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

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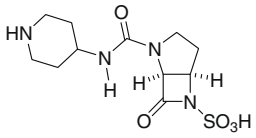
ABSTRACT

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.

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]



Ro48-1256 (1)

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 debenzylation 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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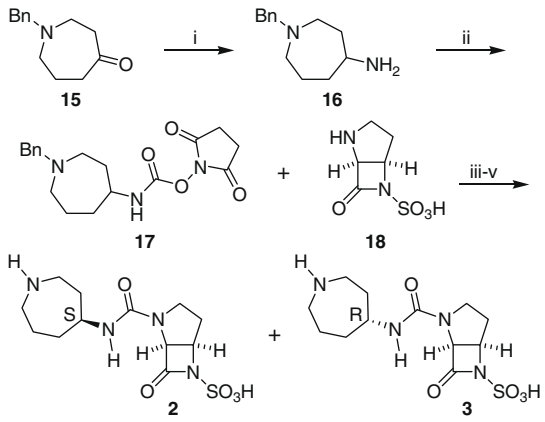


Scheme 1. Reagents and conditions: (i) $Ti(O^iPr_4)$, NH_3, EtOH, then $NaBH_4$, 52%; (ii) *N*,*N*′-disuccinimidyl carbonate, MeCN; (iii) $NaHCO_3$, MeCN, H_2O, 51%; (iv) $Pd(OH)_2$, MeOH, AcOH, H_2O, 40 psi H_2, 20%; (v) HPLC on phenominex synergi polar RP80 column eluted with MeOH/H_2O.

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

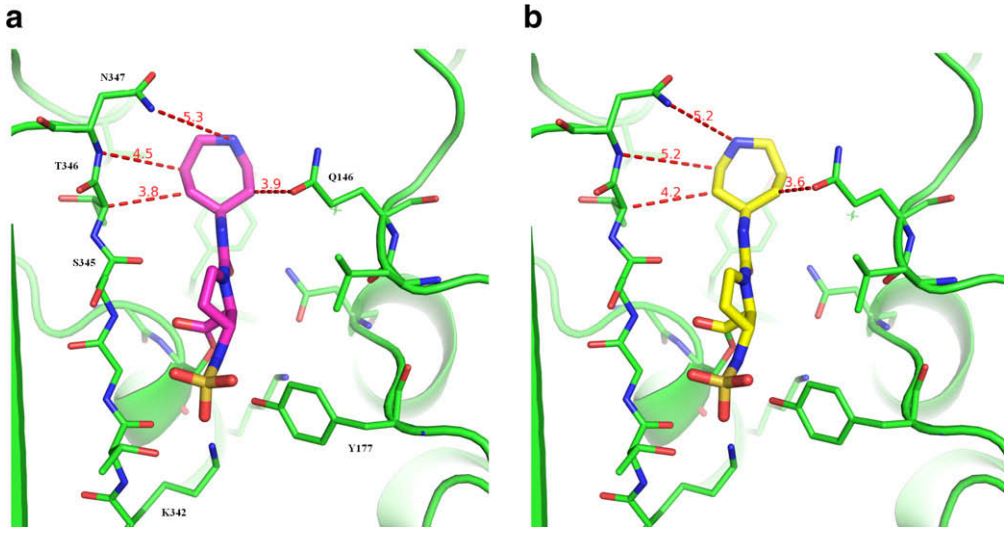
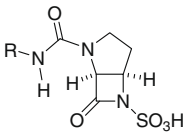
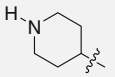
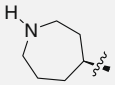
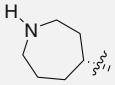
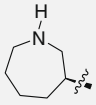
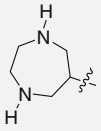
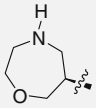
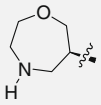
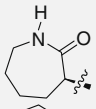
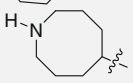
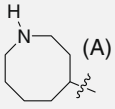
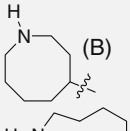
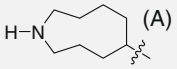
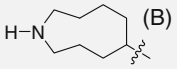


Figure 1. X-ray crystal structures of **2** (a) and **3** (b) in the active site of Amp C.

