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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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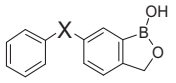
Table 1
β-lactamase inhibition of benzoxaboroles with different linkers



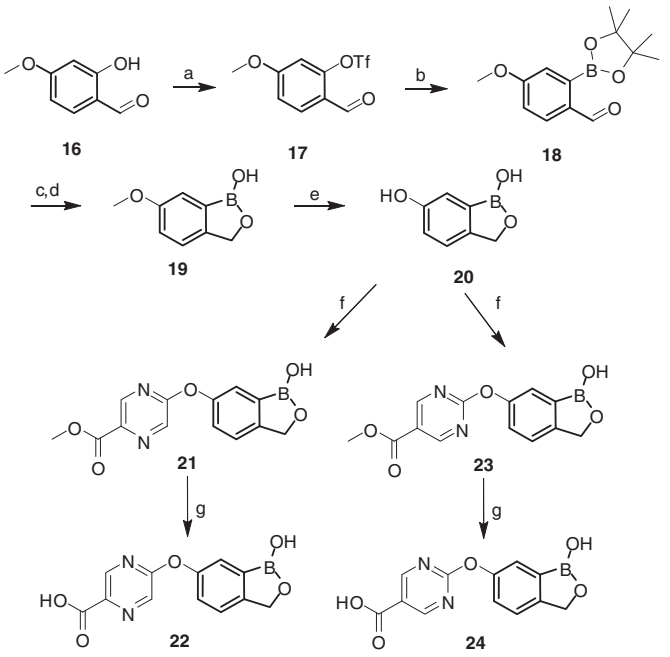
Compd	X	K_i[a] (μM)			
		CTX-M-9a	TEM-1	AmpC P99	CMY-2
1	O	1.89	1.02	0.71	1.51
2	S	>32	1.76	9.09	12.65
3	CH_2	>32	>37	19.79	25.63
4	CO	>32	10.6	28.47	46.94
5	CH(OH)	>32	>37	7.28	8.42
6	SO	>32	4.87	57.98	59.29
7	SO_2	>32	11.23	63.22	108
8	CONH	>32	>37	46.56	>138
9	NHOCO	>32	>37	16	13.8
10	NH	>32	>37	4.73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

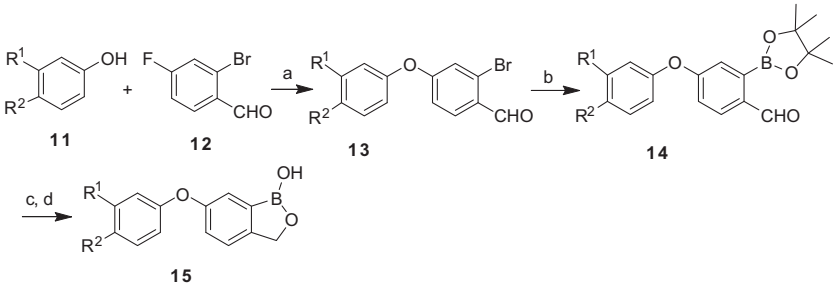


Scheme 2. Synthesis of C6-heteroaryl benzoxaboroles. Reagents and conditions: (a) $(CF_3SO_2)_2O$, pyridine, −10 to 0 °C; (b) bis(pinacolato-diboron), $PdCl_2$(dppf), KOAc, dioxane, 80 °C; (c) $NaBH_4$, MeOH-THF, 0 °C; (d) HCl; (e) BBr_3, DCM, −10 to 0 °C; (f) methyl-5-chloropyrazine-2-carboxylate or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



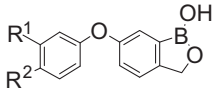
Scheme 1. Synthesis of phenoxy benzoxaboroles. Reagents and conditions: (a) Cs_2CO_3, DMF, 80 °C; (b) bis(pinacolato-diboron), $PdCl_2$(dppf), KOAc, dioxane, 80 °C; (c) $NaBH_4$, MeOH-THF, 0 °C; (d) HCl.

tolerated in CTX-M-9a and TEM-1. However, they demonstrated improved affinity in class C β-lactamase. Compound **15n** was extremely potent against AmpC P99 and CMY-2 with a K_i value in 0.02 μM, suggesting a canonical anion recognition residue around the carboxylate in AmpC P99 and CMY-2, which revealed the importance of this group for recognition by AmpC P99, CMY-2 β-lactamases.

With promising inhibitory activity of **15n** in AmpC P99 and CMY-2, we further replaced the phenyl ring with heterocyclic rings to investigate the effects of lipophilicity as lipophilic compounds tend to be good substrates for efflux pumps. As expected, pyrazine (**22**) and pyrimidine (**24**) carboxylic acids showed very potent inhibition on AmpC P99 and CMY-2 with K_i values in the low nanomolar range. These potent class C β-lactamase inhibitors were then tested for their anti-bacterial inhibition in *E. cloacae* and *Escherichia coli* with a plasmid bearing the CMY-2 class C β-lactamase. As shown in Table 3, compound **22** restored antibacterial activity of ceftazidime from >128 μg/mL to 1 μg/mL against *E. cloacae* P99 expressing AmpC P99 at the concentration of 8 μg/mL of inhibitor. In addition, **22** restored antibacterial activity of ceftazidime to 0.5 μg/mL against *E. coli* expressing CMY-2.

