Structure-based design of inhibitors of metallo β-lactamases: rescuing our current antibiotics

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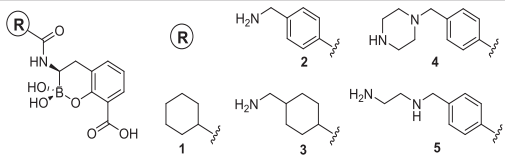
Introduction

The β-lactam antibiotics such as the penicillins and cephalosporins, remain the most important drug class for the treatment of bacterial infections. However, their continued use is jeopardized by the increasing spread of resistance mechanisms, including that mediated by β-lactamases, which, cumulatively, can hydrolyse all classes of β-lactam antibiotics. The serine-β-lactamases (SBLs), classes A, C, and D, likely evolved from the penicillin-binding protein (PBP) targets of β-lactam antibiotics. Inhibitors of the SBLs include clavulanic acid, sulbactam, and tazobactam, which are active against class A β-lactamases, and the recently introduced non-β-lactam β-lactamase inhibitor avibactam, which has a broader spectrum of SBL inhibition activity. These inhibitors have increased the efficacy of β-lactam antibiotics against SBL-mediated resistance in bacteria, but they are inactive against the Zn(II)-dependent class B metallo-β-lactamases (MBLs), which constitute a structural and mechanistically distinct family of enzymes and exhibit considerable heterogeneity, even among themselves. The MBLs are able to hydrolyse all classes of β-lactam except for monobactams. The ability of the MBLs to hydrolyse SBL inhibitors, including avibactam, is a growing problem in the treatment of infections where both SBL- and MBL-mediated cephalosporin and carbapenem resistance have been acquired. To date there are no clinically approved MBL inhibitors. Our work aims to use structure-guided molecular design and organic synthesis in order to identify small molecule inhibitors of β-lactamases that in the form of a co-therapy, have the potential to render resistant bacteria open to the effects of β-lactam antibiotics.

Results

We applied the de novo molecular design programe SPROUT to a crystal structure of NDM-1 (4RAM.pdb) followed by subsequent synthesis, to produce a smal library of cyclic boronate-based inhibitors. Using a fluorogenic assay for MBLs, these were then screened against a representative panel of clinically relevant B1 subfamily MBLs, including IMP-1 (Imipenemase-1), VIM-2 (Verona-Integron-Encoded MBL-2), NDM-1 (New Delhi MBL-1), SPM-1 (São Paulo MBL-1), and the model MBL, BcII from *Bacillus cereus* (Table 1).



	IC_{50} [μM]				
	1	**2**	**3**	**4**	**5**
BcII	7.27	0.30	0.96	0.52	1.14
VIM-2	0.051	0.003	0.011	0.014	0.002
IMP-1	1.44	1.00	1.50	1.21	1.41
NDM-1	2.04	0.029	0.687	0.04	0.004
SPM-1	24.1	16.7	16.0	13.9	36.3
TEM-1	0.001	0.003	0.002	0.0003	0.006
OXA-10	0.33	5.1	0.83	2.26	12.7

Figure 1: X-ray crystal structures of 2 bound to the VIM-2 (left) and OXa-10 (right).

The results imply that cyclic boronates with an aromatic sidechain, positioned analogously to the 6β/7β sidechains of the penicillins/cephalosporins, are potent inhibitors of B1 MBLs. In vitro inhibition of MBLs by the tested cyclic boronates yielded the following rank order of

potency: VIM-2 > NDM-1 > BcII > IMP-1 > SPM-1. Overall, these data identify **2** and **5** as highly potent inhibitors of VIM-2 and NDM-1, the most widely distributed members of the clinically important B1 subfamily. We then used fluorogenic assays to measure the potency of the cyclic boronates against clinically relevant Class A and Class D SBLs, including TEM-1 (Class A) and OXA-10 (Class D). All of the compounds tested were potent TEM-1 inhibitors (IC_{50} 6 →0.3 nM) and compounds with saturated sidechains (**1** and **3**) manifested IC_{50} values < 1 μM against OXA-10.

We were able to verify the mode of binding of these inhibitors to both classes of enzymes via X-ray crystallography (Figure 1).

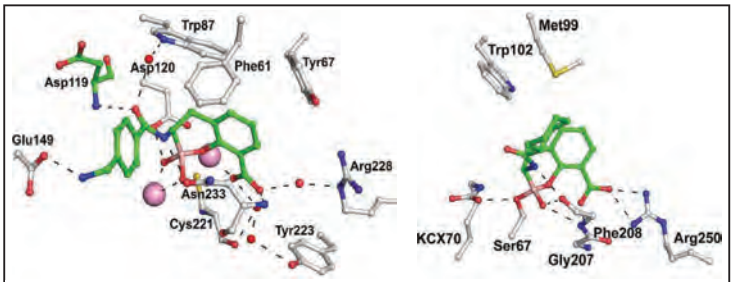


Figure 2: X-ray crystal structures of 2 bound to the VIM-2 (left) and OXa-10 (right).

Since **2** was a potent inhibitor of both enzyme classes in vitro, we next tested its activity against highly resistant Gram-negative bacterial cells (strains of *E. coli* and *Klebsiella pneumoniae,* both carrying the NDM-1 MBL together with multiple SBLs of different classes), alone and in combination with the carbapenem meropenem, Compound **2** alone did not display antibacterial activity against any of the strains tested. However, for all strains carrying the MBL NDM-1, co-administration with **2** reduced the minimal inhibitory concentration (MIC) of meropenem. Clear reductions in meropenem MIC were observed at 10 μg / mL inhibitor, while increasing the concentration of 2 to 25 μg / mL brought the meropenem MIC into the susceptible range (MIC < 8 μg / mL). Strikingly, for the clinical strain *K. pneumoniae* IR16, compound **2**, even at 10 μg / mL, was able to reduce the meropenem MIC from resistant (32 μg / mL) to fully susceptible (MIC ≤ 0.25 μg / mL). Neither compounds **1**, nor **2** showed cytotoxicity in human HEK293 cells when administered at concentrations up to 100 μM.

Publications

Adams P.G., Collins A.M., Sahin T., Subramanian V., Urban V.S., Vairaprakash P., Tian Y., Evans D.G., Shreve A.P. & Montano G.A. (2015) Diblock copolymer micelles and supported films with noncovalently incorporated chromophores: A modular platform for efficient energy transfer. *Nano Lett.* **15**:2422-2428.

Adams P.G., Swingle K.L., Paxton W.F., Nogan J.J., Stromberg L.R., Firestone M.A., Mukundan H. & Montano G.A. (2015) Exploiting lipopolysaccharide-induced deformation of lipid bilayers to modify membrane composition and generate two-dimensional geometric membrane array patterns. *Sci. Rep.* **5:**10331.

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Collaborators

External: C. Schofield (Oxford), J. Spencer (Bristol).