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Letter

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4,5-Disubstituted 6-Aryloxy-1,3-dihydrobenzo[*c*][1,2]oxaboroles Are Broad-Spectrum Serine β-Lactamase Inhibitors

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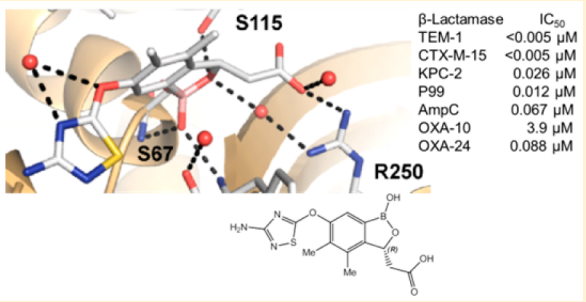
Supporting Information

ABSTRACT: Bacterially expressed β-lactamases are rapidly eroding the clinical utility of the important β-lactam class of antibacterials, significantly impairing our ability to fight serious bacterial infections. This paper describes a study of oxaborole-derived β-lactamase inhibitors in which crystal structures and computational modeling aided in the rational design of analogues with improved spectrum of activity against class A, C, and D enzymes. Crystal structures of two of these inhibitors covalently bound to two different serine β-lactamases, class C *Pseudomonas aeruginosa* AmpC and class D OXA-10, are described herein. Improved physicochemical properties as well as increased activity against an array of β-lactamases resulted in substantial restoration of susceptibility to ceftazidime in *Escherichia coli* and *Klebsiella pneumoniae*.



KEYWORDS: *oxaboroles, β-lactamase inhibitors, Gram-negative infections, structure-guided design*

The rapid rise of resistance to antibiotics among various pathogens is a significant threat to global health.[1] The β-lactams, once trusted stalwarts in our antibacterial armamentarium, are rapidly losing ground due to the expression of bacterial β-lactamases.[2] This resistance can be countered by co-administration with β-lactamase inhibitors (BLIs), but the established BLIs inhibit only a subset of class A enzymes, have limited efficacy against class C and D β-lactamases, and do not inhibit carbapenemases.[3] Whereas the recent approval of avibactam (in combination with ceftazidime) partially addresses this unmet medical need, its activity against class D β-lactamases remains limited. Thus, there remains a need for novel BLIs that are more broadly capable of inhibiting all three classes of serine β-lactamases. In this respect, a recent patent application describing oxaborole-derived BLIs, such as compounds **1** and **5** (Figure 1), presented an interesting starting point.[4,5] Given that boron-based BLIs[6] do not form an acyl–enzyme complex, but rather form a reversible covalent (dative) bond to the active-site serine, they are expected not to be susceptible to hydrolysis by evolving β-lactamases, unlike the clinically used BLIs that are derived from a β-lactam core.

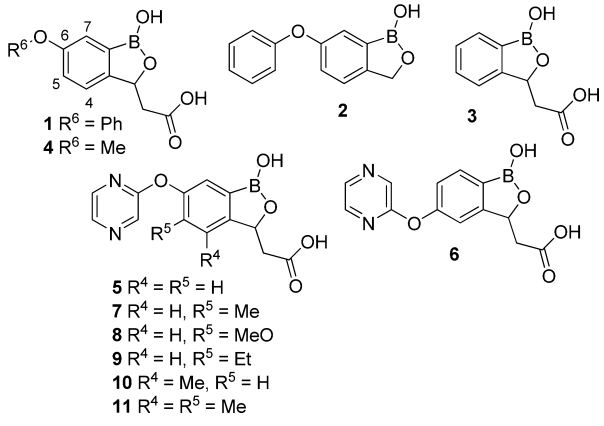
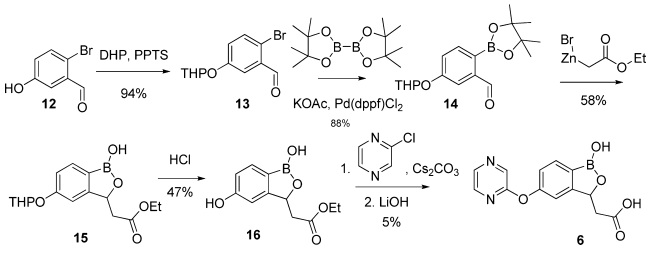


Figure 1. Structures and numbering scheme of oxaborole inhibitors.

