



Side chain SAR of bicyclic β -lactamase inhibitors (BLIs). 1. Discovery of a class C BLI for combination with imipenem

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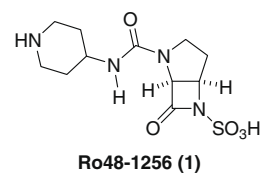
ABSTRACT

Bridged monobactam β -lactamase inhibitors were prepared and evaluated as potential partners for combination with imipenem to overcome class C β -lactamase mediated resistance. The (*S*)-azepine analog **2** was found to be effective in both in vitro and in vivo assays and was selected for preclinical development.

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Carbapenem antibiotics (e.g., imipenem) have played an important role in the treatment of nosocomial bacterial infections for over twenty years.¹ However, some *Pseudomonas* strains have developed resistance to carbapenems via porin loss and expression of β -lactamase enzymes (BLs) that can hydrolyze and inactivate carbapenems.² The hundreds of known BLs are divided into four classes (A–D) based on their structures.³ Class A BLs are a major cause of penicillin resistance in Gram-positive bacteria, while carbapenem resistance in *Pseudomonas* is mediated by class C BLs such as AmpC.⁴ Several β -lactamase inhibitors (BLIs) have been developed that effectively restore antibacterial activity against class A BL-expressing strains when combined with a penicillin (e.g., clavulanate with amoxicillin).⁵ Similarly, addition of an effective class C BLI to a carbapenem antibiotic should restore antibacterial efficacy against class C BL-producing strains. To date, no class C BLI has been brought to market although several have been reported.⁶

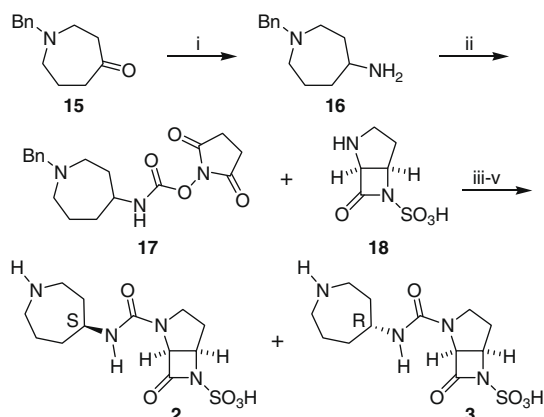
Several years ago, we initiated a program to discover a class C BLI for combination with imipenem/cilastatin (IPM/CIL). Our initial focus was a collaboration with Methylgene based on their phosphonate BLIs.⁷ We subsequently expanded our effort to include analogs of the bridged monobactam BLIs (e.g., Ro48-1256, **1**) previously reported by Roche.^{6b, c}



Molecular modeling studies using a crystal structure of **1** bound in the active site of AmpC as a starting point indicated that there was room for a larger side chain than the piperidine present in **1**. To test this hypothesis, we prepared the seven-membered (azepine) side chain analogs **2** and **3** (Scheme 1). The requisite side chain was prepared from the commercial *N*-benzylazepinone **15**.⁸ Reductive amination of **15** afforded the racemic amine **16** in 52% yield. Reaction of **16** with *N,N'*-disuccinimidyl carbonate afforded the activated carbamate **17**. Coupling of **17** and the chiral bridged monobactam core **18**^{6b,9} followed by debenzoylation and HPLC separation of the diastereomers afforded **2** and **3**. The stereochemistry of the two diastereomers was determined by X-ray crystal structures of the complexes of **2** and **3** bound (separately) in AmpC (Fig. 1).¹⁰

We were pleased to find that the (*S*)-isomer **2** was more active than **1** in our enzyme inhibition assay^{11a,b} and in vitro synergy assay (Table 1).^{11a,c} In addition to allowing assignment of side-chain stereochemistry, the X-ray crystal structures also provided a possible explanation for the differential activity of **2** and **3**. As expected,

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Scheme 1. Reagents and conditions: (i) $\text{Ti}(\text{O}^i\text{Pr})_4$, NH_3 , EtOH, then NaBH_4 , 52%; (ii) N,N' -disuccinimidyl carbonate, MeCN; (iii) NaHCO_3 , MeCN, H_2O , 51%; (iv) $\text{Pd}(\text{OH})_2$, MeOH, AcOH, H_2O , 40 psi H_2 , 20%; (v) HPLC on phenomenex synergis polar RP80 column eluted with MeOH/ H_2O .

the bicyclic cores of **2** and **3** occupy essentially the same position relative to the enzyme since the lactam carbonyl in this part of the molecule forms a covalent bond to the active site serine. Surprisingly, however, the side chain nitrogen atoms of **2** and **3** also occupy the same position relative to the enzyme due to an interaction (via a water molecule) with the oxygen of Y249 below. Because of the fixed locations of the core and the azepine nitrogen, the (*S*)-azepine **2** has three carbon atoms to the left of the binding pocket and two carbon atoms to the right, while the reverse is true of the (*R*) isomer **3** (i.e., the azepine ring is flipped). This results in an increased interaction of the azepine carbons of **3** with Q146 and less freedom of motion for the side chain of this amino acid. In fact, the side chain of Q146 is actually observed in two conformations (~60:40 ratio) in the crystal structure of AmpC with bound **2** while in the crystal structure with **3** bound this side chain is primarily in one conformation. It is possible that the tighter interaction of the hydrophobic azepine carbon atoms of **3** with the polar oxygen of Q146 may account for the increased enzyme IC_{50} observed for this compound. However, it should be noted that the azepine shift is small and it is possible that the observed difference in activity may be due to some other cause. Interestingly, before the X-ray data was available, molecular modeling had qualitatively predicted that **2** would be more active.