In conclusion, we have synthesized and discovered a series of novel benzoxaborole β-lactamase inhibitors. The SAR around C6 benzoxaboroles, which included the influence of linkage, ring and substitution patterns, was established. The most potent class C β-lactamase inhibitor **22** may form productive hydrogen bonding interactions with AmpC though its *p*-carboxylic acid. The same carboxylic acid at *p*-position, however, resulted in diminished class A β-lactamase activity. The SAR described here would be very useful for further design and development of broad-spectrum β-lactamase inhibitors. Further studies to improve spectrum of β-lactamase inhibition are underway and will be reported in due course.

Table 2
β-lactamase inhibition of substituted C6 phenoxybenzoxaboroles



Compound	R^1	R^2	K_i (μM)			
			CTX-M-9a	TEM-1	AmpC P99	CMY-2
1	H	H	1.89	1.02	0.71	1.51
15a	OCH_3	H	1.1	1.4	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_2OH	H	1.92	1.36	8.4	8.43
15e	CH_2NH_2	H	4.18	0.56	3.33	nt[a]
15f	OCH_2Ph	H	8.75	1.08	2.27	74.5
15g	$CH_2N(CH_3)_2$	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_2NH_2	15.1	0.812	13.3	12.3
15l	H	$CH_2N(CH_3)_2$	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

[a] Not tested.

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

	K_i (μM)				MIC (μg/mL)	
	CTX-M-9a	TEM-1	AmpC P99	CMY-2	*E. cloacae* P99AmpC	*E. coli* SYN2549 CMY-2
1	1.89	1.02	0.71	1.51	32	8
15n	>32	18.9	0.02	0.02	8	1
22	>32	20.4	0.02	0.02	1--2	<0.5
24	>32	14.7	0.08	0.07	4–16	<0.5

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9. *Experimental procedure for the preparation of compound* **22**: To a solution of 2-hydroxy-4-methoxy-benzaldehyde **16** (30.0 g, 0.197 mol) and pyridine (77.98 g, 0.986 mol) in dichloromethane (120 mL) was slowly added Tf_2O (83.44 g, 0.296 mol) at −10 to 0 °C over a 2.5-h period. The mixture was stirred at 0 °C for 30 min. Ice-water (150 mL) was added, and the mixture was acidified with diluted hydrochloric acid to pH 2. The resulting mixture was extract with 50% EtOAc/hexanes (2 × 400 mL). The extract was washed with brine, dried and concentrated to dryness to give 51.01 g (91.1%) of compound **17** as pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.13 (s, 1 H), 7.95 (d, J = 8.79 Hz, 1 H), 7.03 (dd, J = 8.79, 2.34 Hz, 1 H), 6.88 (d, J = 2.34 Hz, 1 H), 3.93 (s, 3 H). MS (ESI) m/z = 285 $[M+H]^+$. To a solution of bis(pinacolato)diborane (58.66 g, 0.231 mol) in dioxane (600 mL) was added KOAc (52.33 g, 0.533 mol). After degassed for 15 min, $PdCl_2$(dppf) (13.0 g, 0.0178 mol) and **17** (50.51 g, 0.178 mol) were added to the reaction mixture. The mixture was stirred at 80 °C for 45 min. The reaction was quenched by adding ice-water (400 mL). The resulting mixture was extract with 50% EtOAc/hexanes (2 × 600 mL). The extract was washed with brine, dried and concentrated to dryness. The residue was purified by chromatography on silica gel (EtOAc/hexanes = 1:3) to give 43.48 g (93.2%) of **18** as pale-yellow waxy solid.^{1}H NMR (400 MHz, $CDCl_3$) δ 10.88 (s, 1 H), 8.44 (d, J = 8.50 Hz, 1 H), 7.80 (d, J = 2.64 Hz, 1 H), 7.54 (dd, J = 8.50, 2.64 Hz, 1 H), 4.41 (s, 3 H), 1.91 (s, 12 H). MS (ESI) m/z = 263 $[M+H]^+$. To a solution of **18** (25.0 g, 95.4 mmol) in methanol (160 mL) was slowly added $NaBH_4$ powder (10.82 g, 0.286 mol) at 0–10 °C. After stirred for 1 h at room temperature, the mixture was concentrated to remove one-third of methanol. The resulting mixture was cooled to 0 °C, acidified to pH 3 using diluted hydrochloric acid and diluted to 2-fold with cold water. The white precipitate was collected, washed with 30% MeOH/H_2O, water, and dried to give 11.5 g (73.5%) of **19** as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1 H), 7.29 (d, J = 8.21 Hz, 1 H), 7.23 (d, J = 2.34 Hz, 1 H), 7.03 (dd, J = 8.21, 2.34 Hz, 1 H), 4.90 (s, 2 H), 3.75 (s, 3 H). To a solution of **19** (10.0 g, 61.0 mmol) in dichloromethane (400 mL) was slowly added BBr_3 (134 mL, 1 M in DCM, 0.134 mol) at −10 to −5 °C. The mixture was stirred at 0 °C to room temperature for 3 h. The reaction mixture was poured into ice-water (300 mL). The resulting mixture was extract with EtOAc (600 mL). The extract was washed with brine, dried and concentrated to dryness to give 9.11 g (99.6%) of **20** as off-white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (br s, 1 H), 9.03 (br s, 1 H), 7.16 (d, J = 8.20 Hz, 1 H), 7.08 (d, J = 2.34 Hz, 1 H), 6.86 (dd, J = 8.20, 2.34 Hz, 1 H), 4.90 (s, 2H). MS (ESI) m/z = 151 $[M+H]^+$. To a solution of **20** (0.37 g, 2.47 mmol) in anhydrous DMF (8 mL) were added Cs_2CO_3 (2.01 g, 2.71 mmol) and 5-chloro-pyrazine-2-carboxylic acid methyl ester (0.468 g, 2.71 mmol) at room temperature. After stirring at 90 °C for 1.5 h, the reaction mixture was cooled to 0 °C, diluted with water (10 mL) and acidified to pH 3 using diluted hydrochloric acid. The off-white precipitate was collected, washed with water and dried to give the crude product which was purified by chromatography on silica gel (DCM/MeOH = 40:3) to give 0.47 g (66.5%) of **21**. MS (ESI) m/z = 287 $[M+H]^+$. To a solution of **21** (0.47 g, 1.64 mmol) in methanol (16 mL) was added aqueous LiOH-H_2O (0.345 g in 12 mL of water, 8.21 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 1 h. The reaction mixture was acidified to pH 2 using diluted hydrochloric acid. The white precipitate was collected, washed with water and 30% of EtOAc/hexanes and dried to give 0.392 g (87.9%) of **22**; mp 202–204 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1 H), 8.74 (d, J = 1.2 Hz, 1 H), 8.66 (d, J = 1.2 Hz, 1 H), 7.53–7.50 (m, 2 H), 7.37 (dd, J = 8.4 Hz, 2.0 Hz, 1 H), 5.03 (s, 2 H). MS (ESI) m/z = 271 $[M-H]^-$.
10. *Experimental procedure for the preparation of compound* **24**: To a solution of **20** (0.5 g, 3.33 mmol) in anhydrous DMF (15 mL) were added K_2CO_3 (1.382 g,