Scheme 1. Synthesis of Oxaborole 6



Compounds **1**,[4] **2**,[7] **3**,[8] **4**,[4] and **5**[4] were prepared as described in the literature. Regioisomer **6** was accessible in an analogous manner, starting from 2-bromo-4-hydroxybenzaldehyde **12** as shown in Scheme 1. The 4-hydroxyl was protected with a

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Scheme 2. Synthesis of Oxaborole 11

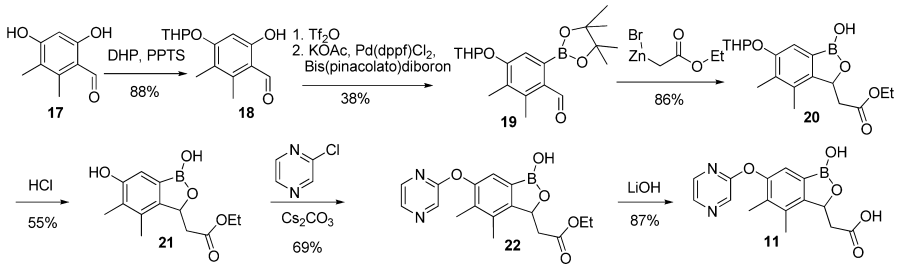
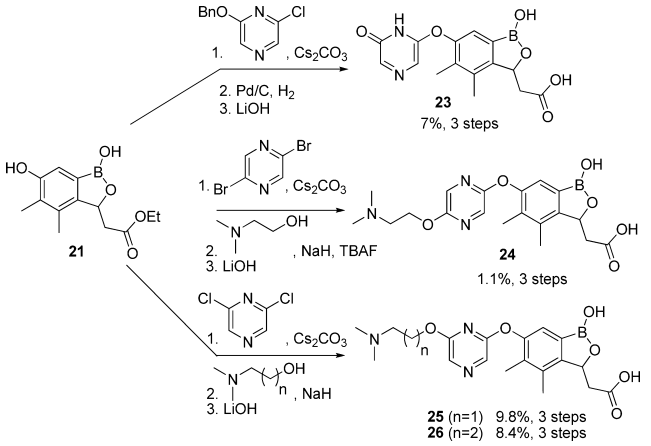


Table 1. Biochemical Activity against Representative β-Lactamases

	IC_{50} (μM)							
	class A			class C		class D		
compd	TEM-1	CTX-M-15	KPC-2	P99[a]	AmpC[b]	OXA-10	OXA-24	OXA-48
tazobactam	0.41	<0.007	39	6.1	3.4	4.6	77	3.6
1	0.18	1	0.29	0.29	4.2	19	4.2	1.7
2	14	35	22	4.6	7.2	>200	85	34
3	22	96	3.7	19	69	>200	17	11
4	1.9	23	1.2	7.3	46	>200	9.1	6.2
5	1.4	2.2	1.4	1.7	7.4	58	3.1	1.1
6	36	84	3.1	30	>200	>200	32	14
7	0.51	0.44	1.9	0.39	4.4	19	0.68	0.4
8	1.5	2.3	2	3.7	20	40	5.1	1.1
9	1.5	1.6	5.3	0.7	6	34	2.2	0.72
10	0.33	0.27	0.23	0.092	1	16	1.1	0.61
11 (rac)	0.23	0.055	0.62	0.041	1	12	1.2	0.5
11 (*R*)	0.15	0.025	0.44	0.026	0.73	8.2	0.83	0.38
11 (*S*)	5.1	4.7	3.6	3.1	69	56	23	1.3

[a]*E. cloacae* ARC3525. [b]*P. aeruginosa* PAO1, PDC-1.

Scheme 3. Synthesis of Pyrazine Variations



tetrahydropyran (THP) group, and the protected aryl bromide **13** was subjected to Miyaura-borylation[9] to give **14**. Addition of (2-ethoxy-2-oxoethyl)zinc(II) bromide to aldehyde **14** and concomitant boronic ester transesterification provided the benzoxaborole core **15**. After acid-catalyzed removal of the THP protective group, the pyrazine ether was formed by arylation of the resulting phenol **16** with chloropyrazine. Basic hydrolysis of the ethyl ester gave target acid **6**.