Once the stereochemistry of the active diastereomer was established, a chiral synthesis of the side chain amine (*S*)-**23** was developed (Scheme 2). Ring expansion of CBZ-piperidone **19**, followed by decarboxylation of **20**, afforded the protected azepinone **21**. Reductive amination of **21** using (*R*)-*t*-butyl sulfinamide according to the general method of Ellman et al.¹² afforded a mixture of diastereomeric sulfinamides **22** that were separated by chiral HPLC. Hydrolysis of the slower eluting diastereomer afforded the desired (*S*)-**23**.

Additional analogs (**4–9**) were prepared to explore the effect of altering the heteroatom and its location (Scheme 3). DIBAL reduction of the seven-membered lactams **24a–c**¹³ afforded the amines **25a–c**. Acylation of the amines followed by condensation of the activated intermediates with the bicyclic core amine **18** afforded the penultimate intermediates, which were deprotected to afford final products **4–6**. Although the overall yields of this sequence were generally low, sufficient material was obtained for evaluation of all three analogs in our in vitro screens. The diastereomeric analog **7** was obtained by the same route starting with the enantiomer of **24c**. Lactam **8** was prepared in 54% overall yield by direct condensation of amide **24a** with N,N' -disuccinimidyl carbonate followed by reaction with **18**. Carbacycle **9** was prepared from aminocycloheptane.

The eight-membered and nine-membered side chains **29**, **30a,b**, and **31a,b** were prepared by ring expansion of azepinone **21** (Scheme 4).

Side chains **29**, **30a,b**, and **31a,b** were then converted to the eight- and nine-membered analogs **10–14** via the standard activation, condensation, and deprotection sequence (Table 2). Note that compounds **11–14** are single diastereomers of undetermined stereochemistry.

As previously noted, the (*S*)-azepine analog **2** was a more efficient inhibitor of the pseudomonal class C β -lactamase AmpC than the piperidine **1**. This improvement in enzyme inhibition was reflected in the increased in vitro synergy with IPM observed for **2**. The (*R*)-azepine analog **3** was less active against the enzyme and exhibited reduced in vitro synergy. Interestingly, the closely related (*S*)-azepine analog **4**, in which the ring nitrogen has been shifted one atom closer to the exocyclic nitrogen, exhibited enzyme inhibition comparable to **2** but much reduced synergy with IPM, perhaps due to reduced cell penetration or increased efflux. Addition of a second nitrogen atom (e.g., **5**) resulted in a substantial reduction in both enzyme inhibition and synergy. Addition of an oxygen atom at the same position (e.g., **6** and **7**), led to a slight

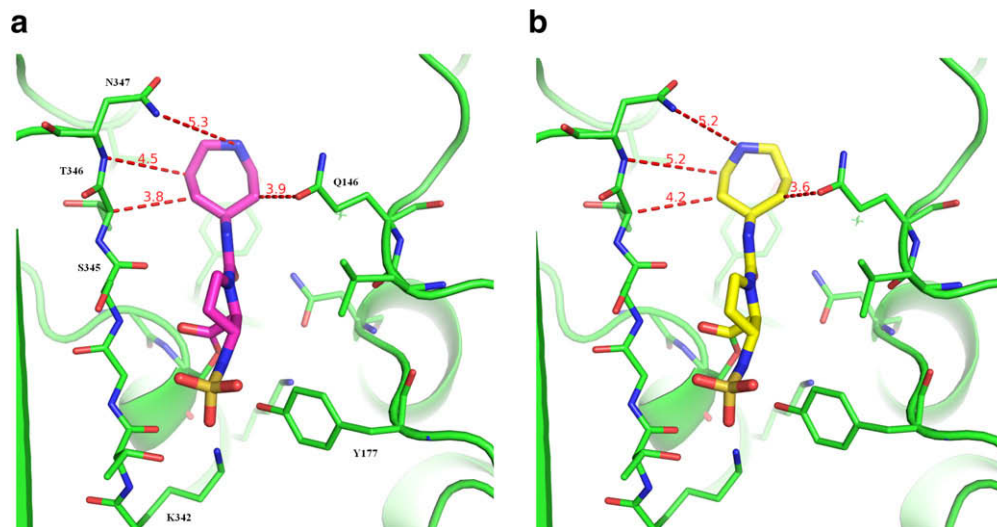


Figure 1. X-ray crystal structures of **2** (a) and **3** (b) in the active site of Amp C.

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