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Synthesis and SAR of novel benzoxaboroles as a new class of β-lactamase inhibitors

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ABSTRACT

A new class of benzoxaborole β-lactamase inhibitors were designed and synthesized. 6-Aryloxy benzoxaborole **22** inhibited AmpC P99 and CMY-2 with K_i values in the low nanomolar range. Compound **22** restored antibacterial activity of ceftazidime against *Enterobacter cloacae* P99 expressing AmpC, a class C β-lactamase enzyme. The SAR around the arylbenzoxaboroles, which included the influence of linker and substitutions was also established.

The β-lactam antibiotics represent the drug of choice to treat Gram-negative bacterial infections. Resistance to the β-lactams in Gram-negative bacteria is predominately through the acquisition of β-lactamases,[1] which act by catalyzing the hydrolysis of the amide bond of the β-lactam ring. The combination of the proliferation of β-lactamases and a poor drug pipeline pose a serious threat to the future treatment of Gram-negative bacterial infections.[2] The β-lactamases can be classified by their structure into four classes (A, B, C and D).[3] Class A, C and D are serine proteases, while class B are metallo-hydrolases. Inhibition of β-lactamases is a common method of treatment in the clinic with the currently marketed inhibitors clavulanic acid, tazobactam, and sulbactam predominately inhibiting class A enzymes. The class C enzymes, AmpC and CMY, which are resistant to these inhibitors, are a significant problem in the clinic. Furthermore, β-lactam-based inhibitors can induce the expression of the chromosomal encoded class C β-lactamase, AmpC, especially in *Enterobacteria* spp., which can lead to development of resistance during therapy.[4] The use of a non-β-lactam based β-lactamase inhibitor would avoid this significant handicap.

Boronic acids inhibitors of β-lactamases have been known since the late 1970s[5] where its empty p orbital forms a covalent bond with the catalytic serine residue of a β-lactamase, thus acting as a serine trap.[6] The boronic acid-serine adduct mimics the transition state and is stabilized by the enzyme active site, therefore locking the enzyme in this inactive state. Since boronic acids are often associated with poor drug-like properties, we have evaluated other boronic acid derivatives as enzyme inhibitors. We have previously reported that benzoxaboroles, a 5-membered boron containing heterocycle fused to an aromatic ring, showed selective inhibition of leucyl-tRNA synthetase by coordinating to *cis*-diols of substrate tRNA in the editing active site.[7] These benzoxaboroles posses significantly better pharmacokinetic properties than boronic acids. Therefore, we set about to use the oxaborole chemical scaffold to design potent and specific β-lactamase inhibitors.

Screening our chemical library of boron compounds against the class A enzyme TEM-1 and the class C enzyme AmpC from *Enterobacter cloacae* P99 identified benzoxaborole, compound **1**, with promising broad-spectrum inhibitory activity (Table 1). Compound **1** is the benzoxaborole with an ether linker at C6 position. Subsequently, we explored the effect of a variety of linkage groups and different substitution pattern at C6 of benzoxaboroles on β-lactamase inhibition. We also synthesized the heteroaryl analogs to investigate the effect of lipophilicity on anti-bacterial activity.

The synthesis of benzoxaboroles with thioether, sulfoxide, and sulfone, amino, amide and carbamate linkage groups (**1–10**) at C6 position has been reported previously.[8] The synthesis of substituted aryloxy benzoxaboroles is outlined in Scheme 1. Nucleophilic substitution of 2-bromo-4-fluorobenzaldehyde (**12**) by phenol gave ether (**13**). Palladium-mediated boronylation provided aldehyde (**14**), followed by reduction with $NaBH_4$ and acid-catalyzed cyclization to the phenoxybenzoxaboroles (**15a–m**).

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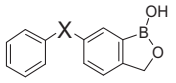
Table 1
β-lactamase inhibition of benzoxaboroles with different linkers



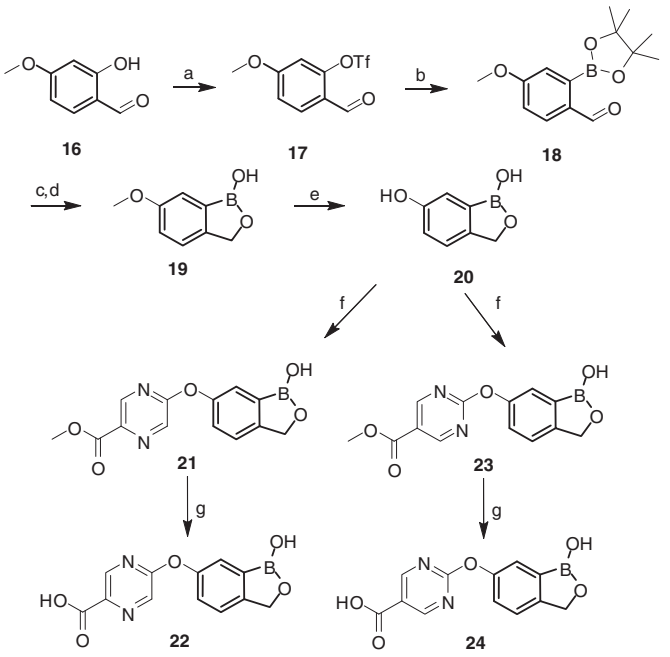
Compd	X	K_i[a] (μM)			
		CTX-M-9a	TEM-1	AmpC P99	CMY-2
1	O	1.89	1.02	0.71	1.51
2	S	>32	1.76	9.09	12.65
3	CH_2	>32	>37	19.79	25.63
4	CO	>32	10.6	28.47	46.94
5	CH(OH)	>32	>37	7.28	8.42
6	SO	>32	4.87	57.98	59.29
7	SO_2	>32	11.23	63.22	108
8	CONH	>32	>37	46.56	>138
9	NHOCO	>32	>37	16	13.8
10	NH	>32	>37	4.73	13.8

[a] All β-lactamases were tested as described in Ref. 11.