The enzymatic inhibition of oxaboroles **1**−**6**, as well as the approved BLI tazobactam, was evaluated against a series of clinically relevant serine β-lactamases, as summarized in Table 1. Tazobactam is a potent inhibitor of class A enzymes CTX-M-15, with an IC_{50} below 7 nM, and TEM-1 (0.41 μM). However, it is a much weaker inhibitor of class C enzymes P99 (6.1 μM) and AmpC (3.4 μM) and only very weakly inhibits the class A carbapenemase KPC-2 (39 μM). Whereas tazobactam shows some class D inhibition of OXA-10 (4.6 μM) and OXA-48 (3.6 μM), it lacks significant OXA-24 activity (77 μM). In contrast to tazobactam, oxaborole **1** demonstrates more evenly distributed activity, with single-digit micromolar activity against the tested class A and class C enzymes while showing modest activity against several class D enzymes. Evaluation of the activity of analogues **2**−**6** provides insights into the key pharmacophoric features required for inhibition of the different classes of β-lactamases. For example, removal of the carboxylic acid (**2**) resulted in a 10−100-fold decrease in inhibition of all enzymes except AmpC. Likewise, removal of the aryloxy substituent (**3**) resulted in a similar decrease in activity. In contrast to the carboxylic acid, the aryloxy moiety was essential for inhibition of AmpC, whereas activity against OXA-24 was less affected when this substituent was removed. Small alkyl ethers in the 6-position, as in compound **4**, regained some inhibitory activity but were not as potent as aryloxy ether **1**. Replacement of the phenyl group with a pyrazine was tolerated (**5**), but moving the aryloxy group from the 6-position to the 5-position was not (**6**).

Docking of the oxaborole **1** into the published AmpC[10,11] and OXA-10[12] structures suggested that there was very little room for substitution in the 7-position. Moreover, the OXA-10 model revealed a lipophilic pocket around the 4- and 5-positions that could be exploited by small lipophilic substituents extending from these positions, suggesting an

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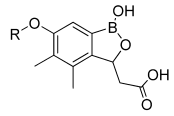
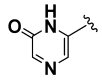
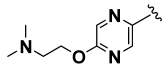
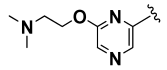
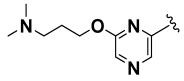
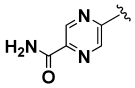
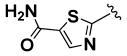
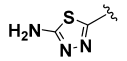
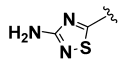
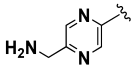
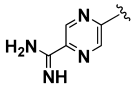
Table 2. Effect of Different Aryloxy Groups on Inhibitory Activity against Representative β-Lactamases

		Class A IC_{50} (μM)			Class C IC_{50} (μM)		Class D IC_{50} (μM)		
Cmpd	R	TEM-1	CTX-M-15	KPC-2	P99[a]	AmpC[b]	OXA-10	OXA-24	OXA-48
23		0.52	0.023	13	0.33	7.3	170	0.11	0.15
24		1.2	0.15	0.9	2.5	85	15	26	7.1
25		0.066	0.26	4.3	0.025	0.55	80	0.91	0.38
26		0.084	0.17	4.4	0.024	0.22	69	0.89	0.41
27		0.16	0.041	0.29	0.023	0.97	3.8	21	0.81
28 (*R*)		0.055	0.18	0.24	0.029	0.87	8.3	8.9	1.5
29		1.8	0.12	0.53	0.11	2	14	11	0.85
30		1.4	0.54	1.3	0.11	3.1	13	9.1	2.3
31		1.9	<0.005	0.37	0.015	0.12	9.5	4.8	1.1
32		0.13	0.016	0.19	0.039	2.3	4.5	6	0.75
33		0.11	<0.005	0.15	0.008	0.053	2.7	0.54	0.46
34		0.23	0.045	0.013	0.21	3.5	3.7	3.9	0.34
35		0.17	<0.007	0.021	0.49	15	2.1	4	1.1
36		0.075	0.012	0.071	0.014	0.44	6.2	3.9	0.31
37 (rac)		0.01	<0.005	0.041	<0.008	0.36	3.1	0.17	0.21
37 (*R*)		<0.005	<0.005	0.026	0.012	0.067	3.9	0.088	0.12
38		0.59	0.036	1	0.62	8.5	8.5	29	12
39		0.44	0.012	0.4	0.12	13	2	26	12

