

Discovery of Novel Benzoxaboroles as a New Class of β -Lactamase Inhibitors

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
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Abstract

The β -lactamase enzymes are the main resistance mechanism to β -lactam antibiotics, especially in Gram-negative bacteria. The continuous development of resistance represents a serious threat to the clinical utility of β -lactams, leading to an urgent medical need for new β -lactamase inhibitors that can be used in combination with β -lactam antibiotics. Boronic acids were previously reported to be inhibitors of β -lactamases. The electron-deficient boron forms a tetrahedral transition state mimic with the catalytic serine residue of β -lactamase, thus acting as a serine trap. Since boronic acids are often associated with poor drug-like properties, we have evaluated other boron derivatives as enzyme inhibitors. Previously we have reported that benzoxaboroles, a 5-membered boron containing heterocycle fused to an aromatic ring, show selective inhibition of leucyl-tRNA synthetase by coordinating to cis-diols of substrate tRNA in the editing active site. Because of the favorable drug-like properties of benzoxaboroles, we set about using this chemical scaffold to design potent and specific β -lactamase inhibitors.

Screening our boron compound collection identified benzoxaborole hits with promising inhibitory activity against a panel of β -lactamase enzymes. SAR optimization of 6-aryloxy benzoxaboroles hits led to low micromolar inhibition of class A β -lactamases, CTX-M-9a and TEM-1, and low nanomolar inhibition of class C β -lactamases, AmpC P99 and CMY-2. Selected compounds restored antibacterial activity of ceftazidime against *Enterobacter cloacae* expressing AmpC P99. We now report the synthesis, crystal structure and discovery of this novel class of drug-like boron-containing inhibitors of β -lactamases.

Table 1: β -lactamase inhibitors with different linkers

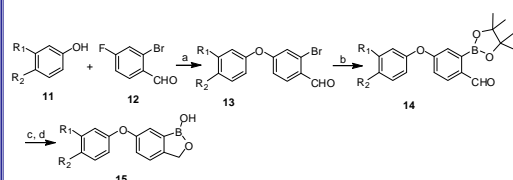


Compound	X	Ki (μ m)			
		CTX-M-9a	TEM-1	AmpC P99	CMY-2
1	S	>32	1.76	0.09	12.65
2	O	1.89	1.02	0.71	1.51
3	CO	>32	>37	19.79	25.63
4	CO	>32	10.6	28.47	46.94
5	CH(OH)	>32	>37	7.28	8.42
6	SO	>32	4.87	57.98	59.29
7	SO ₂	>32	11.23	63.22	108
8	NHCO	>32	>37	46.56	>138
9	OC(=NH)	>32	>37	16	13.8
10	NH	>32	>37	4.73	13.8

• Focused library screening identified compound **1** that had moderate inhibition of TEM-1 (Class A) and AmpC P99, CMY-2 (Class C) β -lactamases.
• Compounds with different linkers suggested that oxygen linker (**2**) was beneficial for inhibition of both classes of β -lactamases.

Synthesis

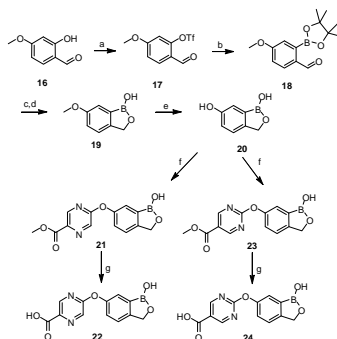
Scheme 1. Synthesis of phenoxy benzoxaboroles



^aReagents and conditions: (a) Cs₂CO₃, DMF, 80 °C; (b) Bis(pinacolato-diboron), PdCl₂(dppf), KOAc, dioxane, 80 °C; (c) NaBH₄, MeOH-THF, 0 °C; (d) HCl.

Synthesis

Scheme 2. Synthesis of heteroaryl benzoxaboroles



^aReagents and conditions: (a) (CF₃SO₂)₂O, pyridine, -10 to 0 °C; (b) Bis(pinacolato-diboron), PdCl₂(dppf), KOAc, dioxane, 80 °C; (c) NaBH₄, MeOH-THF, 0 °C; (d) HCl; (e) BBr₃, DCM, -10 to 0 °C; (f) methyl-5-chloropyrimidine-2-carboxylate or methyl 2-chloropyrimidine-5-carboxylate, K₂CO₃, DMF, 80 °C; (g) LiOH, MeOH-H₂O, 0 °C to rt.

Acknowledgements: The contributions of Decode Biostructures for provision of Ki data and T. Akama, Y Zhang of Anacor for provision of benzoxaborole collection compounds are acknowledged.

