Modification at the β-lactam C6-position for the inhibition of carbapenem-hydrolyzing class D β-lactamases (CHDLs) in Gram-negative bacteria

Deisy Peña, Anthony Stefanelli, Marc Boudreau*

Department of Chemistry, University of New Hampshire, Durham, NH 03824, Unites States.

deisy.penaromero@unh.edu , marc.boudreau@unh.edu

INTRODUCTION AND OBJECTIVES

CARBAPENEM-HYDROLYZING CLASS D β–LACTAMASES (CHDLs). A major impediment in antimicrobial chemotherapy involving β-lactam antibiotics is the presence of bacterial enzymes (β-lactamases) that can hydrolyze the β-lactam bond and inactivate the antibiotic. These enzymes can be grouped into four classes (A-D). Among the most genetically diverse are the class D β-lactamases, which are mostly found in Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, and *Acinetobacter baumannii*. In this class are β-lactamases that exhibit clinical levels of resistance to carbapenem antibiotics, known as carbapenem-hydrolyzing class D β-lactamases (CHDLs).[1]

The active site of class D β–lactamases contains an unusual *N*-carboxylysine modification. A strongly hydrophobic active-site allows the lysine to combine with CO_2, and the resulting carbamate is stabilized by a number of hydrogen bonds (Fig. 1).[1] The carboxylysine is crucial for the catalytic activity of class D β-lactamases (Scheme 1).

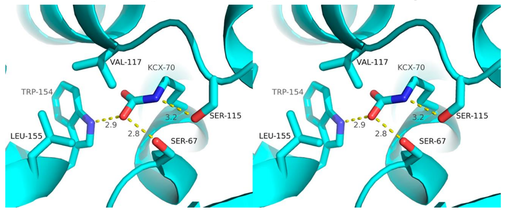
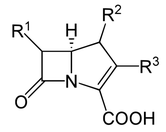
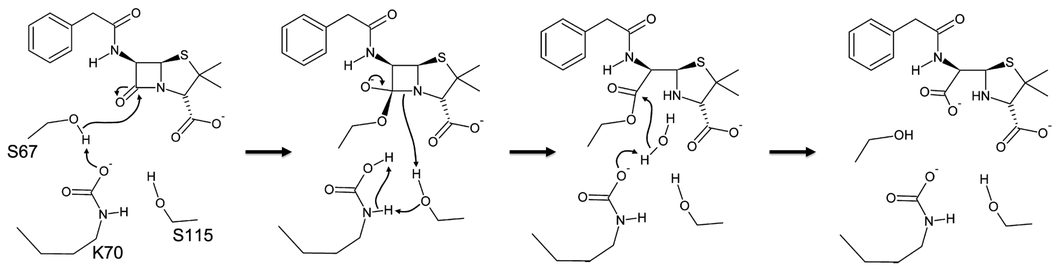


Fig. 1. Stereoview of the active site of the class D β–lactamase OXA-10.[1]

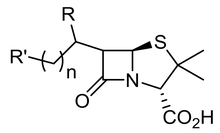


Core structure of carbapenem antibiotics, often used for the treatment of infections caused by multidrug-resistant bacteria



Scheme 1. Proposed β-lactam hydrolysis mechanism of class D β–lactamases. The initial acylation step is aided by carboxy-lysine-mediated deprotonation of S67. The carbamate also activates the deacylating water in the second step.[1]

β-LACTAM DERIVATIVES AS CHDL INHIBITORS. A small molecule that binds strongly to the carboxylate or that promotes its decarboxylation may function as an enzyme inhibitor. Our design principle for our target compounds draws from crystal structures of several class D β-lactamases complexed with β-lactams, including OXA-13 with meropenem,[2] OXA-24 with doripenem,[3] and OXA-10 with 6-hydroxyalkylpenicillanates (Fig. 2).[4,5] Thus, the C6 position is ideal for modification to introduce functional groups that may disrupt lysine carboxylation, by virtue of its proximity to the carboxylate. In this regard, this poster summarizes our efforts on the synthesis of C6-modified penicillanic acid derivatives.