[a]*E. cloacae* ARC3525. [b]*P. aeruginosa* PAO1, PDC-1.

approach to compounds with increased affinity for class D enzymes. Hence, our initial design efforts focused on compounds substituted in the 4- and/or 5-position to take advantage of this pocket and to retain the more balanced spectrum already present in oxaboroles **1** and **5**. Because the enzymatic activities of oxaboroles **1** and **5** were similar and analogues of pyrazine-containing **5** were synthetically more accessible, oxaborole **5** was chosen as the starting point for further analogue design. Analogues of **5** were prepared from the corresponding bromobenzaldehyde (for **8**[13]) following the methods in Scheme 1 or from the corresponding dihydroxybenzaldehyde (for **7**,[14] **9**,[15] **10**,[16,17] and **11**[18]) following the methods shown in Scheme 2, as exemplified by compound **11**. Regioselective THP protection of dimethylresorcinol **17** to give phenol **18** was followed by triflate activation and subsequent Miyaura-borylation to provide aldehyde **19**, which was elaborated to the final compound by following the same route as described for the formation of **6** from **14** (Scheme 1).

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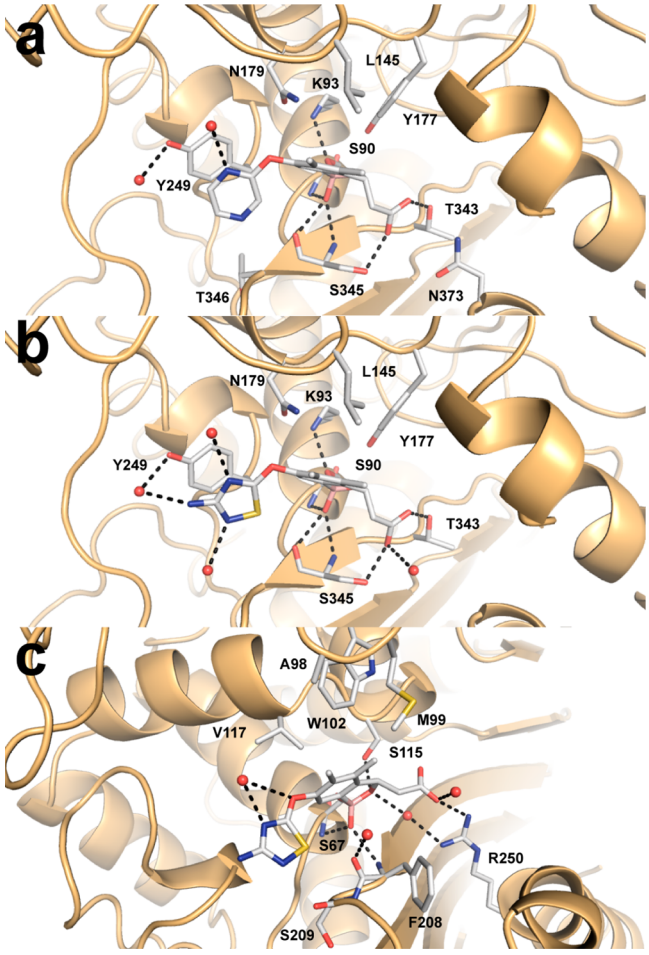

Figure 2. Crystallographic structures of *P. aeruginosa* AmpC and OXA-10 with covalently bound boron-containing inhibitors: (a) AmpC with compound **11**; (b) AmpC with **37**; (c) OXA-10 with **37**. The protein is shown as an orange ribbon. All residues placed within 3.5 Å of the bound compound and the compound itself are shown with white carbon atoms, blue nitrogen atoms, red oxygen atoms, and pink boron atoms. Water molecules are shown as red spheres. Hydrogen bonds are shown as black dashes.