As shown in Scheme 2, benzoxaboroles with heteroaryl at C6 were also synthesized. 2-Hydroxy-4-methoxybenzaldehyde was converted to triflate (**17**) followed by catalytic boronylation to provide compound **18**. Reduction with $NaBH_4$ and acid-catalyzed cyclization to the benzoxaboroles (**19**). Treatment of BBr_3 at −10 to 0 °C for demethylation afforded **20**. Compound **20** was reacted with a series of heteroaryl chloride by nucleophilic substitution. For example, treatment of **20** with methyl 5-chloropyrazine-2-carboxylate in basic condition provided **21**. Subsequently, hydrolysis of the ester with LiOH in methanol and water yielded the corresponding carboxylic acid (**22**).[9] Compounds **23** and **24**[10] were synthesized in similar method as shown in Scheme 2.

To probe the effect of different linker groups on anti-β-lactamase inhibitory effects, those benzoxaboroles were tested for their ability to inhibit both class A (CTX-M-9a and TEM-1) and class C (AmpC P99, CMY-2) β-lactamases. The data shown in Table 1 summarizes the K_i values for those compounds on β-lactamase inhibition.

As shown in Table 1, compound **1** with ether linker showed moderate broad-spectrum inhibition of class A and class C β-lactamases. Converting the hydrogen bond acceptor oxygen to methylene linker (**3**), thioether (**2**) or hydrogen bond donor amino linker (**10**) diminished potency in CTX-M-9a and TEM-1. Carbonyl (**4**), carbinol (**5**), sulfoxide (**6**), and sulfone (**7**) represent the category of linkage groups with a hydrogen bond acceptor at an increased distance from the boron of the benzoxaborole and showed decreased potency. The linker SAR indicated that oxygen is essential for good potency and it may contribute to the potency through a hydrogen bonding interaction with the enzyme. Also the SAR suggests that the length and hydrogen-bonding properties of the linkage group had a significant effect on β-lactamase inhibitory activity.

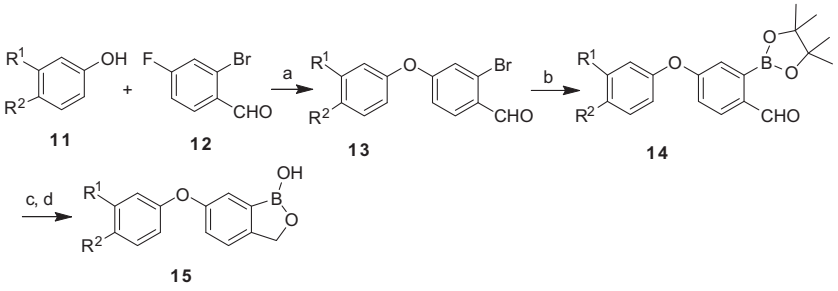


Scheme 2. Synthesis of C6-heteroaryl benzoxaboroles. Reagents and conditions: (a) $(CF_3SO_2)_2O$, pyridine, −10 to 0 °C; (b) bis(pinacolato-diboron), $PdCl_2$(dppf), KOAc, dioxane, 80 °C; (c) $NaBH_4$, MeOH-THF, 0 °C; (d) HCl; (e) BBr_3, DCM, −10 to 0 °C; (f) methyl-5-chloropyrazine-2-carboxylate or methyl 2-chloropyrimidine-5-carboxylate, K_2CO_3, DMF, 80 °C; (g) LiOH, MeOH-H_2O, 0 °C to rt.

Based on these results, C6 phenoxy-benzoxaboroles (**1**) was selected as a template for further SAR exploration. The effect on affinity of different substitution group of **1** on β-lactamase inhibition is summarized in Table 2.

In general, compounds with small substitution at *meta*-position exhibited better inhibitory activity against CTX-M-9a, while maintaining class C activities. Compounds **15a–c** had K_i values about 2–3-fold more potent than **1** in CTX-M-9a, suggesting that small polar group contributed on inhibitory binding. Adding a methylene to amino or hydroxyl group to hydroxymethyl (**15d)** and aminomethyl **(15e**) reduced affinity suggesting the binding geometry was not optimum by increasing the distance from the polar OH or NH_2 group to the enzyme residues. Bulky groups (**15f, 15g**) decreased activity further which is consistent with the above findings.

On the contrary, substitution on *para*-position reduced affinity in CTX-M-9a as compared to *meta*-position. Compounds **15i** and **15j** were about 7-fold less potent than **1**, while corresponding *meta*-substituted analogs (**15a**, **15c**) improved affinity by 2–3-fold, respectively. Interestingly, anionic charged moiety, such as carboxylate on either *meta*- (**15h**) or *para*-position (**15n**) was not



Scheme 1. Synthesis of phenoxy benzoxaboroles. Reagents and conditions: (a) Cs_2CO_3, DMF, 80 °C; (b) bis(pinacolato-diboron), $PdCl_2$(dppf), KOAc, dioxane, 80 °C; (c) $NaBH_4$, MeOH-THF, 0 °C; (d) HCl.