Department of Biochemistry and Molecular Biology

Inhibition of *Klebsiella pneumoniae* carbapenemase 2 (KPC2) by computationally designed peptide macrocycle inhibitors

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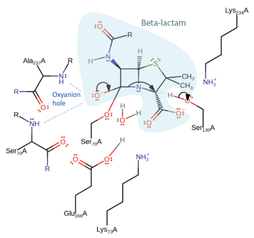
Introduction

Antibiotic-resistant bacterial pathogens present an escalating global health crisis[1].

Klebsiella pneumonia carbapenemase is a β-lactamase enzyme capable of hydrolyzing almost all recently developed β-lactam antibiotics[2]. KPC-2 is present across a range of bacterial pathogens of critical priority, as assessed by the World Health Organization[3]. In the search for new ways to inhibit these enzymes, cyclic boronate structures have shown significant promise[4]. These compounds copy the tetrahedral oxyanion transition of β-lactamase hydrolysis using a boronate to mimic the full charge in the transition state, enabling activity against even highly resistant class-B metallo-β-lactamases[5]



Active site binding in β-lactamases

Mechanism of stabilization of β-lactam in the oxyanion hole of a β-lactamase enzyme[6]

Structural determination of these mechanism-basec inhibitors bound to KPC2 , as well as kinetic analysis of the affinity of these inhibitors , are initial steps in the creation of high-affinity β-lactamase inhibitors to treat bacterial pathogens with this broad resistance enzyme

Objectives

Aim 1: Characterize the functional inhibition of KPC-2 by synthetic macropeptide using nitrocefin kinetic competition assays

Aim 2: Characterize the structure and binding of the macro peptide inhibitors of KPC-2 by crystallization and X-ray diffraction

Methods

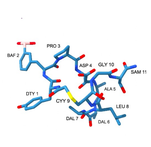
Design of cyclic macropeptide inhibitors

1B		
1	DTY	y
2	BAF	B
3	PRO	P
4	ASP	D
5	ALA	A
6	DAL	a
7	DAL	a
8	GLN	Q
9	CYY	C
10	GLY	G
11	SAM	S

cyclization

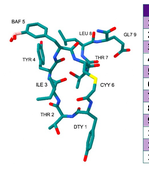
2B		
1	DTY	y
2	BAF	B
3	PRO	P
4	ASP	D
5	ALA	A
6	DAL	a
7	DAL	a
8	LEU	L
9	CYY	C
10	GLY	G
11	SAM	S

cyclization



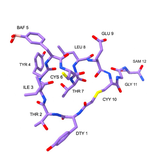
9B		
1	DTY	y
2	THR	T
3	ILE	I
4	TYR	Y
5	BAF	B
6	CYY	V
7	THR	T
8	LEU	L
9	GL7	E
10	CYS	C
11	GLY	G
12	SAM	S

cyclization



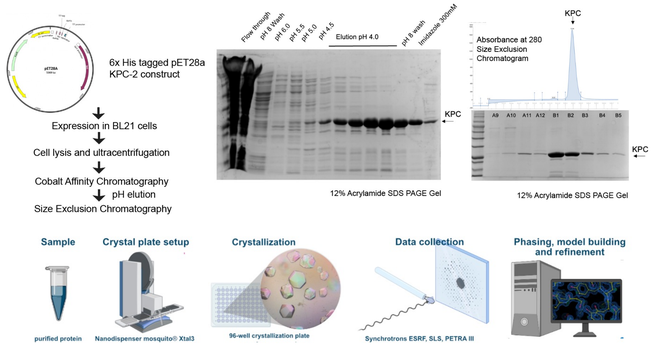
10B		
1	DTY	y
2	THR	T
3	ILE	I
4	TYR	Y
5	BAF	B
6	CYS	C
7	THR	T
8	LEU	L
9	GLU	E
10	CYY	C
11	GLY	G
12	SAM	S

cyclization



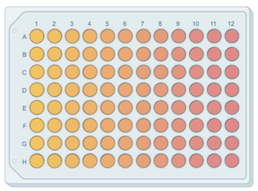
Rosetta Commons, a structure-guided computational design program, was used to design the cyclic macro peptides

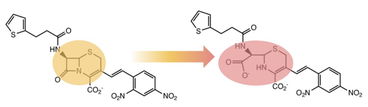
Purification and crystallization of KPC-2



Nitrocefin kinetic assay

A colormetric assay measuring hydrolysis rates calculates change in reaction velocity of β-lactamase with inhibitor, allowing the determination of IC_{50} [7].

