.1

Molecular modeling studies were carried out on thiazepines **20**–**24**. The compounds were docked into the active site of SHV-1, based on the structure of SHV-1 in complex with thiazepine **2**. Results indicated that thiazepine **20**, which differs from thiazepine **2** by substitution of a sulfur atom for a nitrogen and replacement of a cyclopentyl for a phenyl group, interacts similarly with SHV-1 but is ~10-fold less potent , based on a relative comparison of penems **9a** and **1** (Table 1). This decrease in potency is likely due to the heteroatom substitution in the imidazothiazole moiety. Moreover, by restoring the imidazo moiety in thiazepines **21** and **22**, but expanding the central ring, enhancement of the inhibitory potency of these thiazepines over thiazepine **20** was realized. In these thiazepines, similar amino acids interact with **1**, including the Ser130–imidazo N2 interaction.



From a structural perspective, thiazepine **23** offers interesting features in that the central 5-membered ring directs the terminal phenyl ring closer to Val216 while maintaining key interactions with Ser70, Ser130, Asn132 and Ala237.The Val216 distance of 3.7 Å is within the van der Waals interaction energy range (Figure 1a). Thiazepine **24**, in which the phenyl ring is attached to the dihydrothiazepine, offers an alternative way to reach out to Val216. As depicted in Figure 1b, the Val216–S interaction is ~3.7 Å, but in this case the Ser130 H-bonding interaction with the imidazo moiety is lost as a consequence of

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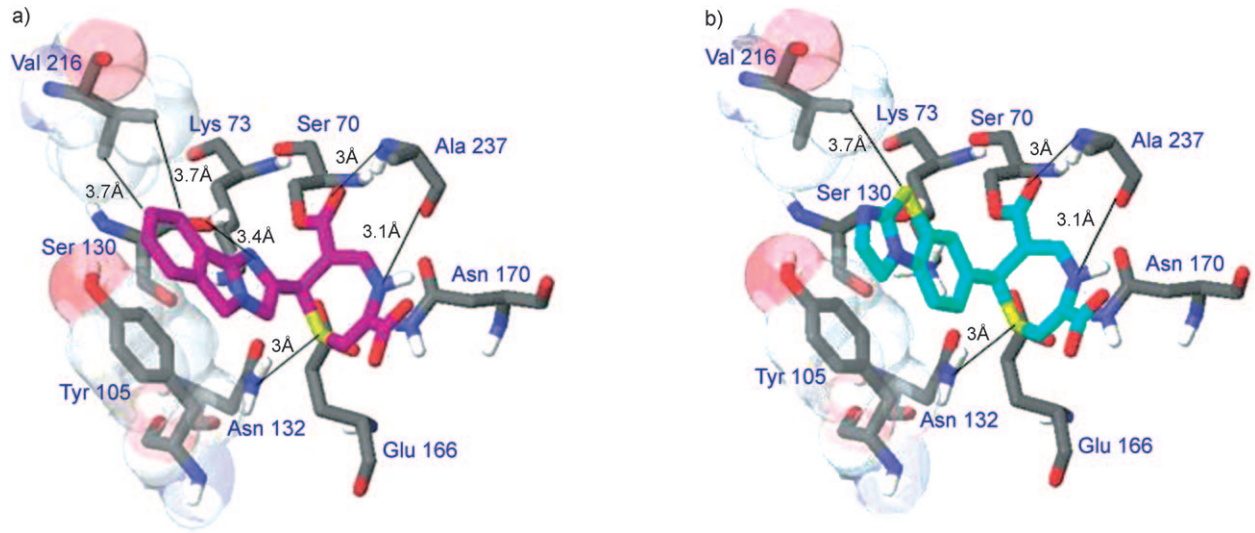

Figure 1. a) SHV-1–thiazepine **23** complex; b) SHV-1–thiazepine **24** complex.