Introduction of a methyl group in the 5-position (**7**) resulted in small increases in inhibitory activity against all enzymes tested, except KPC-2. Further substitution with slightly larger substituents (e.g., methoxy or ethyl, cf. **8** or **9**) resulted in decreased activity, suggesting that the lipophilic pocket was too small to accommodate these larger substituents. Moving the methyl group to the 4-position (**10**) resulted in increased activity against class A and C enzymes, but had little effect on class D activity. Combining substitution in both positions, as illustrated by compound **11**, resulted in improved potency over either substituent individually (compounds **7** and **10**). Chiral HPLC separation of the enantiomers of the final intermediate (ethyl ester **22**) followed by hydrolysis gave access to the two enantiomers of **11**. Evaluation of the individual enantiomers revealed that the majority of the activity of compound **11** resided in a single enantiomer (later determined to be the *R*-enantiomer, vide infra). The more active single enantiomer of **11** showed sub-micromolar activity against all tested isozymes with the exception of OXA-10 (8.2 μM). Attention was then focused on alternate heterocyles to replace the pyrazine and further increase affinity.

Analogues of compound **11** were synthesized from phenol **21** according to the methods discussed in the final steps of Scheme 2 (for **29** and **30**), sometimes requiring straightforward additional transformations after creation of the aryl ether (for compounds **27** and **31**–**39**) as described in the literature.[15] Compound **28** was synthesized following an alternative route (see the Supporting Information). Pyrazinone **23** and dimethylaminoalkoxypyrazines **24**–**26** were obtained according to Scheme 3. Thus, arylation of phenol **21** with benzyloxypyrazine chloride was followed by the removal of the benzyl protective group and ester hydrolysis to give pyrazinone **23**, whereas halide displacement led to dimethylaminoalkoxypyrazines **24**–**26**.

The activity of compounds **23**–**39** containing various aryl ethers is summarized in Table 2. Benzylic substituents and cycloalkyl substituents were poorly tolerated (data not shown). Removal of the pyrazine nitrogen in the 4-position (to give pyridine **27**) or both nitrogens (to give phenyl **28**) was well tolerated and resulted only in a loss of activity against OXA-24. On the other hand, moving the pyrazine nitrogen to give pyrimidines (cf. **29** and **30**) resulted in significant decreases in activity against all isozymes tested, with the exception of KPC-2. Pyrazinone **23** showed 10-fold improved activity against OXA-24, but this improvement was offset by decreased activity against the carbapenemase KPC-2, as well as AmpC and OXA-10. Substitution of the pyrazine with an amide group (**31**) resulted in improved activity against CTX-M-15 and AmpC, but decreased activity against TEM-1.

Replacement of the pyrazine group with a thiazole (**32**) was tolerated. Introduction of an amide group at the 5-position of this thiazole (**33**) again led to substantial improvements against CTX-M-15 and AmpC and additionally resulted in improved activity against OXA-24. In this case, no decrease in TEM-1 activity was observed. Thiadiazole **34** was roughly equipotent to thiazole **32**, whereas the regioisomeric thiadiazole **36** provided improved activity against AmpC. Amino substituents on these thiadiazoles (cf. **35** and **37**) were tolerated and resulted in increased activity against CTX-M-15 and, in the case of **37**, against OXA-24. Separation of enantiomers again showed that the majority of the activity of **37** resided in one enantiomer, again later determined to be the *R*-isomer (vide infra).

A crystal structure of **11** (rac) in complex with *Pseudomonas aeruginosa* AmpC showed the *R*-enantiomer bound to the active site (Figure 2a), supporting assignment of the *R* stereochemistry to the most active enantiomer. The binding mode was as predicted by our model and showed that the catalytic serine S90 formed the anticipated dative bond to the oxaborole boron, stabilized by a hydrogen bond to K93. The OH group of the oxaborole occupied the "oxyanion hole"[19] and made hydrogen bonds to the backbone amides of two serine residues, S90 and S345. The side chain of residue L145 made hydrophobic contacts with the R^4 and R^5 methyl groups. The 6-position pyrazine substituent formed a face-to-face parallel-displaced interaction[20] with the side chain of residue Y249, whereas the oxaborole carboxylate anchored the inhibitor to the active site through hydrogen bonds with the side chains of residues T343 and S345.