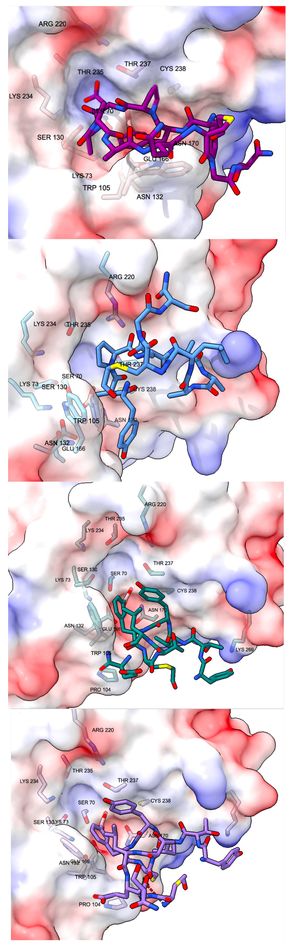


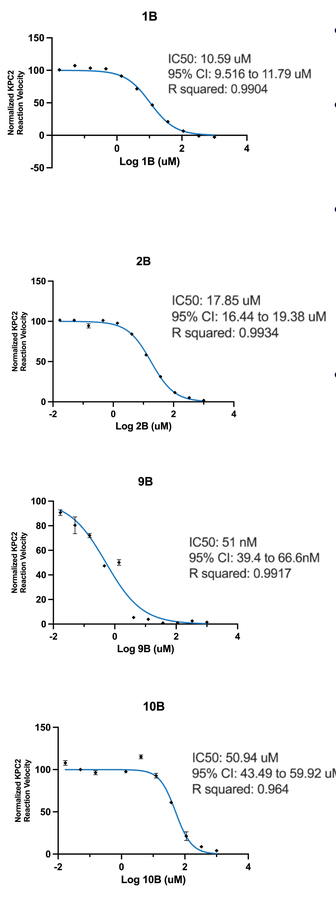


Results

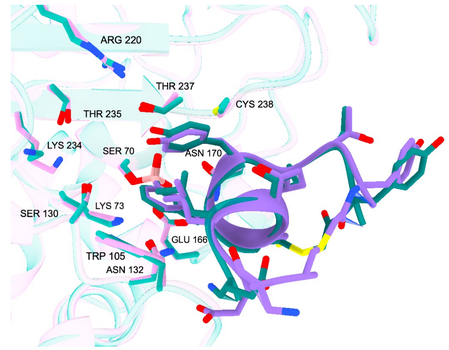
- The designed cyclic peptides bound successfully with varying degrees of affinity, including nanomolar IC_{50} for the best compound, 9B
- 9B and 10B had the highest and lowest relative affinity, respectively; both have the same sequence and similar tertiary structures, but different cyclization linkages
- 1B bound as designed, while 2B adopted a different confirmation than predicted, likely due to bromide from the crystal condition at the active site

Crystallized structures of KPC-2 in complex with cyclic peptide inhibitors





Overlay of inhibitors 9B and 10B binding to KPC-2



9B (teal) bound an order of magnitude stronger affinity than **10B (purple),** with the primary difference in cyclization linkage

Conclusions and Future Directions

- Computationally designed cyclic macropeptide inhibitors can effectively inhibit KPC-2 activity
- All inhibitors functioned as boronate-based transition state mimics and bound to the active site serine in all cases
- While the crystal structures allowed insights into atomic level detail of binding, buffer composition from the crystal conditions contributed to altered binding in 2B, demonstrating how similar data needs to be validated prior to conclusions
- Boronate-based cyclic inhibitors against KPC2 are a promising category of new antimicrobials that will become an effective strategy for combating dangerous multi-drug-resistant bacterial infections.

References

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