10.0 mmol) and 2-chloro-pyrimidine-5-carboxylic acid methyl ester (0.575 g, 3.33 mmol) at room temperature. After stirring at room temperature for 25 h, the reaction mixture was cooled to 0 °C diluted with water (20 mL) and acidified to pH 2 using diluted hydrochloric acid. The white precipitate was collected, washed with water and dried to give 0.678 g of **23**; mp 117–118 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1 H), 9.08 (s, 2 H), 7.44–7.57 (m, 2 H), 7.31–7.40 (m, 1 H), 5.03 (s, 2 H), 3.88 (s, 3 H). MS (ESI) m/z = 287 $[M+H]^+$. To a solution of **23** (0.5 g, 1.75 mmol) in methanol (20 mL) was added aqueous LiOH (0.419 g in 15 mL of water, 17.5 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 1.5 h. After removed most of the methanol, the reaction mixture was cooled to 0 °C and acidified to pH 2 using diluted hydrochloric acid. The white precipitate was collected, washed with water and dried to give the crude product which was purified by chromatography on silica gel (hexane/THF/AcOH = 2:1:trace) to give 0.102 g (21%) of **24**; mp 195–196 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (br s, 1H), 9.04 (s, 2H), 7.51–7.49 (m, 2H), 7.35 (dd, J = 8.4 Hz, 2.0 Hz, 1 H), 5.03 (s, 2 H). MS (ESI) m/z = 273 $[M+H]^+$.

11. All β-lactamases were tested as essentially described by Payne et al *J. Antimicrob. Chemother.* **1991**, *28*, 775–776. with a few modifications. The buffer was 50 mM potassium phosphate pH 7 with 0.2% Triton x-100, and the concentration of nitrocefin was 500 μM for class A β-lactamases and 200 μM for class C β-lactamases. Kinetic data is collected by measuring the rate of change in A_{486} over 30 min. The fraction of enzyme inhibited is determined by dividing the reaction rates in the presence of inhibitor by the reaction rate determined in the absence of inhibitor. Dose–response curves are then generated by plotting log [inhibitor] versus fraction inhibited. IC_{50} values were determined from the dose-response curves by determining the inhibitor concentration required to reduce the maximum inhibitory activity of the compound by 50%. The K_i values were calculated from the IC_{50} using the K_m for nitrocefin for each enzyme and the following equation. $K_i = IC_{50}/(1 + [S]/K_m)$.