Similar binding modes were observed for two additional structures. The crystal structure of **37** (rac) with AmpC (Figure 2b) again showed binding of the *R*-enantiomer, which engaged in similar interactions as observed for compound **11** in AmpC (Figure 2a), with the addition of several water-mediated hydrogen bonds to the heterocycle. Comparison of the binding

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Table 3. Combination MIC with Ceftazidime[a]

	E. coli			*K. pneumoniae*			*C. freundii*	*A. baumannii*	*P. aeruginosa*
BLI	CTX-M-14	KPC-2, OXA-1, TEM-1	CTX-M-15, TEM-1, OXA-1	SHV-18, OXA-2, OKP-6	KPC-2, SHV-11, TEM-1, OXA-9	CTX-M-15, TEM-1, OXA-1, SHV-11	CMY-65, TEM-1	AmpC, OXA-23, PSE-2, OXA-69	VEB-1, OXA-10, AmpC
none	**4**	*8*	16	32	128	>256	128	32	>256
tazobactam	**0.5**	*8*	**0.5**	**2**	64	128	128	32	64
11	**0.25**	**1**	**4**	16	128	>256	**2**	32	>256
11R	**0.5**	**1**	**4**	32	128	>128	**0.5**	32	>128
25	**1**	**4**	16	32	128	>256	32	*16*	>256
26	**1**	*8*	16	32	256	>256	64	32	>256
27	**0.5**	**2**	*8*	64	128	>256	**4**	*16*	>256
28R	**1**	**1**	16	32	128	>128	*8*	128	>128
31	**1**	**2**	**4**	32	>64	>64	32	*16*	>64
32	**0.5**	**0.5**	**4**	32	128	>128	**2**	32	>128
33	**0.5**	**1**	**2**	32	128	>256	16	*16*	>256
34	**1**	**0.25**	**4**	32	128	>128	*8*	32	>128
35	**0.5**	**0.25**	**1**	**4**	16	128	16	*16*	>128
36	**1**	**0.5**	**4**	32	>64	>64	**2**	*16*	>64
37	**0.5**	**0.5**	**2**	**4**	16	>256	**4**	*16*	>256
37R	**0.25**	**0.25**	**1**	*8*	16	256	**1**	*16*	>256
38	**0.25**	**0.25**	**0.5**	16	128	>256	**2**	*16*	>256
39	**0.25**	**0.25**	**1**	16	128	>256	**4**	*16*	>256

[a]The table lists ceftazidime MICs (μg/mL) against pathogens expressing various β-lactamases, in the presence of a fixed concentration (4 μg/mL) of β-lactamase inhibitor. Values are related to the CLSI breakpoints as follows: **bold** indicates sensitive (S), *italics* indicates intermediate (I), and roman indicates resistant (R). The following breakpoints were used for ceftazidime: Enterobacteriaceae, S $\leq$ 4 μg/mL, I = 8 μg/mL, R $\geq$ 16 μg/mL; *P. aeruginosa* and *A. baumannii*, S $\leq$ 8 μg/mL, I = 16 μg/mL, R $\geq$ 32 μg/mL.

mode of **37** with OXA-10 (Figure 2c) and with *P. aeruginosa* AmpC (Figure 2b) revealed a similar orientation of the inhibitor in the two enzymes. Moreover, the structure in Figure 2c clearly shows the complementarity between the lipophilic patch in OXA-10 and the R^4 and R^5 Me groups.

Having identified several compounds with promising broad-spectrum enzymatic activity, we turned our attention to the ability of the oxaborole BLIs to restore activity of ceftazidime against resistant Gram-negative pathogens expressing various β-lactamases. As can be seen in Table 3, the pathogens in this study displayed various levels of resistance to ceftazidime. In the presence of a fixed amount (4 μg/mL) of the marketed BLI tazobactam, activity was improved in two strains of *Escherichia coli*, but tazobactam had no beneficial effect in the KPC-2-expressing strain of this pathogen. Moreover, tazobactam had little effect in *Klebsiella pneumoniae*, restoring susceptibility to ceftazidime in only one strain. No activity was noted in *Citrobacter freundii* and *Acinetobacter baumannii*, and only a negligible effect was observed in *P. aeruginosa*. In the presence of pyrazine **11**, susceptibility to ceftazidime was restored in all *E. coli* strains (including the KPC-2-expressing strain) as well as in *C. freundii*, but no activity was seen in *K. pneumoniae*, *A. baumanni*, and *P. aeruginosa*. Despite the 2-fold difference in concentration of the active moiety, little difference was seen between the racemate and the single enantiomer of **11**, suggesting that both reached the maximum effect at or below 4 μg/mL.