R = H, OH
R' = $B(OH)_2$, H_2N-Ar, HO-Ar, NO_2, CO_2H, SO_3H, CF_3, etc
n = 0-3

Penicillanic acid derivatives

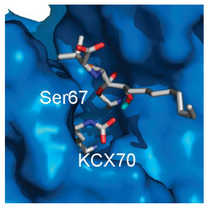
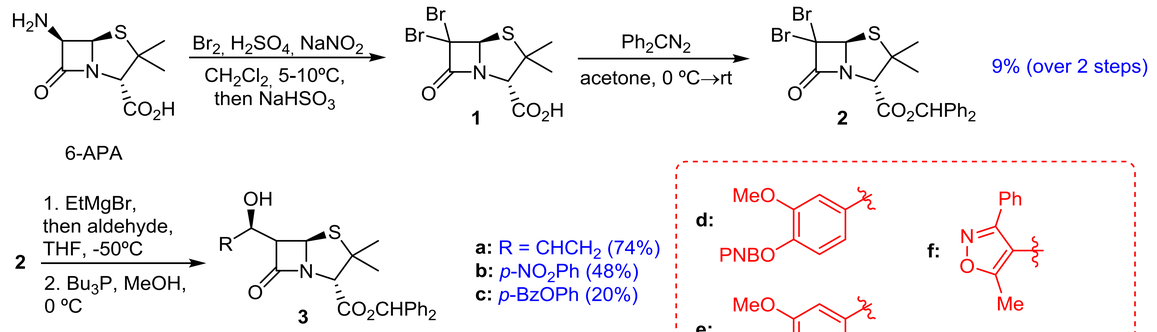
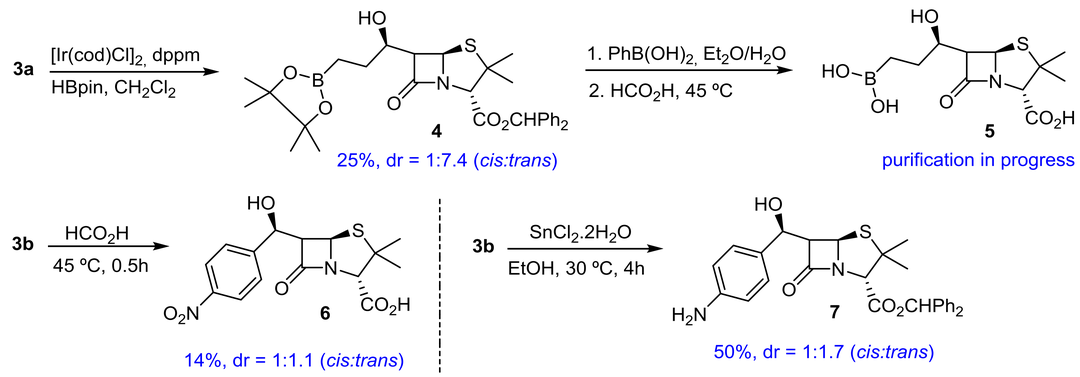


Fig. 2. X-ray structure of the acyl-enzyme species of OXA-10 and 6-β-(hydroxyoctyl)-penicillanate. The hydroxyl group of the C6-functionality is hydrogen-bonded to the *N*-carboxylated lysine.[4]

SYNTHESIS OF PENICILLANIC ACID DERIVATIVES





CONCLUSIONS AND FUTURE DIRECTIONS

- We have designed and synthesized several new penicillanic acid derivatives with the aim to either coordinate to the carboxylysine or prevent lysine carboxylation in CHDLs.
- Our next step will be to 1) evaluate the activity of the new penicillanic acid derivatives by enzymatic assays, 2) establish a structure-activity relationship (SAR), and 3) determine the mechanism of inhibition of active compounds.
- Additionally, we will assess to what extent inhibitors potentiate β-lactam antibiotic activity against CHDL-producing Gram-negative bacteria.

REFERENCES AND ACKNOWLEDGMENTS

1) Leonard, D. A.; Bonomo, R. A.; Powers, R. A. *Acc. Chem. Res.* **2013**, *46*, 2407.
2) Pernot, L.; Frénois, F.; Rybkine, T.; L'Hermite, G.; Petrella, S.; Delettré, J.; Jarlier, V.; Collatz; E.; Sougakoff, W. *J. Mol. Biol.* **2001**, *310*, 859.
3) Schneider, K. D.; Ortega, C. J.; Renck, N.; Bonomo, R. A.; Powers, R. A; Leonard, D. A. *J. Mol. Biol.* **2011**, *406*, 583
4) Maveyraud, L.; Golemi-Kotra, D.; Ishiwata, A.; Meroueh, O.; Mobashery, S.; Samama, J. P. *et al. J. Am. Chem. Soc.* **2002**, *124*, 2461.
5) Testero, S. A.; O'Daniel, P. I.; Shi, Q.; Lee, M.; Hesek, D.; Ishiwata, A.; Noll, B. C.; Mobashery, S. *Org. Lett.* **2009**, *11*, 2515.

6) We thank to the University of New Hampshire for the financial support.