Table 1
Enzyme inhibition and in vitro synergy



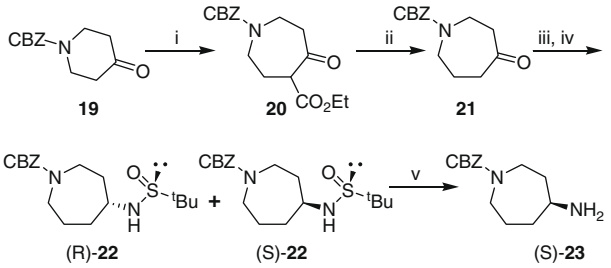
#	R	P.a. AmpC IC_{50}[a] (μM)	Conc. (μM) to restore IPM susc. (CL5701)[b]
1		6.8	21
2		1.0	9.4
3		4.0	25
4		1.9	50
5		17	100
6		0.6	25
7		1.1	50
8		0.8	>100
9		0.4	>100
10		3.4	25
11		1.3	12.5
12		25	>100
13		4	>100
14		5	>100

[a] IC_{50} for inhibition of the hydrolysis of nitrocefin; see Ref. 11 for details.
[b] Concentration of BLI (μM) required to reduce the imipenem (IPM) MIC for *P. aeruginosa* strain CL5701 from 32 μg/mL to the susceptibility breakpoint of 4 μg/mL; see Ref. 11 for details.

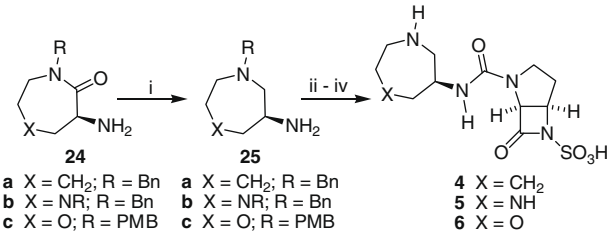
increase in both enzyme activity and synergy. Surprisingly, **8**, the lactam analog of **4**, exhibited improved enzyme inhibition but no synergy at all.

Similarly, analog **9**, which also lacks a basic nitrogen in the side chain, showed improved enzyme inhibition but no synergy. Experiments with these and other analogs in efflux-deleted mutants strongly suggest that a basic nitrogen in the side chain is required to prevent efflux of the BLI.[14] The azocine analogs **10** and **11** showed improved enzyme IC_{50} relative to **1**. Isomer **11** also had better synergy with IPM. Interestingly, **12**, the other side chain diastereomer of **11**, showed reduced enzyme inhibition and no synergy, illustrating the importance of side chain stereochemistry. The two nine-membered diastereomers, **13** and **14**, while reasonably active against the enzyme, had no in vitro synergy with IPM.

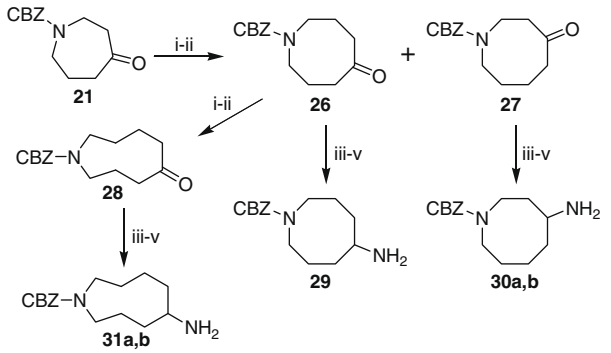
Azepine **2** was evaluated in several in vivo infection models[15] and found to be superior to **1**. For example, in a mouse spleen infection model with IPM-resistant *Pseudomonas aeruginosa*, (CL 5701; MIC 16–32 μg/mL) treatment with 2.5 mpk of IPM/CIL and 2.35 mpk of **2** (1.25 h infusion QID for 1 day) resulted in a 4.6 log reduction (relative to IPM/CIL alone) of bacterial CFU (colony forming units) in the spleen. Treatment with 2.5 mpk of IPM/CIL and 4.7 mpk of **1** (1.25 h infusion QID for 1 day) resulted in only a 1.0 log reduction of bacterial CFU.



Scheme 2. Reagents and conditions: (i) ethyl diazoacetate, $BF_3{\cdot}OEt_2$, Et_2O, 100%; (ii) K_2CO_3, H_2O, reflux, 72%; (iii) (*R*)-*t*BuSONH_2, $Ti(OEt)_4$, $NaBH_4$, THF; (iv) chiral HPLC on OJ column eluted with 15% EtOH/heptane, 27% yield of (*S*)-**22** (slower eluting) and 23% yield of (*R*)-**22** (faster eluting); (v) 4 M HCl/dioxane, MeOH.



Scheme 3. Reagents and conditions: (i) DIBAL 100% (**24a**), 74% (**24b**), 40% (**24c**); (ii) *N*,*N*′-disuccinimidyl carbonate, MeCN; (iii) **18**, $NaHCO_3$, MeCN, H_2O; (iv) $Pd(OH)_2$, MeOH, AcOH, H_2O, 40 psi H_2, 14% (**4**), 23% (**5**), 23% (**6**).