Pyridine **27**, phenyl **28**, and amidopyrazine **31** possessed similar activity as pyrazine **11** in *E. coli*, but were weaker in *C. freundii*. Replacement of the pyrazine in **11** with a thiazole (**32**), amidothiazole (**33**), or thiadiazole (**34** or **36**) resulted in similar activity. Aminothiadiazoles **35** and **37** led to restoration of activity in *E. coli* and improved restoration in *K. pneumoniae*. Compound **37** restored susceptibility in *C. freundii*, whereas compound **35** did not. As observed for pyrazine **11**, little differences were observed between the racemate and single enantiomer for compound **37**, again suggesting that both reached the maximum effect at or below 4 μg/mL. The data in Table 3 suggest that compound **37** is a more potent and a broader spectrum inhibitor than tazobactam, restoring activity of ceftazidime in both an additional *E. coli* and a *C. freundii* strain. However, its poor activity against *K. pneumoniae* and the lack of activity against *P. aeruginosa* and *A. baumannii* remained problematic. We reasoned that perhaps the physicochemical properties of these oxaboroles were not compatible with their adequate accumulation in the periplasm of these pathogens. The introduction of basic amines to these oxaborole acids was explored because Zwitterionic compounds often demonstrate significant improvements in permeation relative to mono-charged species.[21]

Introduction of a basic amine extending from the para-position of the pyrazine was not well tolerated by the class D enzymes or AmpC (cf. compounds **24**, **38**, and **39**, Table 2). Our model of the binding mode suggested that steric hindrance in AmpC was responsible for decreased activity of these compounds and that a vector from the meta-position would encounter fewer steric clashes. Similar trends were previously observed on a related oxaborole scaffold.[5] Indeed, introduction of the basic substituent in the meta-position was better tolerated by class C enzymes as well as OXA-24 and OXA-48, although this resulted in a significant loss of activity against CTX-M-15, KPC-2, and OXA-10 (**25** and **26**). Para-substituted compounds **38** and **39** were able to restore susceptibility to ceftazidime in *E. coli*. The meta-substituted compounds **25** and **26** were significantly less potent in *E. coli*, presumably reflecting their diminished activity against CTX-M and KPC β-lactamases. None of the compounds with basic amines showed

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ACS Infect. Dis. 2015, 1, 310−316

increased potency against *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*.

At this point, compound **37** was identified as having the most promising activity in this series. Although **37** represented a significant improvement over the starting point (**1**), as well as tazobactam, and provided potent restoration of ceftazidime susceptibility in *E. coli* and *C. freundii*, including strains containing KPCs, its activity against other problematic Gram-negative pathogens (*K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*) was deemed insufficient to warrant further development. Despite this disappointing outcome, the data in this paper clearly show that oxaboroles can act as broad-spectrum BLIs. Moreover, the potent restoration of ceftazidime activity in *E. coli* proves that the tetrahedral adduct between an oxaborole and the active site serine can lead to physiologically relevant inhibition of β-lactamases. In this respect, it should be noted that a narrow-spectrum boronate β-lactamase inhibitor (RPX7009) is currently in phase 3 clinical trial in combination with biapenem (Carbavance).[22] The potent enzymatic activity across multiple classes of serine β-lactamases and promising activity in *E. coli* warrant the continued search for oxaborole scaffolds[23] with different physicochemical properties that can lead to improved permeation into additional Gram-negative pathogens, most notably *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.5b00031.

Full experimental details for synthesis of compounds **11**, **28**, and **37**, crystallography, and descriptions of biological assays (PDF)

Accession Codes

The coordinates and structure factors deposited into the Protein Data Bank are under the following codes: 4WYY of AmpC with compound **11**, 4WZ4 of AmpC with compound **37**, and 4WZ5 of OXA-10 with compound **37**.

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Author Contributions

J.C.V. and D.C.M. drafted the manuscript. J.C.V., C.J.E., and S.L. participated in the design and execution of this study. F.Z. and D.C.M. designed and performed the chemical syntheses. A.D.F. and D.B.P. performed the crystal structure determination studies. J.B. executed the majority of enzyme inhibition studies. R.A.G. designed and executed the majority of the MIC restoration studies.

Notes

The authors declare the following competing financial interest(s): The authors are current or former employees of AstraZeneca and may possess AstraZeneca stock and/or stock options.

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