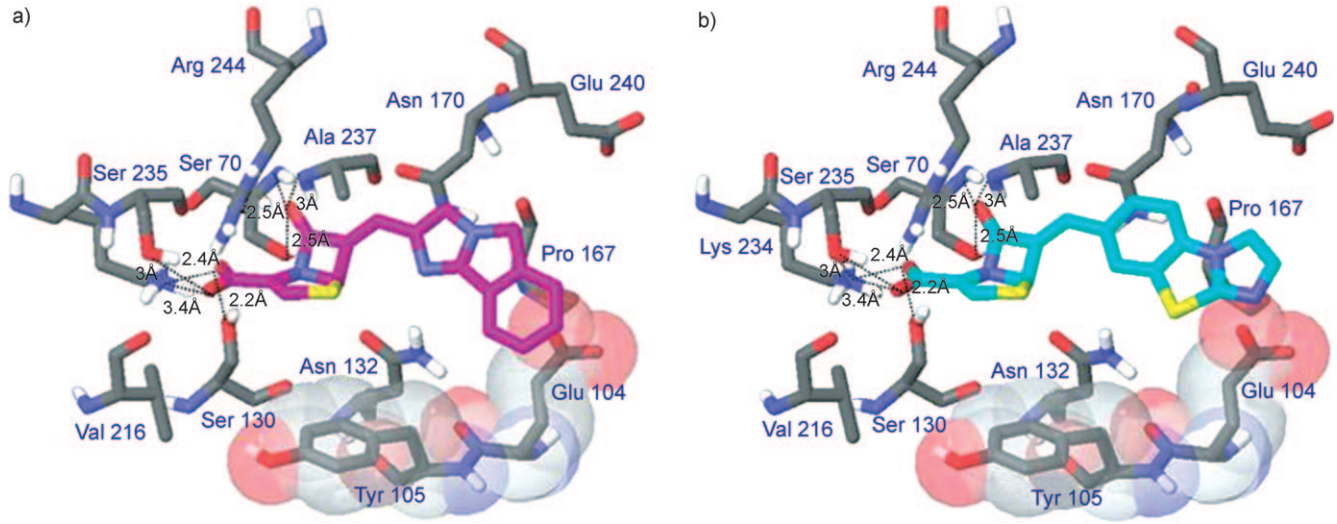

Figure 2. a) TEM-1–penem **9d** complex; b) TEM-1–penem **9e** complex.

introducing the phenyl ring tether with the 7-membered thiazepine ring. Despite the observation that the π–π interaction of Tyr 105 with the heterocyclic bases of both compounds **23** and **24** appear to be weaker than with compounds **20**–**22**, penems **9d** and **9e** are potent inhibitors of both TEM-1 and AmpC enzymes. Interestingly, the AmpC potency tracked well with that of TEM-1 for all inhibitors, but in this study, no attempts have been made to rationalize this finding.

In order to better understand the significance of targeting the Val 216 residue, the penems corresponding to compounds **23** and **24** were docked in the active site of TEM-1.[5,7] Figure 2a indicates that the tricyclic moiety of penem **9d** is removed from both Val 216 and Tyr 105, and therefore does not interact with these residues upon initial binding. This was also found with penem **9e**. As was shown for the [5,5] and [6,5] bicyclic 6-methylidene penems,[8,9] the flexibility in the binding site is largely due to the movement of Tyr 105 (SHV-1) and other residues to accommodate the rearranged thiazepine structures.

Earlier reports on the mechanism of inactivation of clavulanate implicated Val 216 in proton-transfer reactions.[10] This work highlights that new amino acid residues, such as Val 216, that have not been previously targeted, offer alternative possibilities for the design of inhibitors. We should emphasize that some understanding of the mechanism of inhibition for a given class of inhibitors is necessary, for example the penem–thiazepine rearrangement in the 6-methylidene penem class.

In conclusion, the tricyclic penems containing [5,5,6], [5,6,6] and [6,5,5] fused tricycles are potent inhibitors of both class A and C enzymes. This work emphasizes the need to focus on the rearranged product, which is the biologically active species rather than the penem itself. On the basis of modeling studies, new interactions with Val 216, in addition to Tyr 105, were identified for the 1,4-dihydrothiazepines of penems **9d** and **9e**.

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These inhibitors highlight the differences between modeling the penem itself and the rearranged thiazepine product, which is an important consideration for the design of new inhibitors.

Experimental Section

In vitro assay: The enzyme concentrations for TEM-1, and AmpC were 4.3, and 2.1 nM, respectively. A wide range of inhibitor concentrations were prepared in 50 nM PO4, pH 7.0 to include the possible IC_{50} values. The substrate used to initiate the enzyme reaction was nitrocefin at 50 ug/mL in the same buffer as the inhibitor. Initially, the enzyme and inhibitor (20 uL each) were preincubated for 10 min at 25 °C prior to the addition of 160 uL volume of nitrocefin. Initial rates of hydrolysis were monitored for 5 min at 495 nm The inhibitor concentration that caused a 50% reduction in the enzymatic activity (IC_{50}) was determined graphically.

Computational methods: Protein:ligand complexes of the R-enantiomers were modeled by modifying the ligand in 1Q2P. Hydrogen atoms were added in all the models using MOE2007.09 and solvated with the MOE 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. The choice of C7 R isomers is based on the crystal structure of **1** in SHV-1[1] as well as our earlier findings in bicyclic systems as reported by crystallography and computational methods.[8]

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Keywords: β-lactamases · 6-methylidene penems · inhibitors · molecular modeling · stacking interactions

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