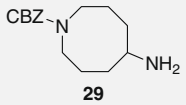
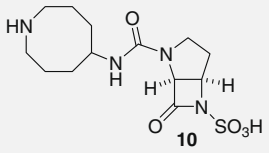
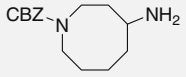
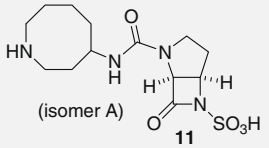
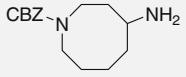
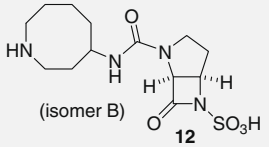
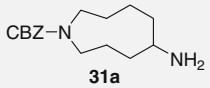
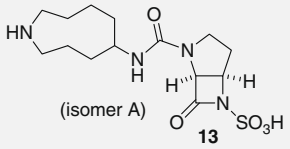
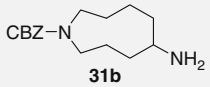
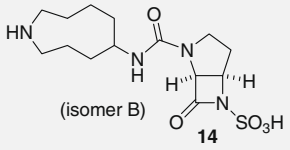


Scheme 4. Reagents and conditions: (i) diazomethane, $BF_3{\cdot}OEt_2$; (ii)K_2CO_3, THF/H_20, 100 °C; (iii) (*R*)-*t*BuSONH_2, $Ti(OEt)_4$, $NaBH_4$, THF; (iv) chiral HPLC; (v) 4 M HCl/dioxane, MeOH.

Table 2
Synthesis of analogs **10**–**14**

Amine → (i - iii) → Product

29; **30a**,**b**; **31a**,**b** → **10** - **14**

Amine	Product	Yield[a]
 **29**	 **10**	8
 **30a**	 (isomer A) **11**	24
 **30b**	 (isomer B) **12**	11
 **31a**	 (isomer A) **13**	10
 **31b**	 (isomer B) **14**	5

Conditions: (i) *N*,*N*′-disuccinimidyl carbonate, MeCN; (ii) **18**, $NaHCO_3$, MeCN, H2O; (iii) $Pd(OH)_2$, MeOH, AcOH, H_2O, 40 psi H_2.

[a] Overall yield (%) for three-step conversion of amine to final product.

Based on its superior in vitro and in vivo activity and acceptable pharmacokinetics (matching imipenem, data not shown), **2** was selected for further development in combination with IPM/CIL for treatment of IPM-resistant *Pseudomonas* infections. Unfortunately, development of **2** has since been terminated due to an inadequate therapeutic margin in subsequent safety studies. Future reports from this laboratory will describe our continuing efforts to identify an optimal BLI for combination with imipenem.

References and notes

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8. Azepinone **15** was purchased from Tyger Scientific (www.tygersci.com).
9. An improved synthesis of **18** was developed by Merck Process Research and will be published separately.
10. X-ray coordinates for **2** (PDB code 2wzx) and **3** (2wzz) have been deposited in the RCSB Protein Data Bank (PDB) database.
11. (a) Blizzard, T.A.; Chen, H.Y.; Wu, J.Y.; Kim, S.; Ha, S.; Mortko, C.; Variankaval, N.; Chiu, A. WO 2008039420. Detailed experimental procedures for the enzyme inhibition assay and the in vitro synergy assay are provided in Example 32 of this patent. For convenience, brief summaries of the protocols for these assays are provided below; (b) *Enzyme inhibition assay*: Hydrolysis of the commercially available substrate nitrocefin by AmpC in the presence of the BLI was measured in a spectrophotometric assay. The enzyme AmpC (from *P. aeruginosa*) and the substrate were dissolved in 100 mM KH_2PO_4 buffer (pH 7) containing 0.005% BSA. The BLI was dissolved in DMSO and serially diluted in a 96-well microplate. The BLI and AmpC were incubated for 40 min at room temperature then the substrate solution was added and the incubation continued for another 40 min. The spectrophotometric reaction was quenched by the addition of 2.5 N acetic acid and the absorbance at 492 nm was measured. The IC_{50} was determined from semi-logarithmic plots of enzyme inhibition versus inhibitor concentration; (c) In vitro synergy assay: The assay determines the concentration of BLI required to reduce the MIC of imipenem by one-half, one-quarter, one-eighth, one-sixteenth and one-thirty-second against resistant bacteria. The BLI was titrated in a serial dilution across a microtiter plate while at the same time imipenem was titrated in a serial dilution down the microtiter plate. The plate was inoculated with the bacterial strain in question then incubated overnight and evaluated for bacterial growth. Each well in the microplate checkerboard contains a different combination of concentrations of the inhibitor and the antibiotic thus allowing determination of synergy between the two.
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13. Lactam **24a** is commercially available (Matrix). Lactams **24b** and **24c** were prepared by condensation of *N*,*N*′-bis-benzyl ethylene diamine and *N*-benzyl-*N*-BOC-ethanolamine, respectively, with the methyl ester of *N*-CBZ-2-aziridine carboxylic acid followed by cyclization of the intermediate amino ester to afford the desired lactam.
14. Young, K. et al., in preparation.
15. Lee, S. et al., in preparation.