Table 2: β -lactamase inhibition of substituted arylbenzoxaboroles

Compound	R1	R2	Ki (μ m)			
			CTX-M-9a	TEM-1	AmpC P99	CMY-2
2	H	H	1.89	1.02	0.71	1.51
15a	OCH ₃	H	1.1	1.14	1.35	2.65
15b	OH	H	0.816	nt ^a	nt ^a	3.12
15c	NH ₂	H	0.69	1.01	2.75	6.09
15d	CH ₂ OH	H	1.92	1.36	8.4	8.43
15e	CH ₂ NH ₂	H	4.18	0.56	3.33	nt ^a
15f	OCH ₂ Ph	H	8.75	1.08	6.42	74.5
15g	CH ₂ N(CH ₂) ₂	H	6.02	0.51	7.72	15.9
15h	COOH	H	22.6	>37	1.44	2.53
15i	H	OCH ₃	14.1	4.34	6	>46
15j	H	NH ₂	14.8	nt ^a	8.73	14.7
15k	H	CH ₂ NH ₂	15.1	0.812	13.3	12.3
15l	H	CH ₂ N(CH ₂) ₂	21.1	3.14	40.63	46.8
15m	H	COOEt	>32	3.11	5.55	nt ^a
15n	H	COOH	>32	18.9	0.02	0.02

^aNot tested

Table 3: Inhibition of β -lactamases by aryl- and heteroarylbenzoxaboroles and Minimum Inhibitory Concentration (MIC; μ g/mL) of ceftazidime in presence of 8 μ g/mL of these β -lactamase inhibitors

Compound	Ki (μ m)				MIC (μ g/mL) *	
	CTX-M9a	TEM-1	AmpC P99	CMY-2	<i>E. cloacae</i> SYN36 AmpC P99	<i>E. coli</i> SYN2549 CMY-2
2	1.89	1.02	0.71	1.51	32	8
15n	>32	18.9	0.02	0.02	8	1
24	>32	14.7	0.08	0.07	4-16	<0.5
22	>32	20.4	0.02	0.02	1-2	<0.5

* The MIC of ceftazidime is 128 μ g/mL with no inhibitor present

Table 4: Pharmacokinetic parameters of **22** in mice plasma

Dosing method ^a	t _{1/2} (h)	t _{max} (h)	C _{max} (μ g/mL)	CL (mL/h/kg)	AUC (h μ g/mL)	F% ^b
iv	0.36	NA	13	2326	4.3	NA
po	0.23	0.25	19.5	NA	8.62	67

^aMice (CD-1, non-fasted) were given a single dose of 30 mg/kg po or 10 mg/kg iv (n = 3 per group).

^bCalculated with non-compartment model and iv bioavailability (F%) is considered to be 100%. NA = not available.

Structure-Activity Relationship (SAR)

- Focused library screening identified compound **1** that had moderate inhibition of TEM-1 (Class A) and AmpC P99, CMY-2 (Class C) β -lactamases.
- Compounds with different linkers demonstrated that oxygen linker (**2**) had better binding affinity than other linkers in inhibiting β -lactamases. It was also shown by X-ray co-crystal structure that the oxygen linker made hydrogen-bond interactions in the active site, which greatly improved inhibition.
- Small, polar substitutions at R₁ improved CTX-M-9a activity while maintaining class C inhibition.
- Carboxylic acid (**15n**) at the R₂ position significantly improved inhibitory activity against AmpC P99 and CMY-2.
- Carboxy-pyrimidine (**24**) and carboxy-pyrazine (**22**) derivatives demonstrated potent class C β -lactamases inhibition. They also restored antibacterial activity of ceftazidime against *Enterobacter cloacae* expressing AmpC P99. Compound **22** also showed good oral bioavailability.

Cocrystal structure of **22** bound at the active site of AmpC P99



- AmpC P99 cocrystal structure (left, ribbons) and catalytic cleft with compound **22** (right, sticks)
- Compound **22** binds covalently to Ser 64 residue of AmpC P99, with the boron group mimicking the negatively charged tetrahedral transition state.
- The oxygen linker forms two hydrogen bonds with the side chains of Gln120 and Asn152, while the terminal carboxylic acid interacts with the main chain NH groups from Ser212 and Gly320, consistent with SAR results for AmpC.
- Resolution 2.02Å, R_{work}=21.1, R_{free}=28.1, RMSD bonds=0.009, RMSD angles=1.27.

Summary

A series of novel benzoxaboroles has been discovered as potent β -lactamase inhibitors. Compound **22** inhibited AmpC P99 and CMY-2 with Ki's in the low nM range. It restored antibacterial activity of ceftazidime against *Enterobacter cloacae* expressing AmpC P99, a Class C β -lactamase enzyme. It displayed good oral bioavailability. Continuing studies to explore more potent inhibitors are ongoing.